

Old Dominion University ODU Digital Commons

Biological Sciences Faculty Publications

Biological Sciences

1997

The Drosophila Melanogaster RAD54 Homolog, DmRAD54, Is Involved in the Repair of Radiation Damage and Recombination

Rolf Kooistra

José B. M. Zonneveld

Anja De Jong


Jan C. J. Eeken

Chris J. Osgood

Old Dominion University, cosgood@odu.edu

See next page for additional authors

Follow this and additional works at: https://digitalcommons.odu.edu/biology_fac_pubs

 Part of the [Biochemistry Commons](#), [Cell Biology Commons](#), and the [Molecular Biology Commons](#)

Repository Citation

Kooistra, Rolf; Zonneveld, José B. M.; Jong, Anja De; Eeken, Jan C. J.; Osgood, Chris J.; Buerstedde, Jean-Marie; Lohman, Paul H. M.; and Pastink, Albert, "The Drosophila Melanogaster RAD54 Homolog, DmRAD54, Is Involved in the Repair of Radiation Damage and Recombination" (1997). *Biological Sciences Faculty Publications*. 162.

https://digitalcommons.odu.edu/biology_fac_pubs/162

Original Publication Citation

Kooistra, R., Vreeken, K., Zonneveld, J. B. M., De Jong, A., Eeken, J. C. J., Osgood, C. J., . . . Pastink, A. (1997). The Drosophila melanogaster RAD54 homolog, DmRAD54, is involved in the repair of radiation damage and recombination. *Molecular and Cellular Biology*, 17(10), 6097-6104. doi:10.1128/MCB.19.9.6269

Authors

Rolf Kooistra, José B. M. Zonneveld, Anja De Jong, Jan C. J. Eeken, Chris J. Osgood, Jean-Marie Buerstedde, Paul H. M. Lohman, and Albert Pastink

The *Drosophila melanogaster* RAD54 Homolog, *DmRAD54*, Is Involved in the Repair of Radiation Damage and Recombination

ROLF KOOISTRA,¹ KEES VREEKEN,¹ JOSÉ B. M. ZONNEVELD,¹ ANJA DE JONG,¹ JAN C. J. EEKEN,¹ CHRIS J. OSGOOD,¹† JEAN-MARIE BUERSTEDDE,² PAUL H. M. LOHMAN,¹ AND ALBERT PASTINK^{1*}

Department of Radiation Genetics and Chemical Mutagenesis, MGC, Leiden University, Leiden, The Netherlands,¹ and Basel Institute for Immunology, Basel, Switzerland²

Received 31 March 1997/Returned for modification 21 May 1997/Accepted 24 June 1997

The *RAD54* gene of *Saccharomyces cerevisiae* plays a crucial role in recombinational repair of double-strand breaks in DNA. Here the isolation and functional characterization of the *RAD54* homolog of the fruit fly *Drosophila melanogaster*, *DmRAD54*, are described. The putative *Dmrad54* protein displays 46 to 57% identity to its homologs from yeast and mammals. *DmRAD54* RNA was detected at all stages of fly development, but an increased level was observed in early embryos and ovarian tissue. To determine the function of *DmRAD54*, a null mutant was isolated by random mutagenesis. *DmRAD54*-deficient flies develop normally, but the females are sterile. Early development appears normal, but the eggs do not hatch, indicating an essential role for *DmRAD54* in development. The larvae of mutant flies are highly sensitive to X rays and methyl methanesulfonate. Moreover, this mutant is defective in X-ray-induced mitotic recombination as measured by a somatic mutation and recombination test. These phenotypes are consistent with a defect in the repair of double-strand breaks and imply that the *RAD54* gene is crucial in repair and recombination in a multicellular organism. The results also indicate that the recombinational repair pathway is functionally conserved in evolution.

Double-strand breaks (DSBs) in DNA are induced by ionizing radiation and by various chemical compounds, such as free radicals and methyl methanesulfonate (MMS). In addition, DSBs arise as intermediates during V(D)J rearrangement in differentiating lymphocytes, transposition events, and meiotic recombination in germ cells. Unrepaired DSBs often lead to cell death or contribute to the formation of chromosomal aberrations such as deletions and translocations. In eukaryotes, DSBs can be repaired via two main pathways: (i) recombinational repair, which is dependent on the presence of an intact duplicate DNA sequence, and (ii) end-to-end rejoining, which is based on rejoining of the two DNA ends (46). The available evidence suggests that both mechanisms are conserved in eukaryotes from yeasts to humans. However, the relative contributions of both mechanisms in repair of DSBs differ considerably between lower and higher eukaryotes. Repair of DSBs in mammals has been investigated with radiation-sensitive rodent cell lines. Several of these cell lines have defects in repairing radiation-induced DSBs and are also strongly impaired in V(D)J rearrangement. Further studies have revealed that these mutants have defects in the end-to-end rejoining pathway (reviewed in references 26, 47, and 63). Repair of DSBs at defined sites in the genome of mammalian cells also occurs more frequently by end-to-end rejoining than by homologous recombination (21, 33). The same observations have been made in experiments studying the fate of linear plasmid molecules in mammalian cells and *Xenopus laevis* oocytes (27, 29,

31). Therefore, DSBs in higher eukaryotes appear to be repaired primarily by end-to-end rejoining mechanisms.

In lower eukaryotes, however, repair of DSBs occurs almost exclusively by recombinational repair. In yeast, a contribution of end-to-end rejoining can be seen only when recombinational repair is inoperative (10, 35, 54). The recombinational repair pathway has been studied extensively in the yeast *Saccharomyces cerevisiae*. The genes involved in this mechanism belong to the RAD52 group (*RAD50*, *RAD51*, *RAD52*, *RAD54*, *RAD55*, *RAD57*, *RAD59*, *MRE11* [*RAD58/XRS4*], and *XRS2*) (3, 12; reviewed in references 19 and 20). A mutation in one of the RAD52 group genes leads to ionizing radiation sensitivity and to defects in mating-type switching and meiotic and mitotic recombination. Among these mutants, the *rad51*-, *rad52*-, and *rad54*-deficient strains have the most severe phenotype. These three mutants are defective in spontaneous and induced mitotic recombination and mating-type switching, and repair of DSBs can hardly be detected (13, 15). In addition, the formation of viable spores is almost completely blocked due to a defect in meiotic recombination in the *rad51*- and *rad52*-deficient strains. The role of Rad54 in meiosis is much less important (23, 44).

With the exception of Rad51, little is known about the precise role of the various gene products in DSB repair. The Rad51 protein is homologous to the bacterial pairing and strand exchange protein RecA (1, 4, 53). In the presence of Rpa, the Rad51 protein also promotes strand exchange activity in vitro (55). Recently, it was shown by Mortensen et al. that Rad52 may also be involved in strand annealing (37). On the basis of its amino acid sequence, the Rad54 protein has been classified as a member of the SNF2/SWI2 subfamily of DNA-dependent ATPases (22). Proteins belonging to this group are characterized by the presence of seven conserved domains, which are also present in DNA and RNA helicases, and have been implicated in transcription, chromosome stability, and DNA repair (16).

* Corresponding author. Mailing address: Department of Radiation Genetics and Chemical Mutagenesis, MGC, Leiden University, Wassenaarseweg 72, 2333 AL Leiden, The Netherlands. Phone: 31-71-5276152. Fax: 31-71-5221615. E-mail: Pastink@rullf2.medfac.leiden.univ.nl.

† Present address: Department of Biological Sciences, Old Dominion University, Norfolk, VA 23529-0266.

