Drosophila IRBP/Ku p70 corresponds to the mutagen-sensitive *mus309* gene and is involved in P-element excision in vivo

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The P family of transposable elements in *Drosophila* transpose by a cut-and-paste mechanism involving double-strand gap repair. We report here that a *Drosophila* mutagen-sensitive mutant, *mus309*, contains a mutation in *IRBP* (inverted repeat binding protein), the *Drosophila* homolog of the mammalian Ku p70 gene. We show that the repair of double-strand DNA breaks after P-element excision is severely reduced in *mus309* mutants using an in vivo assay for P-element transposase activity. In addition, excision products recovered from *mus309* mutant embryos by use of a plasmid-based P-element mobility assay contain large deletions, suggesting that IRBP is involved in the repair of double-strand DNA breaks. Our findings provide the first demonstration that a mutation in the *IRBP* gene affects double-strand DNA break repair and suggest that DNA repair functions are conserved between *Drosophila* and mammals.

[Key Words: DNA repair; P elements; transposition; *Drosophila*; mutagen sensitivity]

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P transposable elements are a family of mobile DNA¹ elements found in *Drosophila melanogaster*. They were originally identified as the causative agents for a group of disorders known as hybrid dysgenesis (for review, see Engels 1989). Full-length, 2.9-kb P elements encode an 87-kD transposase protein that catalyzes transposition (Rio et al. 1986) and a 66-kD repressor of transposition (Misra and Rio 1990). Natural *Drosophila* populations also carry smaller, internally deleted, nonautonomous elements (O'Hare and Rubin 1983), some of which have been shown to repress transposition in vivo (Rasmusson et al. 1993).

All P elements have 31-bp terminal inverted repeats, 11-bp internal inverted repeats, and unique sequences at both P-element termini that are important for transposition (Mullins et al. 1989). The transposase protein specifically binds to sequences within the P element, near the 31-bp inverted repeats (Kaufman et al. 1989). Sequences in the outer half of the 31-bp inverted repeats are specifically recognized by a *Drosophila* protein, IRBP (inverted repeat binding protein) (Rio and Rubin 1988). Genetic and biochemical experiments have outlined a model for transposition in which P elements transpose by a nonreplicative cut-and-paste mechanism involving double-strand gap repair (Engels et al. 1990; Kaufman and Rio 1992). A similar mechanism is used by the bac-

terial transposons Tn7 and Tn10 (Bainton et al. 1991; Benjamin and Kleckner 1989).

In the cut-and-paste model of transposition, sequence information is unidirectionally transferred from a donor site to a target site. When the P element excises, it leaves behind a double-strand break at the donor site (Engels et al. 1990; Kaufman and Rio 1992). Repair of the break must occur to prevent chromosome loss. The template for repair can be the sister chromatid, the homologous chromosome, or an ectopically located P element, which can lead to restoration of the P element at the excision site if the template for repair contains the P-element sequence (Engels et al. 1990). The repair process presumably occurs by *Drosophila*-encoded DNA repair enzymes. Because IRBP interacts specifically with the 31-bp inverted repeats (Rio and Rubin 1988), this protein is a good candidate for a *Drosophila*-encoded protein involved in P-element transposition.

To investigate the possible involvement of IRBP in P-element transposition, the gene encoding IRBP was isolated (Beall et al. 1994). The IRBP amino acid sequence shows significant homology to the p70 subunit of the human Ku autoimmune antigen. Ku was originally identified in patients with several autoimmune disorders (Mimori et al. 1981; Reeves 1992). The mammalian Ku is a heterodimer composed of 70- and 80-kD subunits. Similar to the mammalian Ku protein, IRBP is associated with an 80- to 90-kD protein (Jacoby and Wensink 1994; E.L. Beall and D.C. Rio, unpubl.). The mammalian Ku

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heterodimer can bind to free DNA termini in a sequence-independent manner (Mimori and Hardin 1986; Falzon et al. 1993) as well as site-specifically to internal DNA sites (Knuth et al. 1990; May et al. 1991; Messier et al. 1993). Ku possesses ATPase and DNA helicase activities (Cao et al. 1994; Tuteja et al. 1994). In addition, Ku is the DNA-binding subunit of the DNA-dependent protein kinase (DNA-PK) and can reversibly associate with the 450-kD catalytic component (DNA-PKcs) (Gottlieb and Jackson 1993). DNA-PK is known to phosphorylate a number of nuclear proteins in vitro, including p53, simian virus 40 T antigen (SV40 Tag), and Sp1 (Lees-Miller et al. 1990; Bannister et al. 1993; Gottlieb and Jackson 1993). More recently, radiation-sensitive mutant mammalian cell lines defective for either the Ku 80-kD subunit (*xrs-6/sxi-3*) or the DNA-PKcs protein (*V-3/scid*) have been identified, and both show hypersensitivity to ionizing radiation and exhibit defects in immunoglobulin *V(D)J* recombination (Rathmell and Chu 1994; Taccioli et al. 1994; Blunt et al. 1995; Boubnov et al. 1995; Finnie et al. 1995; Kirchgessner et al. 1995). Interestingly, the Ku p80 mutants display a defect in both signal and coding joint formation during *V(D)J* recombination (Taccioli et al. 1994), whereas coding joint formation is affected primarily in the DNA-PKcs/*scid* mutants (Blunt et al. 1995), suggesting overlapping but distinct roles for the Ku heterodimer and the DNA-PK in *V(D)J* recombination.

Genetic screens have identified several excision or postreplication repair-deficient mutants in *Drosophila* (Boyd and Setlow 1976; Boyd et al. 1976, 1981). One mutation, called *mus302* (for *mutagen-sensitive 302*), causes a severe reduction in the survival of flies that have undergone P-element excision (Banga et al. 1991). Cytogenetic mapping of *Drosophila* polytene chromosomes localized the *IRBP* gene to the right arm of the third chromosome at position 86E2-3 (Beall et al. 1994). Another mutagen-sensitive mutant, *mus309*, was mapped genetically to the same location (Boyd et al. 1981). Here, we report that the *mus309* mutation is a mutation in the *IRBP* gene. In addition, we provide evidence that IRBP is involved in double-strand DNA break repair and P-element transposition.

