

Evidence for Multiple Cycles of Strand Invasion During Repair of Double-Strand Gaps in *Drosophila*

Mitch McVey,^{*,1} Melissa Adams,^{†,1} Eric Staeva-Vieira^{‡,2} and Jeff J. Sekelsky^{†,§,3}

[†]Department of Biology, ^{*}SPIRE Program and [§]Program in Molecular Biology and Biotechnology, University of North Carolina, Chapel Hill, North Carolina 27599 and [‡]Developmental Genetics Program, Skirball Institute of Biomolecular Medicine, New York University School of Medicine, New York, New York 10016

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ABSTRACT

DNA double-strand breaks (DSBs), a major source of genome instability, are often repaired through homologous recombination pathways. Models for these pathways have been proposed, but the precise mechanisms and the rules governing their use remain unclear. In *Drosophila*, the synthesis-dependent strand annealing (SDSA) model can explain most DSB repair. To investigate SDSA, we induced DSBs by excision of a *P* element from the male *X* chromosome, which produces a 14-kb gap relative to the sister chromatid. In wild-type males, repair synthesis tracts are usually long, resulting in frequent restoration of the *P* element. However, repair synthesis is often incomplete, resulting in internally deleted *P* elements. We examined the effects of mutations in *spn-A*, which encodes the *Drosophila* Rad51 ortholog. As expected, there is little or no repair synthesis in homozygous *spn-A* mutants after *P* excision. However, heterozygosity for *spn-A* mutations also resulted in dramatic reductions in the lengths of repair synthesis tracts. These findings support a model in which repair DNA synthesis is not highly processive. We discuss a model wherein repair of a double-strand gap requires multiple cycles of strand invasion, synthesis, and dissociation of the nascent strand. After dissociation, the nascent strand may anneal to a complementary single strand, reinvade a template to be extended by additional synthesis, or undergo end joining. This model can explain aborted SDSA repair events and the prevalence of internally deleted transposable elements in genomes.

THE repair of DNA double-strand breaks (DSBs) is of critical importance for the maintenance of genomic integrity and cell survival. In humans, a number of hereditary disorders are associated with genome instability that results from failure to properly repair spontaneous DSBs that occur during normal DNA metabolism (THOMPSON and SCHILD 2002). DSBs are repaired by proteins that act in both independent and overlapping pathways. These pathways are typically divided into two classes, homologous recombination (HR) and nonhomologous end joining (NHEJ), which differ in their requirement for a homologous DNA template and in the fidelity of repair (reviewed in HABER 2000).

HR encompasses several genetically defined pathways that utilize homology comparison and templated DNA synthesis during the repair process. These pathways are generally conservative and therefore preserve genomic integrity. In contrast, during NHEJ the ends of the break are joined without consulting external homologies, and degradation of the ends prior to ligation can lead to

loss of genetic information. Different organisms use the various DSB repair pathways to different extents, depending on the stage of the cell cycle or the availability of a homologous template. In some instances coupling of HR and end-joining repair mechanisms has been observed (RICHARDSON and JASIN 2000; ADAMS *et al.* 2003). Such observations suggest that although HR and NHEJ are mechanistically distinct, they are both part of one integrated, multicomponent response to DSBs.

Studies in *Drosophila melanogaster* indicate that DSBs remaining after *P*-element transposon excision in mitotic cells are repaired primarily by HR mechanisms (ENGELS *et al.* 1990). A pathway termed synthesis-dependent strand annealing (SDSA) was described to account for the observed recombinational repair products (KURKULOS *et al.* 1994; NASSIF *et al.* 1994). In the canonical SDSA model the 3' end of one side of a DSB invades a homologous template and primes repair DNA synthesis. Newly synthesized DNA is displaced from the template, and the resulting single-stranded DNA can anneal at a region of complementarity. DSB repair by SDSA can result in gene conversion, but does not generally result in reciprocal crossovers. In *Drosophila*, compelling evidence for the SDSA model comes from assays in which repair must traverse a large gap that contains one or more direct repeats (KURKULOS *et al.* 1994; PÂQUES *et al.* 1998; ADAMS

¹These authors contributed equally to this work.

²Present address: New York Academy of Sciences, New York, NY 10021.

³Corresponding author: Department of Biology, CB 3280, 303 Fordham Hall, University of North Carolina, Chapel Hill, NC 27599.
E-mail: sekelsky@unc.edu

et al. 2003). If repair synthesis is primed by both ends of the DSB, then single-stranded DNA that is complementary at the repeat sequences is generated. Annealing at the direct repeats can generate a product that is precisely deleted for one repeat and any intervening sequences.