Homologs of the *RAD50*, *RAD51*, *RAD52*, *RAD54*, and *MRE11* genes have recently been identified in the fission yeast *Schizosaccharomyces pombe* (25, 40–42, 59), *Drosophila melanogaster* (2, 34), chickens (8, 9), mice, and humans (7, 14, 28, 36, 39, 45, 51, 52). However, the function of these homologs in higher eukaryotes remains uncertain, since the murine *RAD51* gene is essential for early embryonic development and since no other mammalian cell mutant defective in one of the RAD52 group genes has been described (30, 61, 65).

To study whether recombinational repair contributes to the repair of DSBs in a higher eukaryote, we isolated the *D. melanogaster* *RAD54* homolog, *DmRAD54*, and examined its function by generating a strain carrying a mutation in this gene. The sensitivity to ionizing radiation and MMS of *DmRAD54*-deficient larvae is strongly increased. In addition, the *DmRAD54* mutant is defective in X-ray-induced mitotic recombination. Together, these data demonstrate a structural and functional conservation of the recombinational repair pathway in a multicellular organism.

MATERIALS AND METHODS

Isolation and analysis of genomic and cDNA clones. Rad54 from *S. cerevisiae* has two stretches of seven identical amino acids at positions 456 to 462 (in helix box II) and 484 to 490 (in helix box III), respectively, in common with the human ERCC6 protein (60). These regions were used to design two degenerate primers for the amplification of a 102-bp genomic fragment. The sequences of the upstream and downstream primers are 5'-CCTGCAGA(A/G)GGICA(C/T)(A/C)GITTIAA(A/G)AA-3' and 5'-CCTGCAG(A/G)TC(A/G)TT(T/C)TG(G/T)ATIGGIGTICC-3', respectively. Alternative nucleotides are given in parentheses, and I stands for inosine. To facilitate subcloning, a *Pst*I restriction enzyme recognition site (underlined) was inserted in both primers. Screening of a *dp cn bw* genomic library (constructed in λ EMBL3 and a gift from A. Blackman) and the Oregon R head cDNA library (the library was constructed in λ EXLX and contains size-selected inserts of 1.2 kb or larger; a gift of B. Hamilton), subcloning of DNA fragments, and other standard molecular techniques were carried out as described previously (48). DNA sequence analysis was performed as described previously (50).

Rapid amplification of 5'-cDNA ends (5' RACE) was performed as specified by the manufacturer (Gibco/BRL). First-strand cDNA synthesis with total RNA from wild-type Amherst M65i flies was carried out with antisense primer Dhr54.7, 5'-GCGGGTGGATGGAAGAGTAC-3'. After dC tailing of the cDNA, the anchor primer and antisense primer Dhr54.15, 5'-TGCTCTCGCTCCAGTTCCTG-3', were used for amplification. The products were reamplified with the anchor primer in combination with a third antisense primer, Dhr54.12, 5'-CGCAGTGGTCCACGTTGGCT-3'. Finally, PCR products were cloned in pCRII (Invitrogen).

The *DmRAD54* allele of the MMS-sensitive A17-11 strain was amplified in four overlapping fragments with *Pfu* polymerase and cloned with the pCR-Script Amp SK(+) cloning kit (Stratagene).

Northern blot analysis and in situ hybridization. Total RNA was extracted from different developmental stages and ovaries by homogenizing in 4 M guanidine thiocyanate–25 mM sodium citrate (pH 7.0)–0.5% sarcosyl–0.1 M 2-mercaptoethanol followed by the addition of 0.1 volume of 2 M sodium acetate (pH 4.0) and 1 volume of phenol. After mixing, 0.2 volume of chloroform-isoamyl alcohol (24:1) was added, and the homogenate was mixed again and left for 15 min on ice. After centrifugation, the RNA was precipitated from the aqueous phase with an equal volume of 2-propanol. The RNA was dissolved in 10 mM Tris-HCl (pH 7.5)–1 mM EDTA, and high-molecular-weight RNA was precipitated for 16 h at –20°C after addition of an equal volume of 8 M LiCl. RNA samples (20 μ g) were size fractionated on a 1% agarose gel containing 0.62 M formaldehyde, transferred to a nylon membrane (Hybond N⁺; Amersham), and hybridized for 16 to 20 h at 42°C with an 800-bp genomic fragment as a probe (48).

Salivary gland chromosome preparations were made of third-instar larvae of wild-type Berlin K. The 800-bp *DmRAD54* genomic fragment was nick translated with biotinylated dUTP and used for in situ hybridization as described previously (17). Signals were visualized with streptavidin-alkaline phosphatase (Bluegene detection system; Gibco/BRL). No counterstaining was used; instead, the preparations were analyzed by combining phase-contrast with conventional light microscopy.

Fly strains and techniques. With the exception of *Df(2)JS17*, all chromosomes and mutations are listed by Lindsley and Zimm (32). The *JS17* deletion [in stock balanced over *SM1*, *In(2LR)SM1 al² Cy cn² sp²*, referred to below as *cn Cy*] was kindly provided by J. Sekelsky and uncovers approximately region 23C to 23E of the second chromosome. By blot hybridization, it was confirmed that the *DmRAD54* gene is located within the *JS17* deletion (data not shown).

For the induction of *DmRAD54* mutations, *cn bw* males were placed in glass vials containing eight layers of glass microfiber paper (Whatman GF/A) soaked with 0.9 ml of 5 mM ethyl methanesulfonate (EMS) in 33 mM sodium phosphate (pH 6.8)–5% sucrose. After a 24-h exposure, the males were mated en masse in bottles to *JS17/cn Cy* virgin females. Single F₁ *cn bw/cn Cy* males were crossed to three to five *JS17/cn Cy* virgin females. After 2 days, the flies were transferred to fresh vials. The larvae in the first vials were treated by adding 0.2 ml of 0.07% MMS in water to the vial. One MMS-sensitive mutant, A17-11, in which no *DmRAD54* mRNA could be detected by Northern blot hybridization, was used for further studies. To determine larval sensitivity to MMS and X rays, A17-11 *cn bw/cn Cy* flies were crossed with *JS17/cn Cy* flies. Two days after the crosses were started, the parental flies were transferred to fresh vials and the larvae were treated with 0.2 ml of the appropriate MMS dilution per vial. To determine the X-ray sensitivity larvae were irradiated at a dose rate of about 1 Gy/min with an Andrex machine operated at 200 kV and 4 mA 1 day after the crosses were started. The offspring of the transferred parental flies were subsequently used as untreated controls. After 10 to 14 days, the numbers of mutant *DmRAD54* (A17-11 *cn bw/JS17*; *Cy⁺ cn⁺* phenotype), heterozygous *DmRAD54⁺* (A17-11 *cn bw/cn Cy*; *Cy cn* phenotype), and hemizygous *DmRAD54⁺* (*JS17/cn Cy*; *Cy cn⁺* phenotype) flies were counted. The ratio between *DmRAD54* mutant and non-mutant flies was calculated by dividing the number of *Cy⁺* flies by the number of *Cy* flies. Finally, the relative sensitivity for a certain dose can be quantified by dividing the ratio of the untreated sample by the ratio of the treated sample. For the higher doses, several experiments were performed and the results were pooled to obtain significant numbers.

Recombination in somatic cells was studied by a somatic mutation and recombination test (SMART). A17-11 *cn bw/cn Cy* and *cn bw (DmRAD54⁺)/cn Cy* males were crossed to *JS17/cn Cy* virgins. After 16 h of egg-laying, the parental flies were removed, and 24 h later the larvae were treated with increasing doses of X rays. At 10 to 14 days after the beginning of egg-laying, the flies were recovered and the different genotypes were counted and inspected with a stereomicroscope for the presence of colorless spots in the eyes due to recombined or mutated *cn bw/cn bw* cells in the otherwise red background eye cells (*cn bw/cn⁺ bw⁺*). Spot frequencies were calculated as the total number of spots per total number of eyes scored.