Results

mus309 mutants are unable to repair double-strand DNA breaks

It has been shown that several *mus* mutants in *Drosophila* are involved in DNA double-strand break repair (Banga et al. 1991). To test whether *mus309* mutants are involved in this process, we used a genetic assay involving P-element transposase-induced chromosome breaks. The *singed-weak* (*sn^w*) allele of the *singed* bristle locus contains two small, tandemly arranged P elements inserted into the 5'-untranslated region of the X-linked *singed* gene (Engels 1979; Roiha et al. 1988) (Fig. 1A) and was used as the source of P elements. When a somatic source of transposase [$\Delta 2-3$ (99B)] is provided (Robertson

Figure 1. Somatic P-element excision assay. (A) A schematic diagram of the assay used to determine the involvement of *mus309* in P-element excision. Two tandemly arranged P elements at the *sn^w* locus are readily mobilized in the presence of P-element transposase. Excision of one element changes the bristle phenotype to singed-extreme (*sn^e*), whereas excision of the other element changes the phenotype to wild-type (*sn⁺*). P-element mobilization is detected as a mosaic bristle pattern in adult flies. (B) The genetic crosses used to determine the involvement of *mus309* in P-element transposition. Male or female survival was determined as the ratio of male or female siblings undergoing P-element mobilization in a *mus309* mutant background relative to *mus309*/TM3 sisters that lacked the *sn^w* elements. *mus309* / + refers to the control cross in which a single, functional copy of *mus309* was present in all progeny. *mus309* without $\Delta 2-3$ refers to the control cross performed in the absence of transposase. For all crosses, non-TM3 progeny containing the *sn^w* chromosome and *mus309*/TM3 female progeny that lacked the *sn^w* chromosome were scored to determine the male or female survival, as diagramed for the experimental cross. (See Materials and methods for details.)

et al. 1988), mobilization of the *sn^w* elements results in a change in the bristle phenotype from *sn^w* to *sn⁺* (wild type) or *sn^e* (*singed-extreme*), which is detected as a mosaic bristle pattern in adult flies (Laski et al. 1986). If *mus309* is involved in the repair of double-strand DNA breaks after a P element is mobilized, a reduction in the number of mosaic males should be observed in a *mus309* mutant background.

Heterozygous males carrying both the *mus309^{D3}* allele and the transposase source ($\Delta 2-3$) on the same third

chromosome were crossed to heterozygous *mus309*^{D2} females carrying the *sn*^w allele (Fig. 1B). It was necessary to perform the test with the *mus309* heteroallelic combination because of the linkage of each *mus309* allele to a recessive lethal mutation(s). Control crosses were performed with isogenic strains that were either heterozygous for the *mus309* mutation or lacked the $\Delta 2\text{-}3(99\text{B})$ transposase (Fig. 1B). The involvement of *mus309* in P-element transposition was determined by calculation of the ratio of male progeny carrying the *sn*^w P-element-containing chromosome relative to their sisters that lacked the *sn*^w chromosome, in the presence of transposase, as depicted in Figure 1B (male survival). We found a threefold reduction in the survival of *mus309* mutant males that lacked the P-element transposase (Table 1A, cf. lines 1 and 2). However, we found an additional sixfold reduction in the survival of *mus309* mutant males containing the *sn*^w chromosome in the presence of transposase (Table 1A, cf. lines 2 and 3). Two *Drosophila* mutants, *mei-41* and *mus302*, showed a similar reduced male survival by this same assay (Banga et al. 1991).

Because the males in this experiment did not have a homologous X chromosome to serve as a template for gap repair following P-element excision, we examined the effect of P-element mobilization in sibling *mus309* mutant females as diagramed in Fig. 1B to determine whether the presence of an homologous sequence would affect the repair of double-strand breaks in *mus309* mutants. Female survival was calculated as described in Figure 1B, and the results are shown in Table 1B. Like the sibling males, there was a slight reduction in the survival of *mus309* mutant females that lacked P-element mobilization (1.3-fold decrease; Table 1B, cf. lines 1 and 2). However, there was an additional 1.9-fold reduction in the survival of *mus309* mutant females that had undergone P-element mobilization (Table 1B, cf. lines 2 and 3). Moreover, all of the surviving *mus309* mutant females had several interesting phenotypes, including extensive white eye color mosaicism (*w*⁻) but normal ommatidial

morphology as well as severe abdominal deformities that are indicative of cell death.

The higher survival of *mus309* mutant females in the *sn*^w assay might result from gap repair following P-element mobilization. However, because *mus309* mutants undergo extensive deletions following a transposase-induced double-strand DNA break (see below), we can suggest an alternative hypothesis. Because the two female X chromosomes are differentially marked (*w*⁺ on the *sn*^w chromosome and *w*¹¹⁸ on the *sn*⁺ chromosome, Fig. 1B), intrachromosomal deletions extending from *sn*^w through the *white* locus in *mus309* mutant females would result in the somatic loss of the *w*⁺ gene on the *sn*^w-containing chromosome. These deletions could lead to the observed *w*⁻ mosaic eye color patches in *mus309* mutant females. Deletions of this sort would result in a higher rate of lethality in *mus309* mutant males because of the loss of essential genes located within the deleted regions. Because all of the *mus309* mutant females, but not the control females that had undergone P-element mobilization, displayed the *w*⁻ eye color mosaicism, it appears that gap repair from a homolog in *mus309* mutant females is severely impaired. These results demonstrate that the *mus309* gene product is required for the repair of P-element-induced double-strand DNA chromosome breaks, both in the presence or absence of a homologous sequence. The finding that *mus309* mutants are hypersensitive to the X-ray mimetic agent methylmethane sulfonate (MMS) (Vogel and Natarajan 1979; Boyd et al. 1981), also supports a role for *mus309* in double-strand DNA break repair.