The DNA synthesis reaction carried out during SDSA is mechanistically distinct from DNA replication that occurs during S phase. In contrast to normal semi-conservative DNA replication in which nascent strands remain associated with template strands, the DNA synthesized during SDSA dissociates from the template and anneals back to the recipient molecule. Among the features of SDSA that are unknown are whether repair synthesis is processive and whether the nascent strand is completely dissociated from the template before annealing. According to the bubble migration model, the nascent strand is extruded from a migrating replication bubble, and processive DNA synthesis continues until sequences complementary to the other end of the break are generated. This model was originally proposed to explain a bacteriophage T4 recombination reaction (FORMOSA and ALBERTS 1986), but has also been used to explain gap repair in *Drosophila* (NASSIF *et al.* 1994).

Alternatively, SDSA may entail a relatively short stretch of repair synthesis, after which the entire synthesis complex is dissociated, releasing the nascent strand to search for a complementary sequence. In the absence of a complementary single strand, the nascent strand may be further extended by a subsequent cycle of strand invasion and synthesis. Consistent with this model is the observation that when a DSB is made adjacent to an array of tandem repeats, both expansions and contractions of the array are observed in the repair products, although the template remains intact (PÂQUES *et al.* 1998). One way that changes in repeat number can come about is if the nascent strand dissociates from the template and subsequently reinvasades, with misalignment of repeats.

Homologous pairing and strand invasion, key steps in repair by SDSA, are promoted by the strand exchange protein Rad51 (reviewed in SUNG *et al.* 2003). Rad51 forms a helical nucleoprotein filament on the 3' end of a resected DSB and catalyzes the ATP-dependent invasion of a homologous duplex. Repair DNA synthesis is subsequently primed by the end of the invading strand. If repair synthesis is not processive, then repeated rounds of Rad51-mediated strand invasion and DNA synthesis may be necessary to replicate across a large gap.

We recently developed a DSB repair assay based on excision of the $P\{w^a\}$ transgene (Figure 1; ADAMS *et al.* 2003). To further probe the mechanism by which repair DNA synthesis proceeds during SDSA and to gain additional insight into the function of DmBlm in repair synthesis, we used this assay to determine the consequences of removing or reducing the amount of DmRad51. DmRad51 is encoded by the *spn-A* locus (STAEVA-VIEIRA *et al.* 2003). Null mutations in this gene

result in female sterility, as a secondary consequence of failure to repair meiotic DSBs. These mutants are hypersensitive to ionizing radiation, suggesting a defect in mitotic DSB repair. We report here the mitotic DSB repair phenotype of *spn-A* mutants. As expected, our results demonstrate an absolute requirement for DmRad51 in DSB repair by SDSA. Nearly all of the DSB repair products recovered from *spn-A* mutants were consistent with repair by an end-joining pathway. Surprisingly, we found that reducing the dose of DmRad51 by half results in an intermediate DSB repair phenotype characterized by a decreased frequency of complete repair by SDSA and short repair synthesis tracts among aborted SDSA repair events. We believe our results are best explained by a model in which repair of the gap created by $P\{w^a\}$ excision requires multiple cycles of strand invasion and repair DNA synthesis.

MATERIALS AND METHODS

Drosophila stocks and genetics: The $P\{w^a\}$ transgene used in this study is described in KURKULOS *et al.* (1994) and ADAMS *et al.* (2003). *spn-A*⁰⁹³ is a nonsense mutation that eliminates detectable DmRad51 protein, and *spn-A*⁰⁵⁷ is a missense mutation that behaves as a genetically null allele (STAEVA-VIEIRA *et al.* 2003). Other genetic components are described in FLY-BASE (2001). The transposase source we used in previous work, $P\{\text{ry}^+, \Delta 2-3\}$ (99B), maps very near *spn-A*. This necessitated the use of a different transposase source in these experiments, $H\{w^+, \Delta 2-3\}$ Hop2.1. All experiments reported here, both wild type and controls, used this transposase source.