Nucleotide sequence accession number. The sequence data reported in this paper have been submitted to the GenBank database under accession no. Y10229.

RESULTS

Isolation and sequence analysis of *DmRAD54*. Two regions of sequence identity between Rad54 from *S. cerevisiae* and the human ERCC6 protein (60), another member of the SNF2/SWI2 subfamily, were exploited to design two degenerate oligonucleotide primers (see Materials and Methods). With total genomic *Drosophila* DNA as a template, a fragment of 102 bp was obtained after amplification in vitro. This product was used to screen cDNA and genomic *Drosophila* libraries. A composite cDNA sequence of 2,727 bp was derived from the analysis of cDNA clones and 5' RACE products. Within this sequence, a large open reading frame between nucleotides 51 and 2406, encoding a putative protein of 784 amino acids, can be recognized. The identity and similarity between the predicted Dmrad54 amino acid sequence and Rad54 homologs from humans, mice, *S. cerevisiae*, and *S. pombe* are extensive and extend almost over their entire length (Fig. 1). In between several conserved regions, additional stretches of amino acids were observed in Rad54 and Rhp54 that were not present in Dmrad54 or the mammalian homologs. At the carboxy terminus, Dmrad54 has an extra stretch of 35 amino acids not present in other Rad54 homologs. Although the overall identity of Dmrad54 to the mammalian and yeast homologs is between 45.8 and 56.8%, the level of identity is most extensive in the so-called helicase region up to the C terminus of the proteins. Among the Rad54 homologs the seven helicase domains are almost identical. The identity in the N-terminal region is somewhat lower (Fig. 1).

To analyze the intron-exon structure of the *DmRAD54* locus, the nucleotide sequence of a region of 3,688 bp, encompassing the *DmRAD54* gene, was determined. Comparison of genomic and cDNA sequences revealed the presence of five introns ranging in size from 56 to 61 bp. The first intron is

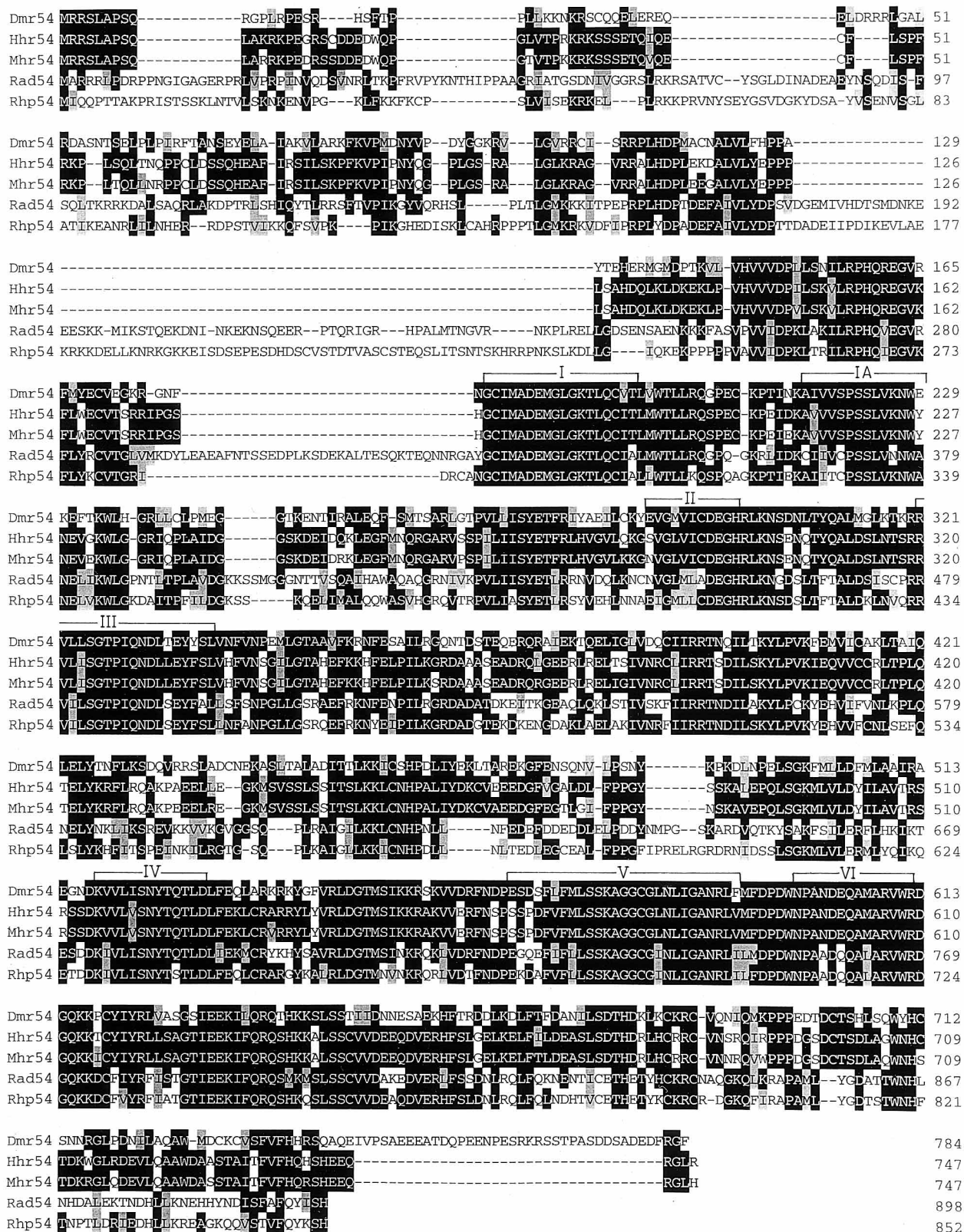


FIG. 1. Amino acid sequence alignment of Rad54 homologs. The alignment was carried out by the Clustal method in the Megalign program of DNASTar. Black boxes indicate identical amino acids, and gray boxes indicate functionally conserved amino acids according to the following groups: M, I, L, and V; D, E, N, and Q; F, Y, and W; A, G, P, S, and T; and H, K, and R. The seven so-called helicase domains are indicated by boxes I to VI. The BESTFIT program was used for a pairwise comparison of the Dmr54 and the other four proteins. The identity of Dmr54 to Hhr54, Mhr54, Rad54, and Rhp54 is 55.8, 56.8, 45.8, and 46.8%, respectively. The levels of similarity of Dmr54 to the other proteins are 18.3, 17.8, 19.2, and 20.8%, respectively. Dmr54, *D. melanogaster* homolog; Hhr54, human homolog; Mhr54, mouse homolog; Rad54, *S. cerevisiae* homolog; Rhp54, *S. pombe* homolog. The Dmr54 sequence was derived from a genomic fragment isolated from a *dp cn bw* library and contains a glutamine at position 373. The cDNA of the Oregon R strain codes for an alanine at this position.

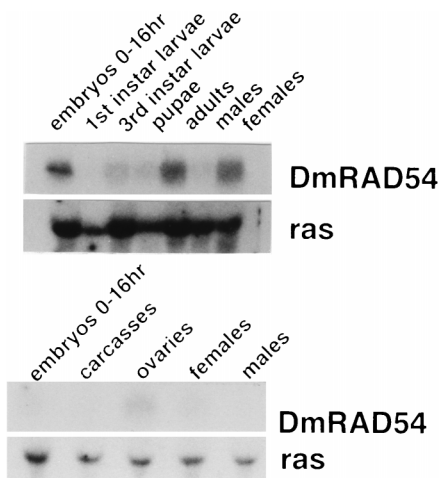


FIG. 2. Expression of *DmRAD54* during *Drosophila* development. Total RNA (20 μ g) from flies of different stages of development, adult flies, and ovaries, and total RNA (24 μ g) from carcasses (equivalent to 20 μ g of ovarian RNA) were electrophoresed on agarose-formaldehyde gels and hybridized with a 800-bp genomic *DmRAD54* probe. The *ras* probe was used as a control for the amount of RNA loaded (38).

located just downstream of the ATG start codon. The sequence context of the start codon at position 707 of the genomic sequence, TGAAATG, weakly matches the *Drosophila* translation initiation consensus (C/A)AA(C/A)ATG (11). Comparison of the genomic and cDNA sequences also revealed substitutions of 16 bp (data not shown). These strain differences result in only one amino acid substitution: a glutamine (Q)-to-alanine (A) change at position 373 of the pro-

tein. The alanine at position 373 is also observed in the mammalian homologs.