IRBP corresponds to *mus309*

We have shown previously that the *IRBP* gene maps to the interval 86E2-3 on the right arm of the third chromosome (Beall et al. 1994). Interestingly, *mus309*, a mutation that causes MMS hypersensitivity and female sterility, was mapped genetically to the same genomic region (Boyd et al. 1981). We wanted to determine whether IRBP is *mus309* by testing for complementation of both *mus309* mutant phenotypes.

We isolated λ genomic DNA clones encompassing the IRBP locus by hybridization with an *IRBP* cDNA clone (E.L. Beall and D.C. Rio, unpubl.). A restriction map of the region is diagramed in Fig. 2A. We constructed several P-element transformation vectors containing genomic fragments encompassing the *IRBP* gene. These include 6.8- and 8.8-kb *Bam*HI fragments, as well as a 13-kb *Sall* fragment (Fig. 2A). We created a transformation vector containing a deletion in the 13-kb genomic fragment spanning the carboxy-terminal half of IRBP (amino acids 266–631) to serve as a negative control (Fig. 2A, bottom line). The transgenes were introduced into the *Drosophila* genome by standard P-element-mediated transformation procedures. Several independent transformants for each of the transgenes were isolated and genetically mapped. Three X-linked or second chromosome transformants for each genomic fragment were

Table 1. Survival of *mus309* mutant males and females after P element mobilization

Genotype	Percent survival ^a	Total ^b
A. Males		
<i>mus309</i> /+	56 ± 7	433
<i>mus309</i> without $\Delta 2\text{-}3$	18 ± 2	439
<i>mus309</i> and $\Delta 2\text{-}3$	3 ± 0.8	415
B. Females		
<i>mus309</i> +	66 ± 1 × 10 ⁻⁴	453
<i>mus309</i> without $\Delta 2\text{-}3$	50 ± 0.2	585
<i>mus309</i> and $\Delta 2\text{-}3$	27 ± 1 × 10 ⁻⁴	608

^a Percent survival is determined as the ratio of *mus309* mutant males/females undergoing P element mobilization vs. *mus309*/TM3 sibling sisters lacking P elements (×100) as diagramed in Fig. 1B for male/female survival. Mean ratios are shown ± s.d.

^b The number of flies scored of the genotypes indicated in Fig. 1B that were used to determine male/female survival.

Figure 2. Complementation of the *mus309* mutant phenotypes. (A) Diagram of the IRBP-containing transgenes used to test for complementation of the *mus309* mutant phenotypes. The top line depicts the 13-kb genomic DNA fragment used to construct the various transgenes, with the IRBP location indicated by the bracket. The restriction endonuclease sites are indicated. (S) *Sall*; (B) *Bam*HI; (A) *Apa*I. The 1.6-kb *Apa*I fragment was removed to create a transgene containing a deletion in the carboxyl terminus of IRBP [amino acids 266–631, bottom line]. A qualitative assessment for the ability of the various transgenes to complement the *mus309* mutant phenotypes is indicated at right. (B) Crosses used to assay for complementation of the *mus309* mutant phenotypes by IRBP-encoding transgenes. To determine whether IRBP complemented the *mus309* MMS sensitivity, the ratio of heteroallelic *mus309* mutant progeny containing an IRBP-encoding transgene to siblings heterozygous for the *mus309* mutation [experimental classes, divide the number of male and female progeny from line 1 by line 2], was compared to the ratio of heteroallelic *mus309* mutant progeny lacking an IRBP-encoding transgene to siblings heterozygous for the *mus309* mutation (control classes, divide the number of male and female progeny from line 1 by line 2) as outlined in the left box. Complementation of the female sterility was determined by crossing heteroallelic *mus309* mutant progeny with or without an IRBP-encoding transgene to wild-type males (w^{1118}) and scoring for progeny as outlined in the right box.

tested for genetic complementation of the *mus309* mutant phenotypes, as diagramed in Figure 2B. The cross outlined in Figure 2B represents the second chromosome transformants. Heterozygous *mus309*^{D2} females were crossed to heterozygous *mus309*^{D3} males containing an IRBP transgene (P[w⁺, IRBP]) and progeny tested for survival on mutagen-containing food, or for female sterility as discussed below. Because each strain contains a single IRBP transgene, siblings lacking the transgene were used as controls for each cross.

Because *mus309* mutant females produce inviable eggs, complementation of female sterility was scored by the presence of larvae in vials after the crossing of heteroallelic *mus309* mutant females, either with or without the transgene, to fertile males (Fig. 2B, right box). For each transformant, 100 females were tested for fertility. We found that the 6.8-kb *Bam*HI fragment did not complement the *mus309* female sterility (E.L. Beall and D.C. Rio, unpubl.). Three different transformants containing either the 8.8-kb *Bam*HI or the 13-kb *Sall* fragments complemented the *mus309* female sterility (Table 2). In

Table 2. Complementation of *mus309* mutant female sterility with IRBP-containing transgenes

Transformant ^a	Transgene	Percent fertility ^b
No transgene		29
34.1	P[w ⁺ , gIRBP8.8]	40
121.3	P[w ⁺ , gIRBP8.8]	49
223.2	P[w ⁺ , gIRBP8.8]	75
No transgene		36
23.1	P[w ⁺ , gIRBP13]	85
30.2	P[w ⁺ , gIRBP13]	62
97.1	P[w ⁺ , gIRBP13]	77
No transgene		36
39.1	P[w ⁺ , gIRBPΔ]	38

^a No transgene refers to isogenic *mus309* strains lacking an IRBP-encoding transgene. Each number represents an independently isolated transgenic line for the fragment size indicated.

^b 100 females were tested for each transformant. Fertility was scored by the presence of larvae in vials after 7 days.

the best case, a 2.5-fold increase in female fertility was observed (cf. no transgene with transformant 223.2 for the 8.8-kb transformants). In addition, we found that an *IRBP* cDNA-containing transformant complemented the *mus309* female sterility (E.L. Beall and D.C. Rio, unpubl.) and that a transformant containing a deletion in the *IRBP* gene would not complement the *mus309* female sterility (Table 2). Taken together, these results demonstrate that an ectopic copy of the *IRBP* gene can partially complement the *mus309* female sterility. Our previous finding that the *IRBP* transcript level is elevated in females and ovaries is consistent with a role for *IRBP* in female fertility (Beall et al. 1994).