Flies were reared on standard cornmeal agar medium at 25°. For the $P\{w^a\}$ assay, single males of the genotype $y w P\{w^a\}/Y; +/CyO, H\{w^+, \Delta 2-3\}; ru st e ca spn-A^{057}/ru h st th cu e ca spn-A^{093}$ (or various combinations of wild-type and mutant *spn-A* alleles) were crossed to four $y w P\{w^a\}$ virgin females in vials, and the eye colors of all Cy^+ female progeny were scored. To recover aberrant repair events for molecular analysis, a single yellow-eyed female from each vial was crossed to *FM7w* males. Genomic DNA was isolated from white-eyed male offspring of this cross. The absence of white-eyed sons was taken to indicate that a particular repair event was associated with a lethal mutation resulting from deletion into the flanking *sd* gene. This was confirmed by crossing to *sd'* males and scoring for a scalloped-wing phenotype.

Molecular analysis of aberrant repair events: Repair synthesis tract lengths were determined as described in ADAMS *et al.* (2003). Briefly, genomic DNA was prepared from single flies according to GLOOR *et al.* (1993). PCR reactions contained 10 mM Tris-HCl, pH 9.0, 50 mM KCl, 2.5 mM MgCl₂, 0.1% Triton X-100, 1.25 μM each primer, 250 μM dNTPs, 2 μl of the genomic DNA prep, and TAQ polymerase in a 20 μl volume. PCR products were analyzed by agarose gel electrophoresis followed by ethidium bromide staining. Positive and negative controls were included in each set of reactions.

Gamma-ray sensitivity: To measure relative survival after exposure to ionizing radiation, embryos were collected overnight on grape agar supplemented with yeast paste and then allowed to age for 3 days, so that larvae were in late second or early third instar. The plate was exposed to gamma radiation in a Gammator 50 ¹³⁷Cs irradiator. Irradiated larvae were transferred to standard food bottles and allowed to develop. Adult progeny were counted through day 18 after egg laying.

RESULTS AND DISCUSSION

The $P\{w^a\}$ assay for DSB repair: In *Drosophila*, most DSBs are repaired through the SDSA pathway (KURKULOS *et al.* 1994; NASSIF *et al.* 1994; ADAMS *et al.* 2003). To determine the requirements for SDSA and to explore mechanisms of DSB repair when SDSA is compromised, we conducted repair assays in flies mutant for *spn-A*, which encodes the *Drosophila* ortholog of the Rad51 strand exchange protein (STAEVA-VIEIRA *et al.* 2003). We used a previously characterized genetic system to create site-specific DSBs and follow their repair (ADAMS *et al.* 2003). This assay makes use of a $P\{w^a\}$ transgene, which carries a *white* gene interrupted by a *copia* retrotransposon. The presence of *copia* reduces expression of *white* so that females with one copy of $P\{w^a\}$ (and in which the endogenous *white* gene is mutant) have yellow eyes, whereas those with two copies have apricot eyes.

Females homozygous for an X chromosome insertion of $P\{w^a\}$ are crossed to males carrying an autosomal P-transposase source. All male progeny inherit an X chromosome carrying $P\{w^a\}$. In the males that also inherit the transposase source, excision of $P\{w^a\}$ occurs throughout development of both the germline and the soma. Somatic excision and repair is evident by mosaicism in the adult eye. Germline DSB repair events are assayed by crossing these males back to females homozygous for $P\{w^a\}$. The paternal X chromosomes that may have experienced excision and repair in the male germline are transmitted to daughters. These daughters also inherit a maternal X chromosome with an intact $P\{w^a\}$. Eye color is scored in daughters that did not inherit transposase, thereby ensuring the stability of the repair event, and repair events are analyzed molecularly in their sons.

During the excision reaction, transposase cuts at both ends of the P element. Because both ends are required for cutting, transposase is believed to make both cuts in a coordinated fashion (SVOBODA *et al.* 1995; BEALL and RIO 1997). The $P\{w^a\}$ used in this assay is on the male X chromosome, so the only available template for homologous repair is the sister chromatid. Thus, the lesion remaining after excision may behave like a single DSB in end-joining pathways or like a 14-kb gap in repair by homologous recombination pathways (Figure 1). Repair by SDSA can lead to restoration of an intact $P\{w^a\}$ transgene, an event that is indistinguishable from a failure of the element to excise at all (Figure 1B). However, if repair synthesis is initiated from both ends of the break and proceeds through the *copia* long terminal repeat (LTR) sequences, these LTRs may anneal, resulting in a P element that has only a single LTR at the *copia* insertion site. Flies inheriting this type of repair event have red eyes (KURKULOS *et al.* 1994). Thus, the percentage of red-eyed progeny recovered provides a direct measure of the frequency of successful repair by SDSA. Repair by other pathways, including aborted