Expression pattern of *DmRAD54*. To examine the expression of *DmRAD54* during *Drosophila* development, total RNA was isolated from different developmental stages and hybridized with an 800-bp genomic probe. Hybridization could be detected with an RNA species of approximately 3 kb at all stages of development. A relatively high level of *DmRAD54* RNA was observed in embryos and in adult flies (Fig. 2A). The enhanced expression in adults is specific for female flies. Hybridization with RNA isolated from ovaries and the remaining carcasses indicated that the *DmRAD54* gene is specifically expressed in the ovaries (Fig. 2B). The expression level in the carcasses is comparable to that of the male flies.

Generation of *DmRAD54*-deficient flies. To isolate *DmRAD54* mutant fly strains, the chromosomal location of the *DmRAD54* gene was determined by in situ hybridization. Hybridization was observed in region 23D/E on the left arm of the second chromosome (data not shown). The *Rrp1* gene, which codes for a protein with apurinic/apyrimidinic endonuclease activity (49), has also been localized in the same region (56). Male flies homozygous for the recessive *cinnabar* (*cn*) and *brown* (*bw*) markers on the second chromosome were treated with EMS to randomly induce mutations. These treated males were crossed to females heterozygous for a second chromosome balancer carrying multiple inversions, a dominant *Curly* (*Cy*) and several recessive markers including *cn*. From the offspring of this cross, *cn bw/cn Cy* males were selected and crossed to *JS17/cn Cy* females. The *JS17* deletion uncovers the *DmRAD54* gene on the second chromosome. This cross produces four different combinations of the second chromosome: *cn Cy/cn Cy*, which is unviable, and *cn bw/cn Cy*, *JS17/cn Cy*, and *cn bw/JS17* in a 1:1:1 ratio (Fig. 3). By setting up the second cross with only one

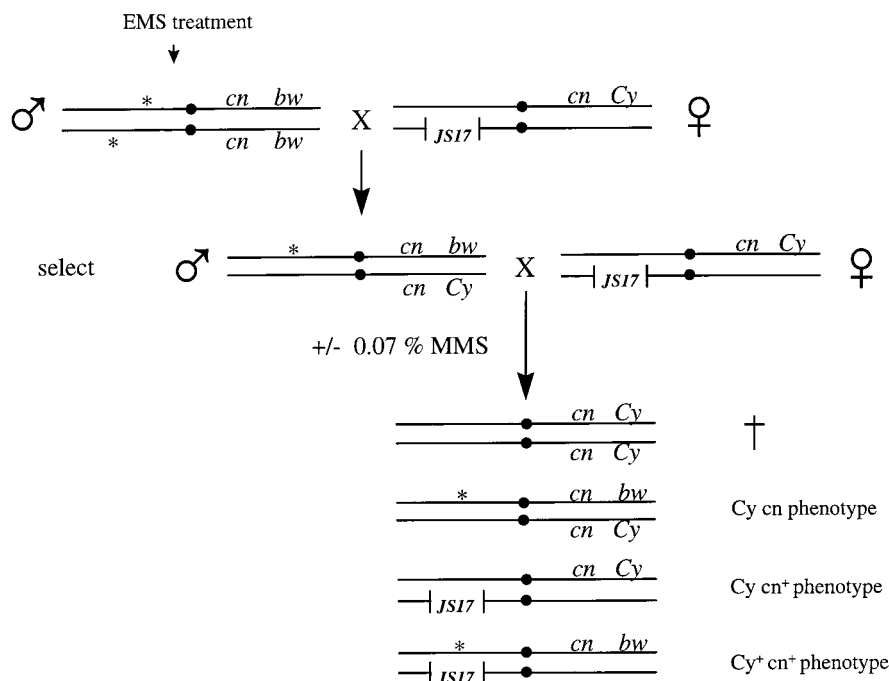


FIG. 3. Scheme used for the isolation of *DmRAD54* mutants. Genotypes of the second chromosomes of the parental and F_1 generations are indicated. The marker mutations used are as follows: *cn*, *cinnabar*; *bw*, *brown*; and *Cy*, *Curly*. The chromosome designated *JS17* contains a deletion of the 23C to 23E region. The first cross was performed to obtain *cn bw/cn Cy* males carrying random induced mutations (indicated by the asterisks). The second cross was used to determine the larval sensitivity for MMS and X rays. See Materials and Methods for details.

TABLE 1. X-ray sensitivity of A17-11 *cn bw/JS17*^a

Dose (Gy)	No. of control flies		Ratio-C (Cy ⁺ /Cy)	No. of X-rayed flies		Ratio-X (Cy ⁺ /Cy)	Sensitivity ^d
	Cy ⁺ ^b	Cy ^c		Cy ⁺ ^b	Cy ^c		
3	76	125	0.61	82	158	0.52	1.17
6	94	181	0.52	27	137	0.20	2.64
9	74	140	0.53	2	73	0.03	19.29

^a Parental flies were allowed to lay eggs on two vials; one was X-ray irradiated, and the other was used as a control.

^b The number of A17-11 *cn bw/JS17* flies (Cy⁺ phenotype).

^c The number of A17-11 *cn bw/cn Cy* and *JS17/cn Cy* flies (Cy phenotype).

^d Sensitivity is calculated by Ratio-C/Ratio-X.

male fly together with several females, it is possible to test individual EMS-treated *cn bw* chromosomes for enhanced MMS sensitivity in combination with *JS17* (see Materials and Methods). A defective *DmRAD54* gene will presumably result in an enhanced MMS sensitivity, as in *rad54* yeast mutants. As a result of the enhanced MMS sensitivity, the number of *cn bw/JS17* flies will be reduced in comparison to their heterozygous *cn bw/cn Cy* and *JS17/cn Cy* brothers and sisters when treated with MMS. In 19 experiments, in which a total of 8,167 chromosomes were tested, 69 lethal strains, 11 semilethal strains, and 10 strains with an enhanced MMS sensitivity were isolated (details will be published elsewhere).

Characterization of a *DmRAD54* mutant. Total RNA isolated from the MMS-sensitive strains was analyzed by Northern blot hybridization. In one strain, A17-11, no *DmRAD54* mRNA could be detected. The other nine strains showed normal levels of *DmRAD54* RNA in comparison with wild-type flies (data not shown). Sequence analysis of the A17-11 *DmRAD54* allele and comparison with the sequence of the wild-type allele of the *cn bw* strain revealed only a G-to-A transition at the splice acceptor site of the second intron (data not shown). This type of alteration is consistent with the fact that EMS induces mainly GC-to-AT transitions (43). The presence of a splice mutation in the A17-11 mutant might lead to the formation of unstable *DmRAD54* mRNA, explaining why no *DmRAD54* transcripts could be detected by blot hybridization. Most probably, this type of mutation results in a null phenotype.