We also tested whether *IRBP*-containing transgenes could complement the MMS sensitivity in heteroallelic *mus309* mutant flies. We determined the threshold level of MMS that allows for the survival of a few heteroallelic *mus309* mutant flies to be between 0.045% and 0.055% MMS (Fig. 3, shaded boxes). We tested several concentrations of MMS surrounding this threshold level for complementation of the MMS sensitivity by scoring for survival of heteroallelic *mus309* mutant flies in the presence or absence an *IRBP*-containing transgene when grown on mutagen-containing food (Fig. 2B, left box). We found that the 6.8-kb *Bam*HI transgene did not complement the MMS sensitivity (E.L. Beall and D.C. Rio, unpubl.). However, we found that three different 8.8- and 13-kb transgenes complemented the MMS sensitivity. A representative for each transgene is shown in Figure 3. In addition, an *IRBP* cDNA-containing transgene complemented the MMS sensitivity (E.L. Beall and D.C. Rio, unpubl.). At nearly every concentration of MMS tested, there was an increase in the number of heteroallelic *mus309* mutant flies carrying an *IRBP* transgene, with the best increase in survivability at or near the threshold level. For both the 8.8- and 13-kb genomic fragments, a

maximum sevenfold increase in the survivability of *IRBP*-encoding transgenic flies was observed. Differences in the level of complementation between transgenes are likely the result of differences in the *IRBP* expression levels because of position effects at the insertion site. Differences in transgene expression levels are common and have been seen with the *Drosophila rosy* and *C/EBP* genes (Spradling and Rubin 1983; Rorth 1994). In addition, transformants containing a deletion in the *IRBP* gene were not able to survive above background levels at any concentration of MMS tested (Fig. 3, P[gIRBPΔ]). The finding that both female sterility and MMS sensitivity in *mus309* mutant flies can be complemented by several independent *IRBP*-containing transgenes, and that a transgene lacking a complete copy of *IRBP* no longer complement either mutant phenotype, demonstrates that *IRBP* is *mus309*.

IRBP rescues the DNA repair defect displayed by *mus309* mutant embryos

Because *IRBP* is known to interact with the P element 31-bp inverted repeats (Rio and Rubin 1988), and *mus309* mutants fail to accurately repair P-element-containing X chromosomes in the presence of active transposase, we directly examined the products of DNA repair following P-element excision in *mus309* mutant embryos. We used a P-element mobility assay in which excision events can be detected by antibiotic resistance selection in bacteria after recovery of the transposition products from *Drosophila* embryos (Rio et al. 1986; O'Brochta et al. 1991). In this assay a reporter plasmid containing a P-element insertion into the kanamycin resistance gene was used to detect excision events in the presence of transposase (pISP-2/Km, Fig. 4). We used a plasmid containing the transposase gene with the third intron removed (PΔ2-3) under the control of the *Drosophila* actin promoter as a

Figure 3. Complementation of *mus309* MMS sensitivity by *IRBP*-encoding transgenes. Fifteen pairs of flies for each transgene were allowed to lay for 48 hr before the parents were removed and mutagen applied to the food. All classes of progeny were counted until 17 days after the cross was initiated. For each concentration of MMS, ~300 flies were counted. Complementation was determined from the cross indicated in Fig. 2B (left box). The percent survival was calculated as the number of heteroallelic *mus309* mutant progeny with (hatched bar) or without (shaded bar) an ectopic copy of *IRBP* relative to siblings that were heterozygous for the *mus309* mutation $\times 100$. Three independent transformants were tested for each transgene, and one representative for each transgene tested is diagramed. [gIRBP] Genomic fragment encompassing the *IRBP* locus of the size indicated; [gIRBPΔ] genomic fragment with a deletion in the carboxyl terminus of *IRBP*.

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Figure 4. Plasmid-based P-element mobility assay. Schematic diagram of the assay. The excision indicator plasmid (pISP-2/Km) was injected alone or in conjunction with the P-element transposase-encoding plasmid (PΔ2-3), in the presence or absence of the IRBP-encoding plasmid, into w^{1118} (wild-type) or *mus309* mutant embryos (see Materials and methods for details). Excision events were determined by the gain in kanamycin resistance (Kan^R) after plasmid recovery from injected embryos and electroporation into *E. coli*.

source of transposase. In addition, as a source of IRBP in the assay, we used a plasmid containing the *IRBP* cDNA under the control of the *Drosophila* actin promoter. If IRBP is required for P-element excision *in vivo*, then the observed reporter plasmid excision frequency in the presence of P-element transposase may be reduced in *mus309* mutant embryos and should be restored by a cDNA encoding IRBP.

We injected the reporter plasmid alone or in combination with the transposase plasmid, as well as with or without the IRBP plasmid, into either wild-type or *mus309* mutant, preblastoderm embryos by standard microinjection techniques. Plasmid DNA was recovered and introduced into bacteria. Excision events were detected by the gain of kanamycin resistance (Fig. 4). Because the P element interrupts the coding region of the kanamycin-resistance gene, only excisions that restore the open reading frame will be detected in this assay. In fact, we will only recover excised plasmids that contain small deletions that do not extend into the kanamycin-resistance gene or promoter region. We found nearly a 20-fold reduction in the recovery of excised plasmids in *mus309* mutant embryos when compared to wild-type (w^{1118}) embryos (Table 3, cf. lines 3 and 7). When the plasmid encoding IRBP was injected with both the re-

porter and transposase source, we found a 10-fold increase in the excision frequency in *mus309* mutants (Table 3, cf. lines 7 and 8), which represents ~30% of the wild-type excision frequency (Table 3, cf. lines 4 and 8). In separate experiments in which the concentration of IRBP was doubled, up to 50% of the excision frequency was restored (E.L. Beall and D.C. Rio, unpubl.). Similar frequencies have been reported for the mammalian Ku p80 subunit in restoring coding joint formation during *V(D)J* recombination in the *xrs-6* mutant cell line (Taccioli et al. 1994). It is interesting to note that we found a stimulation in the excision frequency in wild-type embryos when IRBP was injected with both the reporter and transposase source (Table 3, cf. lines 3 and 4). Increased levels of IRBP may protect the broken DNA ends from exonuclease digestion after the P element excises (see Discussion). The finding that the mammalian Ku p70 subunit alone has DNA end-binding activity (Messier et al. 1993) is consistent with this hypothesis.