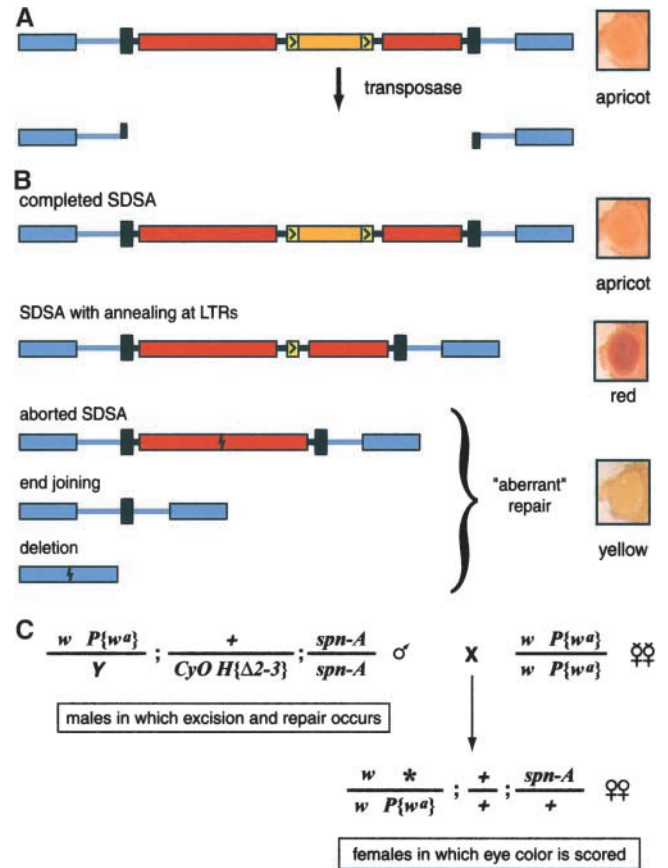


FIGURE 1.— $P\{w^a\}$ excision creates double-strand breaks that are repaired to produce distinct classes of products. (A) $P\{w^a\}$ inserted into an intron of *scalloped* (blue). P-element inverted repeats (black) flank the *white* gene (red), which contains a *copia* retrotransposon (orange) that has 276-bp long terminal repeats (LTRs, yellow). Expression of transposase in males carrying $P\{w^a\}$ causes excision, resulting in a double-stranded gap with 17-nucleotide noncomplementary 3' overhangs. (B) The mechanism of double-strand gap repair can be inferred from progeny eye color. Completed SDSA, resulting in restoration of $P\{w^a\}$, gives apricot-colored eyes in females containing a maternally inherited copy of $P\{w^a\}$. SDSA with annealing at the LTRs produces a product with increased *white* expression, resulting in flat red eyes. Aberrant repair results in loss of *white* expression and yellow-eyed progeny. (C) Cross scheme used to analyze repair events genetically and molecularly. Males with transposase and an X-linked $P\{w^a\}$ insertion are crossed to females homozygous for the same insertion, and eye colors are scored among daughters that did not inherit the transposase. Asterisk indicates the $P\{w^a\}$ element that may have excised and been repaired. Some molecular analyses are performed on these females, and others are performed on their sons.

SDSA or end joining, results in loss of *white* sequences, which in our assay manifests as progeny with yellow eyes (due to the intact copy of $P\{w^a\}$ inherited from the mother). For simplicity, we refer to this class collectively as “aberrant repair.” An important feature of our assay is that these aberrant repair events can be characterized molecularly to gain insight into their origins.

DmRad51 is required for SDSA: We conducted the

TABLE 1
Repair of $P\{w^a\}$ excisions in wild type and in *spn-A* mutants

Experiment	Relevant genotype ^a		Progeny ^b			
	Maternal	Zygotic	<i>n</i>	% apricot	% red	% yellow
1	+ / +	+ / +	3,624	84	6	10
2	<i>spn-A</i> / +	<i>spn-A</i> / <i>spn-A</i>	9,502	80	0	20
3	<i>spn-A</i> / +	<i>spn-A</i> / +	10,414	80	3	17
4	<i>spn-A</i> / +	+ / +	1,687	82	2	16
5	+ / +	<i>spn-A</i> / +	2,221	80	6	14

^a Zygotic indicates the genotype of the males in which excision and repair occurred; maternal indicates the genotype of the mothers of these males. Only the genotype with respect to *spn-A* is shown.

^b Female progeny of males in which excision and repair took place.