In addition to the splice mutation, the A17-11 *cn bw* chromosome contains a second, recessive lethal mutation. For this reason, A17-11 *cn bw* homozygous strains are not viable. To obtain *DmRAD54*-deficient flies, the A17-11 mutation has to be combined with the *JS17* deletion. The A17-11 *cn bw/JS17* male flies are viable and fertile, whereas the A17-11 *cn bw/JS17* females are viable but not fertile. The females do lay eggs, but these do not hatch. Microscopic analysis indicates that early development is normal in the newly laid eggs.

***DmRAD54* mutant flies are sensitive to ionizing radiation and MMS.** To determine the sensitivity of the A17-11 *cn bw/JS17* flies to ionizing radiation and MMS, A17-11 *cn bw/cn Cy* males were crossed to *JS17/cn Cy* females (Fig. 3). After 1 day, the eggs were given X-ray treatment, or after 2 days of egg-laying, they were treated with MMS. Approximately 2 weeks later, the number of Cy⁺ flies (A17-11 *cn bw/JS17*) and the number of Cy flies (heterozygous for A17-11 or *JS17*) was determined and their ratio was calculated. This ratio is expected to be 0.5 according to Mendelian laws. In the untreated controls, the ratio varied between 0.47 and 0.61 (Tables 1 and 2). If a *DmRAD54* mutation results in an increased sensitivity to DNA-damaging agents, this ratio of 0.5 will decrease with increasing dose. The sensitivity of A17-11 *cn bw/JS17* flies can

TABLE 2. MMS sensitivity of A17-11 *cn bw/JS17*^a

MMS concn (%)	No. of control flies		Ratio-C (Cy ⁺ /Cy)	No. of MMS-treated flies		Ratio-M (Cy ⁺ /Cy)	Sensitivity ^d
	Cy ⁺ ^b	Cy ^c		Cy ⁺ ^b	Cy ^c		
0.01	167	346	0.48	153	287	0.53	0.91
0.03	186	355	0.52	109	344	0.32	1.65
0.07	164	348	0.47	9	96	0.09	5.03
0.09	238	435	0.55	3	91	0.03	16.60

^a Parental flies were allowed to lay eggs on two vials; one was MMS treated, and the other was used as a control.

^b The number of A17-11 *cn bw/JS17* flies (Cy⁺ phenotype).

^c The number of A17-11 *cn bw/cn Cy* and *JS17/cn Cy* flies (Cy phenotype).

^d Sensitivity is calculated by Ratio-C/Ratio-M.

be quantified by dividing the Cy⁺/Cy ratio of the untreated sample by the Cy⁺/Cy ratio of the treated sample (X-ray or MMS treatment). For example, a sensitivity of 5.03 obtained for a dose of 0.07% MMS (Table 2) means that the A17-11 *cn bw/JS17* flies are 5.03 times more sensitive for this dose than are the heterozygous *cn Cy/JS17* or *cn Cy/A17-11 cn bw* flies. For low doses (3 Gy of X irradiation or 0.01 to 0.03% MMS), there was no significant increase in sensitivity of the A17-11 *cn bw/JS17* flies. However, when exposed to higher doses (6 to 9 Gy of X irradiation or 0.07 to 0.09% MMS), the A17-11 *cn bw/JS17* flies showed a clearly enhanced sensitivity: 2.6 to 19.3 times and 5.0 to 16.6 times, respectively (Tables 1 and 2).

***DmRAD54* mutant flies have a defect in radiation-induced mitotic recombination.** To investigate if *DmRAD54* is involved in mitotic recombination, a SMART was performed. By using this assay, genetic alterations in somatic cells can be measured (reviewed in reference 6). The flies used for this test are heterozygous for the *cn* and *bw* markers. A mutagenic event in larval cells affecting both the wild-type *cinnabar* and *brown* gene will give rise to a daughter cell homozygous for the *cn* and *bw* markers (Fig. 4). Clonal expansion of mutated cells results in spots in the eyes of the adult fly that do not contain pigment any more and can be scored microscopically. An example of a fly containing nonpigmented spots in one of the eyes is shown in Fig. 5. In this study, 24- to 40-h-old A17-11 *cn bw/JS17* or *cn bw/JS17* larvae were irradiated with different doses of X rays. After 10 to 14 days, hatched flies were isolated and inspected for the presence of spots. Although *Drosophila* larvae are more X-ray resistant than are embryos, 24- to 40-h-old larvae were chosen for this experiment since the frequency of induced spots is higher due to an increased number of target cells. The number of spots per eye in the *cn bw/JS17* control group was enhanced after treatment with X rays. With increasing dose, a clear dose response was observed (Table 3). In the A17-11 *cn bw/JS17* mutant flies, no enhancement in spot frequency was observed. At all doses used, the frequency of spots is identical to the frequency in untreated animals. These results demonstrate a defect in induced mitotic recombination in the *DmRAD54*-deficient flies after exposure to radiation.

DISCUSSION

There are two main types of DSB repair: end-to-end rejoining and recombinational repair. In *S. cerevisiae* and *S. pombe*, recombinational repair predominates. Yeast cells are capable of end-to-end rejoining, but this process is much less efficient than the recombinational repair mechanism. Only in a Rad52-deficient strain is a contribution of the other mechanism(s) observed (10, 35, 54). Much less is known about the relative

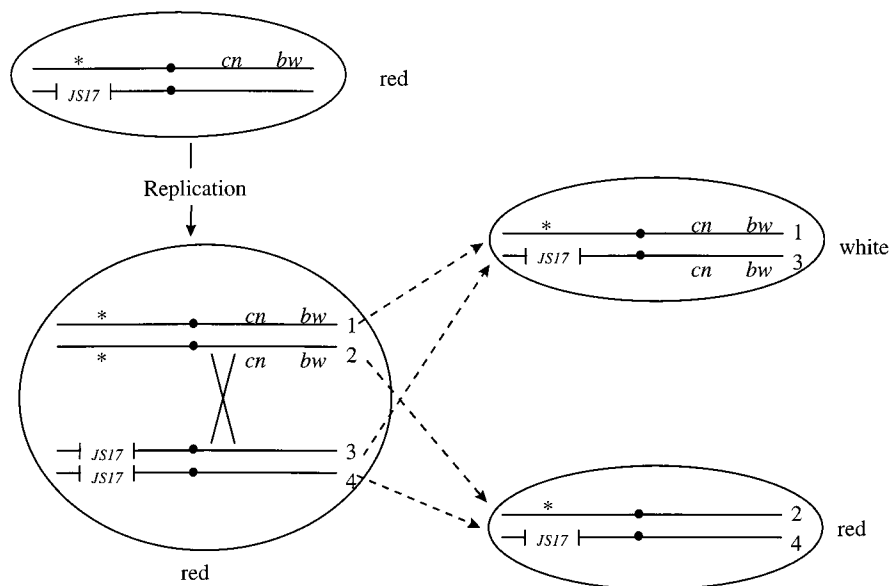


FIG. 4. SMART protocol. Recombination events in larval A17-11 *cn bw/JS17* cells between the centromere and the *cinnabar* gene on the second chromosome followed by cosegregation of chromatids 1 and 3 result in a daughter cell which is homozygous for the *cn* and *bw* marker mutations. Cosegregation of chromatids 1 and 4 will give rise to two heterozygous daughter cells. The *cinnabar* and *brown* genes are required for biosynthesis of the brown and red eye pigments, respectively. Proliferation of a mutant cell may result in patches of mutant tissue in the eye which can be scored as colourless spots. As a control, *cn bw/JS17* larvae were used. The A17-11 mutation is indicated by an asterisk.

contribution of the recombinational repair mechanism to the repair of DSBs in higher eukaryotes. To study the role of the RAD52 group genes in repair and recombination in a multicellular organism, we isolated the *Drosophila RAD54* homolog, *DmRAD54*, and a strain carrying a mutation in this gene.