To determine the nature of the excision products, we analyzed the DNA recovered from ampicillin/kanamycin-resistant plasmids after excision in both wild-type and mutant embryos by restriction endonuclease digestion. We found that a DNA fragment encompassing the excision site was highly variable in size in plasmids recovered from *mus309* mutant embryos, suggesting a defect in the correct processing of the DNA termini after the excision event. In addition, plasmids in which IRBP was injected with both the reporter and transposase source showed a restoration in the uniformity of the fragment size, similar to the size found from wild-type embryos, suggesting that IRBP was restoring the defect in the *mus309* mutant embryos (E.L. Beall and D.C. Rio,

Table 3. Frequency of P element excision in embryos

Embryo type	Plasmid injected ^a	(Amp + Kan)/Amp ($\times 10^5$) ^b
w^{1118}	pISP-2/Km	0.40 $\pm$ 0.3
w^{1118}	pISP-2/Km + IRBP	0.27 $\pm$ 0.06
w^{1118}	pISP-2/Km + PΔ2-3	5.2 $\pm$ 1.0
w^{1118}	pISP-2/Km + IRBP + PΔ2-3	6.5 $\pm$ 0.2
<i>mus309</i> ^{D2 D3}	pISP-2/Km	0.31 $\pm$ 0.3
<i>mus309</i> ^{D2 D3}	pISP-2/Km + IRBP	0.28 $\pm$ 0.3
<i>mus309</i> ^{D2 D3}	pISP-2/Km + PΔ2-3	0.21 $\pm$ 0.3
<i>mus309</i> ^{D2 D3}	pISP-2/Km + IRBP + PΔ2-3	2.1 $\pm$ 0.3

All injections were performed with the same set of flies. One hundred seventy embryos were injected for each plasmid mixture. DNA was recovered, and 35 embryo equivalents electroporated into *E. coli* to score for P element excision events as diagrammed in Fig. 4.

^a (pISP-2/Km) The P element-containing reporter plasmid; (IRBP) the IRBP cDNA-encoding plasmid pBSKS(+)|PacIRBP; (PΔ2-3) the transposase-encoding plasmid pBSKS(+)|PacKP-SΔ2-3.

^b The ratio of excised plasmids vs. the total number of plasmids recovered, including the reporter pISP-2/Km, the transposase-, and IRBP-encoding plasmids. Mean ratios are shown $\pm$ s.d. for experiments that were performed at least three times.

unpubl.). DNA sequence analysis of these plasmids confirmed our findings from the restriction endonuclease analysis (Fig. 5). We found that 50% of the kanamycin-resistant plasmids recovered from *mus309* mutant embryos in the absence of IRBP had extensive regions of the kanamycin-resistance gene deleted [Fig. 5, sequence block 2 (*mus*)], whereas <10% of the plasmids recovered from wild-type embryos had minor deletions [Fig. 5, blocks 1 and 3 (*w¹¹¹⁸* and *w* + IRBP)]. More importantly, plasmids recovered from *mus309* mutant embryos in the presence of IRBP had sequences comparable to sequences recovered from wild-type embryos [Fig. 5, cf. sequence block 3 (*M* + IRBP) with block 1 (*w¹¹¹⁸*)].

Nearly all of the excision products recovered from wild-type embryos contained both copies of the 8-bp target site duplication and up to 7 nucleotides derived from the P-element 31-bp inverted repeat sequences (Fig. 5, blocks 1 and 4). Similar findings have been reported previously in the sequence analysis of P-element excision products from both a plasmid-based assay (Rio et al. 1986; O'Brochta et al. 1991) and from chromosomal excision events (Takasu-Ishikawa et al. 1992; Staveley et al. 1995). It is possible that transposase makes a staggered cleavage at each 5' end of the 8-bp target site. Gap repair from another P-element-containing molecule can result in P-element-derived sequences left at the donor site after excision (see Discussion). A clear picture regarding the mechanism of P-element excision will re-

quire a better understanding of the exact nature of the DNA cleavages made by P-element transposase.

Our results demonstrate that the processing of double-strand DNA breaks after P-element excision is severely affected in *mus309* mutant embryos. In addition, the finding that the IRBP cDNA can partially restore the excision frequency and totally restore the precision of donor site repair in *mus309* mutant embryos shows that the DNA repair defect observed in *mus309* mutant embryos is caused by a mutation in the *IRBP* gene.

Discussion

Drosophila proteins are required for P-element transposition

Several prokaryotic transposons demonstrate a requirement for host-encoded proteins to facilitate transposition (for review, see Berg 1989; Kleckner 1989; Pato 1989). Here, we show an important role for the *Drosophila* protein IRBP in double-strand DNA break repair after P-element transposition. We found that a DNA repair-deficient *Drosophila* mutant, *mus309*, is unable to repair P-element-induced double-strand DNA breaks and that *IRBP* is *mus309*. In addition to repair of site-specific cleavages after P-element mobilization, our results suggest that IRBP is necessary for the repair of DNA breaks induced by DNA-damaging agents such as MMS.

Figure 5. DNA sequence analysis of excised plasmids recovered from *w¹¹¹⁸* (wild-type) and *mus309* mutant embryos. A partial nucleotide sequence of pISP-2/Km (reporter) is shown, including the 0.6-kb P element (shaded box), the 31-bp inverted repeats, the 8-bp target site duplication (black-boxed letters), and 88-bp surrounding the excision site. The right 8-bp target site duplication is missing 1 nucleotide, as has been described previously (O'Brochta et al. 1991). (.) Deletions in the above sequences. The first sequence block (*w¹¹¹⁸*) represents sequences of Kan^R plasmids recovered from wild-type embryos in the presence of transposase only. The second sequence block (*mus*) represents sequences of Kan^R plasmids recovered from *mus309* mutant embryos in the presence of transposase only. The third sequence block (*M* + IRBP) represents sequences of Kan^R plasmids recovered from *mus309* mutant embryos after coinjection of the IRBP-containing plasmid. The fourth sequence block (*W* + IRBP) represents sequences of Kan^R plasmids recovered from wild-type embryos after coinjection of the IRBP-containing plasmid.