$P\{w^a\}$ assay described above in wild-type males and in males carrying null mutations in *spn-A*, the gene that encodes DmRad51 (STAEVA-VIEIRA *et al.* 2003). Wild-type males had mosaic eyes containing patches of red-pigmented cells (data not shown), confirming that excision of $P\{w^a\}$ and repair by SDSA occurred during development of somatic tissues. In contrast, the eyes of homozygous *spn-A* mutant males had a complete lack of red patches, suggesting a defect in SDSA. To characterize individual repair events, we crossed these males to females homozygous for $P\{w^a\}$ and scored female progeny that did not receive transposase (Figure 1C). The majority (84%) of the female progeny from wild-type males had apricot-colored eyes (Table 1, experiment 1). Most of these were probably derived from germline cells in which $P\{w^a\}$ did not undergo excision, but some likely resulted from excision followed by a repair event that restored the entire $P\{w^a\}$ element. Six percent of the progeny of wild-type males had red eyes, representing repair by SDSA with LTR annealing. PCR analysis confirmed that the red-eye phenotype resulted from this type of repair event (data not shown). The remaining 10% had yellow eyes, indicating some form of aberrant repair. The percentages of red- and yellow-eyed progeny from wild-type males are elevated compared to our previous report (ADAMS *et al.* 2003). For technical reasons, we used a different source of *P* transposase in these experiments (see MATERIALS AND METHODS), and we believe that the difference is due to elevated transposase expression or activity causing a higher rate of $P\{w^a\}$ excision.

In contrast to the results obtained with wild-type males, we recovered no red-eyed progeny from *spn-A* mutant males (Table 1, experiment 2). This result demonstrates that, as expected, repair of double-strand breaks by the SDSA pathway requires DmRad51. Although *spn-A* mutants are extremely hypersensitive to ionizing radiation during development (STAEVA-VIEIRA *et al.* 2003), we did not detect any decrease in viability, fertility, or fecundity of *spn-A* males that had both $P\{w^a\}$ and transposase. This suggests that a single DSB can be repaired effi-

ciently in the absence of DmRad51. The 80% of progeny with apricot eyes are presumably all derived from germline cells that did not experience an excision of $P\{w^a\}$, whereas the recovery of 20% of the progeny with yellow eyes provides a good estimate of the frequency of excision in this assay.

DmRad51 is required for initiation of repair DNA synthesis: We characterized the structures of the aberrant repair events recovered in yellow-eyed progeny. We used a PCR-based assay to determine whether SDSA was initiated and to measure the lengths of repair DNA synthesis tracts from one end of the DSB. As in our previous experiments (ADAMS *et al.* 2003), most aberrant repair events generated in wild-type flies had initiated repair synthesis: 82 of 85 (96%) independent events analyzed had evidence for at least 5 nucleotides of synthesis and 45 (53%) had evidence for at least 2400 nucleotides of synthesis (Figure 2). We stress that these numbers are based on analysis of yellow-eyed females only, which represent only about half of all repair events. In the other half, SDSA is completed, giving rise to progeny with red or apricot eyes. All of these repair events are associated with long synthesis tracts.

Most of the aberrant events generated in *spn-A* mutants had no detectable synthesis tracts. We did detect synthesis in 7 of 116 (6%) independent events analyzed. These tracts were usually less than a few hundred base pairs long, and none were as long as 0.9 kb. We attribute these rare, short synthesis tracts in *spn-A* mutants to maternally deposited DmRad51 (see below). However, we cannot exclude the possibility that they represent a small amount of DmRad51-independent repair synthesis.

We also attempted to amplify each aberrant repair event using PCR primers flanking the DSB site. We obtained products from 115 of 116 independent repair events derived from *spn-A* mutants. The sizes of these PCR products were consistent with little or no repair synthesis and little or no degradation of the ends. In contrast, we obtained PCR products from only 5 of 85 (6%) repair events generated in wild-type males. The