The *DmRAD54* gene was cloned with degenerate primers. The 784-amino-acid Dmrad54 protein strongly resembles its

counterparts from *S. cerevisiae* and *S. pombe* and from mammals. The overall homology is 65 to 75% (Fig. 1). To generate fly strains deficient for *DmRAD54*, randomly mutagenized male flies were crossed to females heterozygous for the *JS17* deletion, which uncovers the *DmRAD54* gene, and the offspring was screened for enhanced MMS sensitivity. In 1 of the 10 probable *DmRAD54* mutants isolated, no *DmRAD54* mRNA could be detected by blot hybridization. Sequence analysis showed the presence of a G-to-A transition in the splice acceptor site of the second intron in the *DmRAD54* allele of this mutant. This mutation most probably gives rise to a null phenotype. Heterozygous A17-11 *cn bw/JS17* males carrying the splice mutation in one second chromosome and a deletion including the *DmRAD54* gene on the other second chromosome develop normally and are fertile. The females are also viable, and eggs are deposited, but these do not hatch. The infertility of *DmRAD54*-deficient females could suggest a role for the *DmRAD54* gene product during development or in meiotic recombination which only occurs in females in *Drosophila*. Analysis of newly laid eggs showed a normal early development, indicating an important role for the DmRad54 protein in later development. The increased level of *DmRAD54* mRNA observed in oocytes and early embryos could suggest that *DmRAD54* mRNA is maternally loaded in the oocytes to

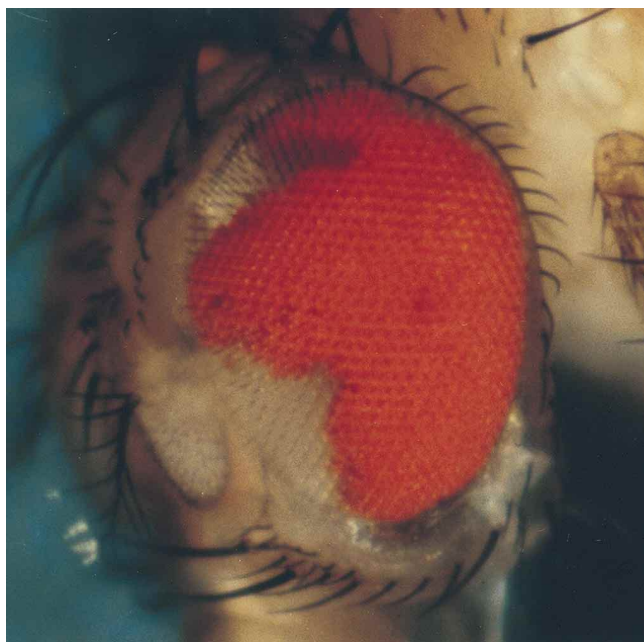


FIG. 5. Spots of mutant tissue in the eye of an adult fly. During development of *cn bw/cn⁺ bw⁺* embryos, recombination/mutation events lead to homozygous *cn bw/cn bw* precursor cells, which after proliferation give rise to patches of colorless mutant tissue against a background of red *cn bw/cn⁺ bw⁺* cells.

TABLE 3. SMART results

X-ray dose (Gy)	Control (<i>cn bw/JS17</i>)		Mutant (A17-11 <i>cn bw/JS17</i>)	
	No. of spots/ no. of eyes analyzed	Ratio	No. of spots/ no. of eyes analyzed	Ratio
0	4/300	0.013	6/300	0.020
3	11/268	0.041	4/246	0.016
6	17/242	0.070	5/244	0.021
9	41/352	0.117	5/236	0.021

support cell proliferation during embryonic development. In the yeast *S. pombe*, the Rhp54 protein is also involved in replication, in addition to playing a role in the repair of radiation-induced DSBs (41). The regulated expression of the mouse and human *RAD51* gene during the cell cycle and the inviability of *RAD51*-deficient mice also indicate an important role for the RAD52 group genes in cell proliferation (18, 30, 57, 58, 61, 64).

In the A17-11 *cn bw/JS17 DmRAD54* mutant flies, a strongly increased sensitivity to ionizing radiation and MMS was observed. At the highest radiation dose used, the survival of this *DmRAD54* mutant was reduced by a factor of 19 in comparison to that of the heterozygous siblings. At a dose of 0.09% MMS, the sensitivity was increased by a factor of 16. These results strongly indicate that in higher eukaryotes the recombinational repair pathway also contributes significantly to the repair of exogenously induced DNA damage. Since heterozygous wild-type A17-11 *cn bw/cn Cy* females have to be used in the crosses because of the female sterility of the hemizygous null mutant, maternally derived transcripts may contribute to the repair of DNA damage in the treated larvae. As a consequence, the increase in sensitivity of the mutant larvae to X rays or MMS may even be underestimated.

The same cross as depicted in Fig. 3 was also used to study mitotic recombination in A17-11 *cn bw/JS17* mutant and *cn bw/JS17* nonmutant flies by the SMART (Fig. 4). Loss of heterozygosity (LOH) of both the *cn* and *bw* markers in mitotically dividing cells results in patches of mutant tissue in the eyes of the adult fly, which can be recognized as colorless spots (see Fig. 5). The great majority of these LOH events are due to mitotic recombination. This conclusion is based on several observations, including (i) the absence of LOH in male flies when X-chromosomal markers are used and (ii) the strong reduction in LOH in females carrying an inversion chromosome (6, 62). Treatment of 24- to 40-h-old *cn bw/JS17* control larvae with increasing doses of X rays results in an increase in the number of spots with increasing doses. In the A17-11 *cn bw/JS17 DmRAD54* mutant flies, no induction of spots was observed. The spots in the untreated sample may be spontaneous recombination events in very early embryos, when the maternally derived *DmRAD54* mRNA is still present, or may be due to other mechanisms such as chromosome loss. These results demonstrate that *DmRAD54* is required for X-ray-induced recombination in mitotically dividing cells. The defect in radiation-induced mitotic recombination and the strongly enhanced radiation sensitivity closely resemble the phenotype of an *S. cerevisiae rad54* mutant, indicating an important role for the *RAD54* homolog in DSB repair and recombination in *Drosophila*. The results presented in this paper also provide convincing evidence that the RAD52-dependent recombinational repair pathway is functionally conserved from yeasts to higher organisms.

The *Drosophila mus309* mutant has a defect in the *Ku70* gene, which is involved in end-to-end rejoining of DSBs. Heteroallelic *mus309* flies display increased MMS sensitivity and show a reduction in the repair of DSBs after P-element excision (5, 24). The phenotypes of the *DmRAD54* mutant and the *mus309* mutant suggest that in *Drosophila* both DSB repair mechanisms are involved in break repair. Construction and analysis of a *mus309/DmRAD54* double mutant may give an indication of the relative contribution of end-to-end rejoining and recombination in DSB repair.

ACKNOWLEDGMENTS

The work described in this paper was supported by the Dutch Cancer Foundation (project RUL 94-774) and by the J. A. Cohen Institute,

Interuniversity Research Institute for Radiopathology and Radiation Protection (IRS; project 4.4.12). The Basel Institute for Immunology was founded and is supported by F. Hoffmann-LaRoche & Co.

We thank H. Vrieling for critically reading the manuscript. We are grateful to the other members of the *Drosophila* group for stimulating discussions and moral support. We also thank R. Kanaar and J. H. Hoeijmakers for their interest.