Because IRBP shows significant protein sequence homology to the 70-kD subunit of the mammalian Ku autoimmune antigen (Beall et al. 1994) our findings provide the first *in vivo* analysis of a Ku 70-kD homolog and demonstrate its involvement in P-element transposition and DNA repair. These results are consistent with the participation of the Ku heterodimer in double-strand DNA break repair and immunoglobulin *V(D)J* recombination in mammalian cells (Rathmell and Chu 1994; Taccioli et al. 1994), as well as its DNA-binding characteristics (Mimori and Hardin 1986; Falzon et al. 1993). It is unclear whether Ku has a direct or indirect role in the repair of double-strand DNA breaks. Ku may simply be protecting the broken ends from exonuclease activity as seen with the gam protein of phage Mu (Akroyd et al. 1986; Thaler et al. 1987). Alternatively, Ku may promote the joining of double-strand DNA breaks by modulating the activity of other repair proteins, such as the human replication protein A (RPA), through its interaction with DNA-PK (Gottlieb and Jackson 1993). RPA is required *in vitro* for mammalian nucleotide excision repair (Aboussekhra et al. 1995), and the p34 subunit of RPA is efficiently phosphorylated by DNA-PK in a DNA-dependent manner (Brush et al. 1994; Pan et al. 1994). In addition, p34 becomes phosphorylated in mammalian cells that have been exposed to ionizing or ultraviolet irradiation, possibly linking RPA phosphorylation to a delay in cell-cycle progression until the DNA damage can be repaired (Liu and Weaver 1993; Carty et al. 1994).

The kinase recognition sequences for DNA-PK have been identified for the substrate p53 and were found to be either serine or threonine residues when adjacent to glutamine (Lees-Miller et al. 1992). There are seven of these potential phosphorylation sequences in the first 144 amino acids of the P-element transposase protein (Rio et al. 1986). We found that an amino-terminal fragment of the transposase protein encompassing these seven potential phosphorylation sites is phosphorylated by the mammalian DNA-PK only in the presence of double-stranded DNA (Y.M. Mul, E.L. Beall, and D.C. Rio, unpubl.). Furthermore, DNA-PK activity has been reported in *Drosophila* (Finnie et al. 1995). It is conceivable that the breaks in DNA after cleavage by transposase may trigger the phosphorylation of transposase by Ku/DNA-PK to allow release of the complex from the DNA and/or direct repair of the breaks by the *Drosophila* repair machinery. It is not known, however, what role, if any, phosphorylation of the transposase protein may have in transposition.

Our finding that large deletions occur after a P element excises in the absence of IRBP (Fig. 5) supports a role for IRBP similar to the mammalian Ku protein in double-strand DNA break repair. Addition of IRBP in the injection assay restores the joining of the DNA termini, as demonstrated both by excision frequency (Table 3) and DNA sequence analysis (Fig. 5). Because IRBP is associated with an 80- to 90-kD protein corresponding to the mammalian Ku 80-kD subunit (Jacoby and Wensink 1994; E.L. Beall and D.C. Rio, unpubl.), we are not sure whether IRBP alone is providing the repair functions or

whether there is a free pool of the 80- to 90-kD subunit present that can interact with IRBP. Recently, the mammalian Ku 80-kD subunit was reported to be unstable in the absence of the 70-kD subunit (Satoh et al. 1995). In addition, the mammalian Ku 70-kD subunit alone can bind to DNA in a manner similar to the p70/p80 heterodimer (Messier et al. 1993; Wang et al. 1994). These findings, along with the observation that IRBP binds site specifically to the 31-bp inverted repeats (Rio and Rubin 1988), suggest that IRBP alone may have a function in repair of P-element-induced double-strand DNA breaks. It is interesting to note that the mammalian Ku heterodimer possesses both ATPase and DNA helicase activities (Cao et al. 1994; Tuteja et al. 1994). It is possible that one function for IRBP may be to unwind the DNA helix to facilitate interaction with RPA and trigger extension of the broken ends by the polymerase during gap repair.

P-element excision mechanism

A mechanism for P-element transposition that is based on several genetic and biochemical experiments has been proposed. In this model, a double-strand DNA break is left at the donor site after a P element is excised (Engels et al. 1990; Kaufman and Rio 1992). Following excision, templated repair from either a homologous chromosome, sister chromatid, or ectopic sequence located elsewhere in the genome leads to restoration of the original target sequence, restoration of the P element at the excision site, or partial restoration of the P-element sequence (Engels et al. 1990). It is thought that the gap repair after a P element is excised may occur after DNA synthesis (during late S or G₂ in the cell cycle), when the sister chromatid may serve as a template for repair of the DNA break (Engels et al. 1990). However, the exact nature of the initial cleavage events prior to excision of the P element from the donor site is unknown. It is not known whether transposase makes a staggered or blunt-ended cleavage during excision.

Our plasmid sequencing data show that in nearly all plasmids analyzed from *w¹¹¹⁸* embryos, both target site duplications and up to 7 additional P element 31-bp inverted repeat-derived nucleotides are left behind at the donor site (Fig. 5). Similar findings have been reported previously in the sequence analysis of excised P elements with a similar plasmid excision assay (Rio et al. 1986; O'Brochta et al. 1991), or from P elements excised from chromosomal locations (Takasu-Ishikawa et al. 1992; Staveley et al. 1995). In the sequence analysis of excision events from chromosomally located P elements at the vestigial (*vg*) locus, however, the majority of cases showed that 15–19 bp derived from the 31-bp inverted repeats remained at the excision site (Staveley et al. 1995). The difference between the extent of the sequence remaining at the donor site may reflect a difference in the assays.