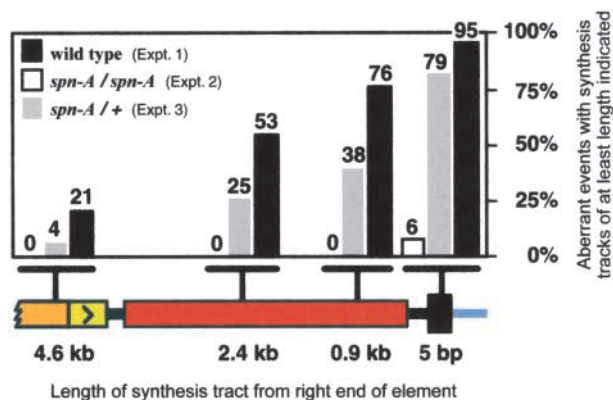


FIGURE 2.—Reduced dosage of *spn-A* results in short repair synthesis tracts. DNA extracted from male progeny of yellow-eyed females was analyzed by PCR. The diagram represents the right side of the $P\{w^a\}$ element. Transposase makes a DSB in the inverted repeat (black box). Sequences internal to the element (to the left) are present only if they have been introduced by repair synthesis. Bars represent percentage of aberrant events in which we obtained evidence for a synthesis tract *at least* as far as the $P\{w^a\}$ primer indicated. For example, 96% of the aberrant events from wild-type males had evidence for at least 5 bp of synthesis, whereas only 76% had evidence for tracts of at least 0.9 kb. Therefore, 20% of the repair events stopped between these two markers. Aberrant repair events from different males, which are therefore independent of one another, were analyzed for each genotype. $n = 83$ (wild type), 113 (*spn-A/spn-A*), and 117 (*spn-A/+*).

sizes of those products were consistent with synthesis tracts up to 2 kb in length. Most of the remaining repair events involved repair synthesis tracts too long to be amplified under the conditions used in this experiment.

Repair synthesis tracts are short when DmRad51 is limiting: No defects have been reported for *rad51* heterozygosity in yeast or mice. However, when we assayed repair of DSBs caused by $P\{w^a\}$ excision in *spn-A* heterozygous males, we observed a prominent defect in the ability to complete SDSA repair (Table 1, experiment 3). The percentage of red-eyed progeny (representing repair by SDSA) from *spn-A* heterozygous males was reduced 50% relative to wild type, whereas the percentage of yellow-eyed progeny (representing aberrant repair) increased. This outcome is significantly different from both wild type and *spn-A* homozygous mutants ($P < 0.0001$ by chi-square test).

To determine the defect in repair when DmRad51 is limiting, we quantified repair synthesis tract lengths among aberrant repair events (Figure 2). We found evidence for the initiation of repair synthesis in 79% of the aberrant repair events produced by *spn-A* heterozygotes. However, the synthesis tracts were, on average, much shorter than those produced in a wild-type background. Thus, *spn-A* heterozygotes are able to initiate SDSA, but this process often aborts prior to complete restoration of $P\{w^a\}$, and repair proceeds by end joining. Repair junctions from *spn-A* heterozygotes were

similar in structure to those obtained from wild-type and *spn-A* homozygotes (M. McVEY and J. SEKELSKY, unpublished data).

STAEVA-VIEIRA *et al.* (2003) found that homozygous *spn-A* mutants are weakly sensitive to ionizing radiation during embryonic stages, but exhibit increased sensitivity in subsequent larval stages. They attributed this to a maternal supply of DmRad51 that can function during embryonic development, but that is progressively lost with increasing developmental age. The *spn-A* heterozygous males used in experiment 3 were derived from mothers that were also heterozygous for *spn-A*, so it is possible that a reduced maternal contribution of DmRad51 could account for the SDSA defect we observed in this experiment. Indeed, STAEVA-VIEIRA *et al.* (2003) found that ovaries of *spn-A* heterozygous females had less DmRad51 than ovaries of wild-type females, although they did not detect any effect of heterozygosity on female fertility.

We conducted additional assays to determine whether the haplo-insufficiency we observed was maternal or zygotic in origin. First, we recovered repair products from genotypically wild-type males whose mothers were heterozygous for *spn-A* mutations (Table 1, experiment 4). We observed an SDSA defect similar to that observed in experiment 3—a reduction in the number of red-eyed progeny, an increase in the number of yellow-eyed progeny, and shorter synthesis tracts among the yellow-eyed progeny. In experiment 5, we recovered repair products from *spn-A* heterozygous males whose mothers were wild type for *spn-A*. These males showed only a modest defect in SDSA, with an increase in the percentage of yellow-eyed progeny, but no decrease in the percentage of red-eyed progeny.

The results described above indicate that the decreased ability to generate long synthesis tracts during SDSA repair is due primarily to decreases in the maternal supply of DmRad51. Nonetheless, the differences between experiments 3 and 4 are statistically significant ($P = 0.04$), suggesting that zygotic heterozygosity for *spn-A* impairs DSB repair to some extent. However, we did not detect any difference in sensitivity to ionizing radiation between wild-type and *spn-A* heterozygous larvae at doses that are completely lethal to *spn-A* homozygotes (Table 2). The excision assay described here allows us to detect differences in the type of DSB repair, rather than the absolute ability to repair a DSB. Indeed, *spn-A* homozygotes are extremely sensitive to killing by ionizing radiation, whereas they are perfectly capable of repairing a single DSB, albeit only by end-joining pathways.