REFERENCES

- Aboussekhra, A., R. Chanet, A. Adjiri, and F. Fabre. 1992. Semidominant suppressors of Srs2 helicase mutations of *Saccharomyces cerevisiae* map in the *RAD51* gene, whose sequence predicts a protein with similarities to prokaryotic RecA proteins. *Mol. Cell. Biol.* **12**:3224–3234.
- Akaboshi, E., Y. Inoue, and H. Ryo. 1994. Cloning of the cDNA and genomic DNA that correspond to the *recA*-like gene of *Drosophila melanogaster*. *Jpn. J. Genet.* **69**:663–670.
- Bai, Y., and L. S. Symington. 1996. A Rad52 homolog is required for *RAD51*-independent mitotic recombination in *Saccharomyces cerevisiae*. *Genes Dev.* **10**:2025–2037.
- Basile, G., M. Aker, and R. K. Mortimer. 1992. Nucleotide sequence and transcriptional regulation of the yeast recombinational repair gene *RAD51*. *Mol. Cell. Biol.* **12**:3235–3246.
- Beall, E. L., and D. C. Rio. 1996. *Drosophila* IRBP/Ku p70 corresponds to the mutagen-sensitive *mus309* gene and is involved in P-element excision in vivo. *Genes Dev.* **10**:921–933.
- Becker, H. J. 1976. Mitotic recombination, p. 1019–1087. In M. Ashburner and E. Novitski (ed.), *The genetics and biology of Drosophila*, vol. 1c. Academic Press, Ltd., London, United Kingdom.
- Bendixen, C., I. Sunjevaric, R. Bauchwitz, and R. Rothstein. 1994. Identification of a mouse homologue of the *Saccharomyces cerevisiae* recombination and repair gene, RAD52. *Genomics* **23**:300–303.
- Bezzubova, O., H. Schmidt, K. Ostermann, W.-D. Heyer, and J.-M. Buerstedde. 1993. Identification of a chicken *RAD52* homologue suggests conservation of the *RAD52* recombination pathway throughout the evolution of higher eukaryotes. *Nucleic Acids Res.* **21**:5945–5949.
- Bezzubova, O., A. Shinohara, R. G. Mueller, H. Ogawa, and J.-M. Buerstedde. 1993. A chicken *RAD51* homologue is expressed at high levels in lymphoid and reproductive organs. *Nucleic Acids Res.* **21**:1577–1580.
- Boulton, S. J., and S. P. Jackson. 1996. *Saccharomyces cerevisiae* Ku70 potentiates illegitimate DNA double-strand break repair and serves as a barrier to error-prone DNA repair pathways. *EMBO J.* **15**:5093–5103.
- Cavener, D. R. 1987. Comparison of the consensus sequences flanking translational start sites in *Drosophila* and vertebrates. *Nucleic Acids Res.* **4**:1353–1361.
- Chepurayana, O. V., S. A. Kozhin, V. T. Peshekhonov, and V. G. Korolev. 1995. RAD58(XRS4)—a new gene in the RAD52 epistasis group. *Curr. Genet.* **28**:274–279.
- Contopoulou, C. R., V. E. Cook, and R. K. Mortimer. 1987. Analysis of DNA double strand breakage and repair using orthogonal field alternation gel electrophoresis. *Yeast* **3**:71–76.
- Dolganov, G. M., R. S. Maser, A. Novikov, L. Tosto, S. Chong, D. A. Bressan, and H. J. Petrini. 1996. Human Rad50 is physically associated with human Mre11: identification of a conserved multiprotein complex implicated in recombinational DNA repair. *Mol. Cell. Biol.* **16**:4832–4841.
- Eckardt-Schupp, E. Personal communication.
- Eisen, J. A., K. S. Sweder, and P. C. Hanawalt. 1995. Evolution of the SNF2 family of proteins: subfamilies with distinct sequences and functions. *Nucleic Acids Res.* **23**:2715–2723.
- Engels, W. R., C. R. Preston, P. Thompson, and W. B. Eggleston. 1986. In situ hybridization to *Drosophila* salivary chromosomes with biotinylated DNA probes and alkaline phosphatase. *Focus* **8**:6–8.
- Flygare, J., F. Benson, and D. Hellgren. 1996. Expression of the human RAD51 gene during the cell cycle in primary human peripheral blood lymphocytes. *Biochim. Biophys. Acta* **1312**:231–236.
- Friedberg, E. C., G. C. Walker, and W. Siede. 1995. DNA repair and mutagenesis. ASM Press, Washington, D.C.
- Game, J. C. 1993. DNA double-strand breaks and the *RAD50-RAD57* genes in *Saccharomyces*. *Semin. Cancer Biol.* **4**:73–83.
- Godwin, A. R., R. J. Bollag, D.-M. Christie, and R. M. Liskay. 1994. Spontaneous and restriction enzyme-induced chromosomal recombination in mammalian cells. *Proc. Natl. Acad. Sci. USA* **91**:12554–12558.
- Gorbalenya, A. E., and E. V. Koonin. 1993. Helicases: amino acid sequence comparisons and structure-function relationships. *Curr. Opin. Struct. Biol.* **3**:419–429.
- Haynes, R. H., and B. A. Kunz. 1981. DNA repair and mutagenesis in yeast, p. 371–414. In J. Strathern, E. W. Jones and J. R. Broach (ed.), *The molecular biology of the yeast Saccharomyces cerevisiae: life cycle and inheritance*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
- Jacoby, D. B., and P. C. Wensink. 1994. Yolk protein factor 1 is a *Drosophila* homolog of Ku, the DNA-binding subunit of a DNA-dependent protein kinase from humans. *J. Biol. Chem.* **269**:11484–11491.