Previous models attempting to explain the occurrence of the extra P-element-derived sequences at the donor site propose either a staggered cleavage within the P-element termini (Staveley et al. 1995) or the formation of

a hairpin intermediate during excision (Takasu-Ishikawa et al. 1992). Neither model can adequately account for the variation in the sequences left behind at the donor site or the retention of the integrity of the excised P-element termini. Occasionally P-element excision events lead to extensive deletions of DNA sequences flanking the insertion site. Perhaps *mus309* mutants will allow for the creation of enlarged deletions extending from the insertion site into the flanking DNA to facilitate the generation of null mutations in nearby *Drosophila* genes. Our results from both the *sn^w* experiments and the plasmid excision experiments support this possibility. The *w⁻* eye color mosaicism observed in *mus309* mutant females that have undergone P-element mobilization can be explained by the creation of large deletions surrounding the excision site in the absence of *mus309*/IRBP.

On the basis of these data and a model for transposition in plants (Saedler and Nevers 1985), we propose that the cleavage pattern made by transposase is staggered, with precise cleavages made at each 5' end of the target site duplication and 3' end of the P element 31-bp inverted repeat. Precise excision would result from the annealing of the staggered ends in the absence of gap repair. However, gap repair using the free 3' termini would lead to the copying of information from another P-element-containing molecule. Depending on the length of the segment copied, a replacement of the target site duplication, along with sequences extending into the transposon termini, would be observed. This model would explain the observed excision products, including the retention of both 8-bp target site duplications (Fig. 5, block 3, line M + IRBP-9), the copying of sequences from a single 31-bp inverted repeat (Fig. 5, block 1, *w¹¹¹⁸*-1, 6, and 4), as well as the copying of sequences from both 31-bp inverted repeats (Fig. 5, block 1, *w¹¹¹⁸*-3, 5, 7, and 9). In addition, if an exonuclease were to bind to the free 3' end prior to polymerase binding, small deletions would be generated (Fig. 5, block 4, *w* + IRBP-3). A similar staggered cleavage pattern has been observed for the prokaryotic transposon Tn7 (Bainton et al. 1991). Once the exact nature of the DNA cleavages by transposase is determined, a more precise mechanism explaining the excision reaction can be formulated.

Conservation of repair mechanisms in eukaryotes

Ku homologs have been reported in several organisms, including mouse, monkey, hamster, and *Drosophila* (Paillard and Strauss 1991; Falzon and Kuff 1992; Beall et al. 1994; Jacoby and Wensink 1994; Rathmell and Chu 1994). In addition, a putative Ku homolog has been reported in the budding yeast, *Saccharomyces cerevisiae* (Feldmann and Winnacker 1993). DNA-PK activity has been reported for mouse, hamster, *Xenopus*, and *Drosophila* through the use of in vitro peptide kinase assay (Finnie et al. 1995). Mutations in either the Ku 80-kD or DNA-PK genes for mouse, hamster, and humans show extreme hypersensitivity to ionizing radiation and display defects in immunoglobulin *V(D)J* recombination in

mammalian tissue culture cells (Rathmell and Chu 1994; Blunt et al. 1995; Lees-Miller et al. 1995; Peterson et al. 1995). Our finding that the *Drosophila* Ku p70 mutant *mus309* has similar phenotypic defects as the mammalian mutant cell lines strongly suggests that the functions of the Ku/DNA-PKs proteins are conserved in eukaryotes.

P elements have been used extensively as vectors for germ-line transformation in *Drosophila*. This procedure allows for the integration of single copies of DNA segments into the genome without duplications or rearrangements. In addition, P elements have been used for insertional mutagenesis studies and for *lacZ* enhancer trapping. It would be useful for P-element transposition to occur in other organisms in which gene manipulation is difficult. Previous experiments have demonstrated that P-element transposase can catalyze excision events in mammalian and yeast cells by a plasmid excision assay (Rio et al. 1988). However, attempts at detecting reinsertion of P elements into a second target DNA segment (forward transposition) have been unsuccessful. DNA repair is required at the donor site after P-element excision and at the insertion site after integration. It is possible that the heterologous mammalian and yeast Ku complexes cannot function at the target site during integration or are unable to modify transposase activity appropriately following excision. The finding that the *Drosophila* IRBP and the mammalian and yeast Ku p70 subunits are only 23% and 18% identical, respectively (Beall et al. 1994), suggests that the required *Drosophila* factors for transposition may have diverged to an extent that no longer allows productive transposition in these heterologous organisms, even though there is a general functional conservation of the Ku subunits and DNA-PK between *Drosophila* and mammals. Perhaps introduction of the *Drosophila* genes into heterologous cell types may permit forward transposition.

Materials and methods

Drosophila strains

The *Drosophila* strains *w¹¹¹⁸* and *w¹¹¹⁸*; *TM3/CxD* are as described (Ashburner 1989). The strains *FM7a y^{31d} sc⁸ w^a vB* [Bloomington Stock Center], *y sn^w* [Roiha et al. 1988], *TM3 ry^{RK}/ry⁵⁰⁶* [Karess and Rubin 1984], and *Sb ry⁵⁰⁶ P[ry⁺ Δ2-3][99B]/TM6B* [Robertson et al. 1988] were used in the *sn^w* assay. The strains *w¹¹¹⁸*; *st mus309^{D2}/TM3* and *w¹¹¹⁸*; *mus309^{D3} ry/TM3* were made by the crossing of *w¹¹¹⁸*; *TM3/CxD* females to *st mus309^{D2}/TM3 Sb Ser* or *mus309^{D3} ry/TM3 Sb Ser* [Boyd et al. 1981] males and selection of white-eyed, stubble bristle, straight-winged flies, and then mating these progeny back to *w¹¹¹⁸*; *TM3/CxD* females to obtain a balanced stock. Flies were raised on standard cornmeal, molasses, and yeast medium at 25°C.