Decreased tract length suggests that DNA synthesis during SDSA is not highly processive: Decreasing the amount of DmRad51 has a clear effect on the ability to repair accurately just one double-stranded gap, resulting in shorter repair synthesis tracts. It is possible that DmRad51 has a direct effect on processivity or repair

TABLE 2

Survival of *spn-A* homozygotes and heterozygotes after exposure to ionizing radiation during larval development

Genotype ^a	No. of adults (relative survival) ^b			
	0 rad	1000 rad	2000 rad	3000 rad
Cross 1: <i>spn-A</i> ⁰⁹³ / <i>TM3</i> ♀♀ × +/+ ♂♂				
+/ <i>TM3</i>	1424	629	1027	1108
<i>spn-A</i> /+	1360 (1.0)	695 (1.16)	1042 (1.06)	1035 (0.98)
Cross 2: <i>spn-A</i> ⁰⁹³ / <i>TM3</i> ♀♀ × <i>spn-A057</i> /+ ♂♂				
+/ <i>TM3</i>	453	568	599	795
<i>spn-A</i> / <i>spn-A</i>	544 (1.0)	71 (0.10)	0 (0.0)	0 (0.0)

^a Progeny from two different genotypic classes were counted for each cross.

^b The number in parentheses is the relative survival of the mutant genotype, relative to the control genotype. The ratio between these classes in unirradiated controls is taken as a relative survival of 1.0.

synthesis, but extensive genetic and biochemical studies of Rad51 in other organisms have not detected such a role (reviewed in SUNG *et al.* 2003). The well-characterized function of Rad51 is to catalyze the strand invasion reaction that is a prerequisite for repair DNA synthesis; after strand invasion, Rad51 is dissociated from the invading strand (SOLINGER *et al.* 2002). Thus, it is difficult to envision a model in which DmRad51 has a direct effect on synthesis tract length.

We favor an alternative model in which decreasing the level of DmRad51 results in some strand invasion attempts being unsuccessful. For human Rad51, *in vitro* homologous pairing and strand transfer require approximately one monomer per three nucleotides of ssDNA, and decreasing the amount of hRad51 below this stoichiometry abolishes detectable strand invasion (BAUMANN and WEST 1997). If repair DNA synthesis is highly processive, a single successful strand invasion reaction should be sufficient for gap repair. This hypothesis predicts that decreasing the amount of DmRad51 would result in fewer synthesis tracts, but those tracts that occur should be the same length in wild-type and mutant flies. We did observe fewer synthesis tracts when DmRad51 was limiting, but those tracts were shorter than tracts from wild-type flies. We believe this result is best explained by a model in which repair synthesis is not highly processive. According to this model, repair of a large gap requires multiple cycles of strand invasion and synthesis. A decreased probability of successful strand invasion in each cycle would result in an overall decrease in synthesis tract length, consistent with our observations. It is unknown whether the single-stranded region generated by synthesis and dissociation remains single stranded. If so, it may be that additional DmRad51 is needed after each cycle, so that later cycles have a greater probability of failure to achieve strand invasion than earlier cycles.