25. Jang, Y. K., Y. H. Jin, E. M. Kim, F. Fabre, S. H. Hong, and S. D. Park. 1994. Cloning and sequence analysis of *rhp51⁺*, a *Schizosaccharomyces pombe* homolog of the *Saccharomyces cerevisiae* *RAD51* gene. *Gene* **142**:207–211.
26. Jeggo, P. A., G. E. Taccioli, and S. P. Jackson. 1995. Menage à trois: double strand break repair, V(D)J recombination and DNA-PK. *Bioessays* **17**:949–957.
27. Jeong-Yu, S., and D. Carroll. 1992. Test of the double-strand break repair model of recombination in *Xenopus laevis* oocytes. *Mol. Cell. Biol.* **12**:112–119.
28. Kanaar, R., C. Troelstra, S. M. A. Swagemakers, J. Essers, B. Smit, J.-H. Franssen, A. Pastink, O. Bezzubova, J.-M. Buerstedde, B. Clever, W.-D. Heyer, and J. H. J. Hoeijmakers. 1996. Characterization of human and mouse homologs of the *Saccharomyces cerevisiae* double-strand DNA break repair gene *RAD54*. *Curr. Biol.* **6**:828–838.
29. Lehman, C. W., J. K. Trautman, and D. Carroll. 1994. Illegitimate recombination in *Xenopus*: characterization of end-joined junctions. *Nucleic Acids Res.* **22**:434–442.
30. Lim, D.-S., and P. Hastay. 1996. A mutation in mouse *rad51* results in an early embryonic lethal that is suppressed by a mutation in *p53*. *Mol. Cell. Biol.* **16**:7133–7143.
31. Lin, F.-L. M., K. Sperle, and N. Sternberg. 1990. Repair of double-stranded DNA breaks by homologous DNA fragments during transfer of DNA into mouse L cells. *Mol. Cell. Biol.* **10**:113–119.
32. Lindsley, D. L., and G. G. Zimm. 1992. The genome of *Drosophila melanogaster*. Academic Press, Inc., San Diego, Calif.
33. Lukacsovich, T., D. Yang, and A. S. Waldman. 1994. Repair of a specific double-strand break generated within a mammalian chromosome by yeast endonuclease I-SceI. *Nucleic Acids Res.* **22**:5649–5657.
34. McKee, B. D., X.-J. Ren, and C.-S. Hong. 1996. A *recA*-like gene in *Drosophila melanogaster* that is expressed at high levels in female but not male meiotic tissues. *Chromosoma* **104**:479–488.
35. Milne, G. T., S. Jin, K. B. Shannon, and D. T. Weaver. 1996. Mutations in two ku homologs define a DNA end-joining repair pathway in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **16**:4189–4198.
36. Morita, T., Y. Yoshimura, A. Yamamoto, K. Murata, M. Mori, H. Yamamoto, and A. Matsushiro. 1993. A mouse homolog of the *Escherichia coli* *recA* and *Saccharomyces cerevisiae* *RAD51* genes. *Proc. Natl. Acad. Sci. USA* **90**:6577–6580.
37. Mortensen, U. H., C. Bendixen, I. Sunjevaric, and R. Rothstein. 1996. DNA strand annealing is promoted by the yeast Rad52 protein. *Proc. Natl. Acad. Sci. USA* **93**:10729–10734.
38. Mozer, B., R. Marlor, S. Parkhurst, and V. Corces. 1985. Characterization and developmental expression of a *Drosophila ras* oncogene. *Mol. Cell. Biol.* **5**:885–889.
39. Muris, D. F. R., O. Bezzubova, J.-M. Buerstedde, K. Vreeken, A. S. Balajee, C. J. Osgood, C. Troelstra, J. H. J. Hoeijmakers, K. Ostermann, H. Schmidt, A. T. Natarajan, J. C. J. Eeken, P. H. M. Lohman, and A. Pastink. 1994. Cloning of human and mouse genes homologous to *RAD52*, a yeast gene involved in DNA repair and recombination. *Mutat. Res.* **315**:295–305.
40. Muris, D. F. R., K. Vreeken, A. M. Carr, B. C. Broughton, A. R. Lehmann, P. H. M. Lohman, and A. Pastink. 1993. Cloning the *RAD51* homologue of *Schizosaccharomyces pombe*. *Nucleic Acids Res.* **12**:4586–4591.
41. Muris, D. F. R., K. Vreeken, A. M. Carr, J. M. Murray, C. Smit, P. H. M. Lohman, and A. Pastink. 1996. Isolation of the *Schizosaccharomyces pombe* *RAD54* homologue, *rhp54⁺*, a gene involved in the repair of radiation damage and replication fidelity. *J. Cell Sci.* **109**:73–81.
42. Ostermann, K., A. Lorentz, and H. Schmidt. 1993. The fission yeast *rad22* gene, having a function in mating-type switching and repair of DNA damages, encodes a protein homolog to Rad52 of *Saccharomyces cerevisiae*. *Nucleic Acids Res.* **21**:5940–5944.
43. Pastink, A., E. Heemskerk, M. J. M. Nivard, C. J. van Vliet, and E. W. Vogel. 1991. Mutational specificity of ethyl methanesulfonate in excision-repair proficient and deficient strains of *Drosophila melanogaster*. *Mol. Gen. Genet.* **229**:213–218.
44. Petes, T. D., R. E. Malone, and L. S. Symington. 1991. Recombination in yeast, p. 407–521. In J. Broach, J. Pringle, and E. Jones (ed.), The molecular and cellular biology of the yeast *Saccharomyces*: genome dynamics, protein synthesis, and energetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
45. Petrini, J. H. J., M. E. Walsh, C. DiMare, X.-N. Chen, J. R. Korenberg, and D. T. Weaver. 1995. Isolation and characterization of the human *MRE11* homologue. *Genomics* **29**:80–86.
46. Price, A. 1993. The repair of ionizing radiation-induced damage to DNA. *Cancer Biol.* **4**:61–71.
47. Roth, D. B., T. Lindahl, and M. Gellert. 1995. How to make ends meet. *Curr. Biol.* **5**:496–499.
48. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
49. Sander, M., K. Owenhaupt, W. S. Lane, and A. Rich. 1991. Cloning and characterization of Rrp1, the gene encoding *Drosophila* strand transferase: carboxy-terminal homology to DNA repair endo/exonucleases. *Nucleic Acids Res.* **19**:4523–4529.
50. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-termination inhibitors. *Proc. Natl. Acad. Sci. USA* **74**:5463–5467.
51. Shen, Z., K. Denison, R. Lobb, J. M. Gatewood, and D. J. Chen. 1995. The human and mouse homologs of the yeast *RAD52* gene: cDNA cloning, sequence analysis, assignment to human chromosome 12p12.2-p13, and mRNA expression in mouse tissues. *Genomics* **25**:199–206.
52. Shinohara, A., H. Ogawa, Y. Matsuda, N. Ushio, K. Ikeo, and T. Ogawa. 1993. Cloning of human, mouse and fission yeast recombination genes homologous to *RAD51* and *recA*. *Nat. Genet.* **4**:239–243.
53. Shinohara, A., H. Ogawa, and T. Ogawa. 1992. *RAD51* protein involved in repair and recombination in *S. cerevisiae* is a RecA-like protein. *Cell* **69**:457–470.
54. Siede, W., A. A. Friedl, I. Dianova, F. Eckardt-Schupp, and E. C. Friedberg. 1996. The *Saccharomyces cerevisiae* Ku autoantigen homologue affects radiosensitivity only in the absence of homologous recombination. *Genetics* **142**:91–102.
55. Sung, P., and D. L. Robberson. 1995. DNA strand exchange mediated by a RAD51-ssDNA nucleoprotein filament with polarity opposite to that of RecA. *Cell* **82**:453–461.
56. Szakmary, A. Personal communication.
57. Taki, T., T. Ohnishi, A. Yamamoto, S. Hiraga, N. Arita, S. Izumoto, T. Hayakawa, and T. Morita. 1996. Antisense inhibition of the *RAD51* enhances radiosensitivity. *Biochem. Biophys. Res. Commun.* **223**:434–438.
58. Tashiro, S., N. Kotomura, A. Shinohara, K. Tanaka, K. Ueda, and N. Kamada. 1996. S phase specific formation of the human Rad51 protein nuclear foci in lymphocytes. *Oncogene* **12**:2165–2170.
59. Tavassoli, M., M. Shayeghi, A. Nasim, and F. Z. Watts. 1995. Cloning and characterization of the *Schizosaccharomyces pombe rad32* gene: a gene required for repair of double-strand breaks and recombination. *Nucleic Acids Res.* **23**:383–388.
60. Troelstra, C., A. van Gool, J. de Wit, W. Vermeulen, D. Bootsma, and J. H. J. Hoeijmakers. 1992. ERCC6, a member of a subfamily of putative helicases, is involved in Cockayne's syndrome and preferential repair of active genes. *Cell* **71**:939–953.
61. Tsuzuki, T., Y. Fujii, K. Sakumi, Y. Tominaga, K. Nakao, M. Sekiguchi, A. Matushuro, Y. Yoshimura, and T. Morita. 1996. Targeted disruption of the Rad51 gene leads to lethality in embryonic mice. *Proc. Natl. Acad. Sci. USA* **93**:6236–6240.
62. Vogel, E. W. 1992. Tests for recombinagens in somatic cells of *Drosophila*. *Mutat. Res.* **284**:159–175.
63. Weaver, D. T. 1995. What to do at an end: DNA double-strand-break repair. *Trends Genet.* **11**:388–392.
64. Yamamoto, A., T. Taki, H. Yagi, T. Habu, K. Yoshida, Y. Yoshimura, K. Yamamoto, A. Matsushiro, Y. Nishimune, and T. Morita. 1996. Cell cycle-dependent expression of the mouse *Rad51* gene in proliferating cells. *Mol. Gen. Genet.* **251**:1–12.
65. Zdzienicka, M. Z. 1995. Mammalian mutants defective in the response to ionizing radiation-induced DNA damage. *Mutat. Res.* **336**:203–213.