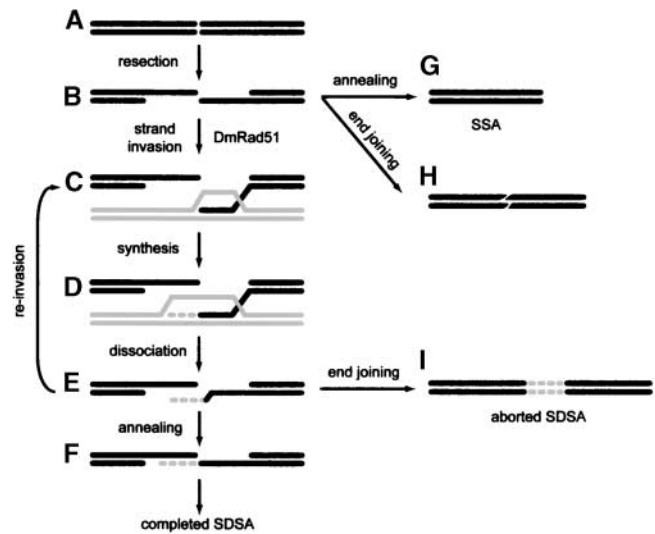


FIGURE 3.—Model for homologous repair of DSBs in *Drosophila*. The ends of a DSB (A) are resected to generate single-stranded 3' ends (B). An end can invade a homologous duplex (C) to prime repair synthesis (D). Dissociation of this complex (E) results in a product with a 3' end extended beyond the position of the initial DSB. This sequence can anneal to a complementary sequence at the other end of the break (F). If complementary sequences have not been generated, the end can reinvade a homologous duplex to be further extended by an additional episode of synthesis. If the break was made between direct repeats (not shown), annealing can take place without any synthesis, through the single-strand annealing (SSA) pathway (G). DSBs can also be mended by an end-joining mechanism. In the absence of DmRad51, this occurs with no repair synthesis (H), but in wild-type flies, end joining can take place after some repair synthesis (I).

We cannot determine the length of repair synthesis in a typical cycle, but our results suggest that the synthesis step is not highly processive. We detected repair synthesis in only 11 of 113 aberrant repair events generated in *spn-A* homozygous mutants. Only 2 of these had evidence for synthesis from both the left and the right ends, so most of these events probably involved only a single synthesis episode. The tracts remaining after end joining were usually <200 bp. Although the end-joining process may remove some sequence from the nascent strand, we speculate that a typical repair synthesis event involves only a few hundred nucleotides of synthesis. In the case of a simple DSB, this would be sufficient to generate single-stranded DNA that is complementary to the other resected end of the break. The *copia* LTRs in our assay are 275 bp long and, given that we obtain a substantial number of products in which these LTRs have annealed, this length must be sufficient for efficient annealing.

A model for DSB repair in *Drosophila* is presented in Figure 3. First, the ends of the DSB are resected to generate 3' single-stranded overhangs (Figure 3B). A 3' overhang can invade a homologous duplex DNA in the DmRad51-mediated reaction (Figure 3C). After a small amount of repair synthesis (Figure 3D), the nascent

strand is dissociated from the template (Figure 3E). If the amount of repair synthesis is sufficient to generate ssDNA complementary to the other end of the DSB, the complementary regions can anneal to one another (Figure 3F). If repair synthesis was not sufficient to generate ssDNA complementary to the other end of the break, as in the case of the double-strand gap remaining after transposable element excision, additional cycles of strand invasion, synthesis, and dissociation can proceed. Following annealing, any overhangs can be trimmed off and single-strand gaps filled, to generate a ligatable repair product.

The intermediates depicted in Figure 3, B and E, are structurally equivalent. As described above, either of these can be used in a strand invasion reaction to extend the 3' end by repair synthesis. Alternatively, complementary single-stranded sequences in these intermediates can anneal. For the intermediate in Figure 3B, this will be possible only when the DSB was made between direct repeats that are uncovered by resection, as in the repair process termed single-strand annealing (Figure 3G; FISHMAN-LOBELL *et al.* 1992; PRESTON *et al.* 2002).

The intermediates in Figure 3, B and E, can also undergo a third fate: end joining. End joining without repair synthesis is rare in wild-type flies, but is the most common pathway for DSB repair in the absence of DmRad51 (Figure 3H). In contrast, many of the repair events we recovered from wild-type flies are best explained by end joining after one or more episodes of repair synthesis (aborted SDSA, Figure 3I). The experiment with *spn-A* homozygotes indicates that 20% of the progeny in these crosses are derived from cells that had experienced excision of *P*{*w^o*} and repair of the resulting break. In wild-type males, therefore, half of the repair events (the 10% of progeny with yellow eyes) are from aborted SDSA. It is unknown what conditions cause the cell to abandon SDSA in favor of end joining.

The products of aborted SDSA are internally deleted *P* elements. In natural populations of *D. melanogaster*, most *P* elements are internally deleted (O'HARE and RUBIN 1983). These internally deleted elements do not encode functional transposase, so they are nonautonomous. The accumulation of such nonautonomous elements is thought to limit the length of time that DNA transposons remain active within a genome. Similarly, maize *Ds* elements, which are nonautonomous copies of the *Ac* transposable element, appear to arise after aborted SDSA (RUBIN and LEVY 1997; YAN *et al.* 1999), and most of the ~300,000 DNA transposon sequences that were identified in the draft sequence of the human genome are nonautonomous "fossils" (LANDER *et al.* 2001). We speculate that these nonautonomous elements arise through aborted SDSA coupled to end joining. This type of repair may therefore be an important force in genome evolution.

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