Recombinant DNA

The *P[w⁻, gIRBP]* elements used in germ-line transformation experiments were constructed by use of *pW8* P-element transformation vector [Klemenz et al. 1987]. DNA isolated from λ FIX genomic clones encompassing the *IRBP* locus was cleaved

with *Sall* to generate a 13-kb fragment and inserted into *XhoI*-cleaved pW8. The 13-kb-containing plasmid was then cleaved with *BamHI* to yield a 6.8- or 8.8-kb fragment that was inserted into *BamHI*-cleaved pW8 to generate the 6.8-kb- or 8.8-kb-containing transformation vectors. The transformation vector containing a deletion in the *IRBP* gene was constructed as follows: The pW8-gIRBP 13-kb *Sall* fragment-containing plasmid was cleaved with either *XbaI* and *ApaI* to generate a 4.1-kb fragment or *Sall* and *ApaI* to generate a 7.3-kb fragment. Both fragments were inserted into pW8 cleaved with *XhoI* and *XbaI* to generate a vector containing a 1.6-kb deletion eliminating the carboxyl terminus of IRBP (amino acids 266–631).

The plasmids used in the P-element excision assay were constructed as follows: pISP-2/Km was derived from pISP-2 (Rio et al. 1986) in which pISP-2 was cleaved with *PstI* and treated with T4 DNA polymerase. Plasmid pKm109-9 (Reiss et al. 1984) was cleaved with *BamHI* and *HincII* and treated with mung bean nuclease and the *NPTII* (neomycin phosphotransferase kanamycin resistance gene) fragment inserted into the flush pISP-2 vector, in place of the *lacZ* α fragment. pHSX-PAC KP-S Δ 2-3 was prepared with pHSX (Jones and Rubin 1990), a 2.6-kb actin 5C fragment from pPac (Ashburner 1989), a fragment of the transposase gene carrying an amino-terminal portion chemically re-synthesized to alter codon usage (D.C. Rio, unpubl.), and a carboxy-terminal fragment carrying the IVS3 (intervening sequence 3) deletion, Δ 2-3 (Laski et al. 1986). pHSX-PAC KP-S Δ 2-3 was cleaved with *NotI* and a 6.5-kb fragment containing the *Drosophila* actin promoter and the transposase gene was inserted into *NotI*-cleaved pBSKS(+) (Stratagene) to make the transposase helper, pBSKS(+)PAC KP-S Δ 2-3. To make the *IRBP* expression plasmid, pNB40 9-1 (Beall et al. 1994) was cleaved with *HindIII* and filled with the Klenow fragment of DNA polymerase I. The plasmid was then cleaved with *NotI* to generate a 2.2-kb fragment containing the *IRBP* cDNA. In addition, pPac (Ashburner 1989) was cleaved with *BamHI*, filled with the Klenow fragment of DNA polymerase I, and cleaved further with *NotI*, to generate a 2.6-kb fragment containing the *Drosophila* actin promoter. Both fragments were inserted into *NotI*-cleaved pBSKS(+), generating pBSKS(+)PAC IRBP.

P-element transformation

Transformation of w^{1118} strains was by standard procedures (Spradling 1986), except that the helper plasmid pHS Δ 2-3 was used (D.C. Rio, unpubl.). Isolines were established from individual independent transformants. Flies from each line were crossed to balancer strains, after genetically mapping the chromosome into which the element had integrated to establish balanced stocks. DNA blot hybridization was used to confirm that each strain contained a single, intact P-element insertion.

sn^w assay

The w^{1118} ; *mus309*^{D3} *ry* Δ 2-3(99B)/TM3 stock was made by the crossing of *mus309*^{D3} *ry*/TM3 *Sb Ser* females to *Sb ry*⁵⁰⁶ P[ry⁺ Δ 2-3(99B)/TM6B] males and selection of females that were ry⁺, *Sb*, non-TM6B. These females were crossed to TM3 *ry*⁵⁰⁶ males, and males that were ry⁺, *Sb*⁺ were crossed back to TM3 *ry*⁵⁰⁶/ry⁵⁰⁶ females to create a balanced stock. Progeny from individual males were tested for the presence of the *mus309*^{D3} allele by the crossing of males to *st mus309*^{D2}/TM3 *Sb Ser* females and testing for sensitivity to killing by MMS as described (Boyd et al. 1976). The presence of Δ 2-3(99B) was verified by the crossing of males to *y sn*^w females, and scoring *Sb*⁺ male progeny for a mosaic bristle pattern, indicating the presence of transposase. The w^{1118} chromosome was introduced by

the crossing of *mus309*^{D3} *ry* Δ 2-3(99B)/TM3 males to w^{1118} ; TM3 *CxD* females and the crossing of white-eyed, *Sb* males to ry⁺, *Sb* females, and selection of only white-eyed, *Sb* progeny in subsequent generations. For the control crosses, isogenic w^{1118} ; Δ 2-3 *Ki P*⁺/TM3 males were used for *mus309*^{D3} +, and w^{1118} ; *mus309*^{D3} *ry*/TM3 males were used for “without Δ 2-3” (see Fig. 1B). Each was made from non- w^{1118} stocks with the w^{1118} ; TM3 *CxD* stock as described above such that the stocks are isogenic except for the non-TM3 third chromosome.

The *y sn*^w/FM7a; *st mus309*^{D2}/TM3 stock used in the assay was made as follows: *y sn*^w females were crossed to FM7a males, and *y sn*^w/FM7a female progeny crossed to *y sn*^w; TM3/*CxD* males. *y sn*^w/FM7a; TM3 females were crossed to *y sn*^w; *CxD* males to create *y sn*^w/FM7a; TM3/*CxD*. Females from this stock were crossed to *st mus309*^{D2}/TM3 *Sb Ser* males, and yellow, *sn*^w, *Sb*, non-*CxD* males crossed back to *y sn*^w/FM7a; TM3/*CxD* females. non-*CxD* progeny were mated to create the *y sn*^w/FM7a; *st mus309*^{D2}/TM3 balanced stock.

In the assay, crosses were performed with five males and five virgin females per vial. Typically, 10–15 vials for each experimental cross were tested, and progeny scored until 17 days after initiation of the cross. All classes of progeny were scored. The male or female survival was determined as the ratio of male or female *mus309* mutant progeny that had undergone P-element mobilization to *mus309*^{D3} + female progeny that lacked the *sn*^w chromosome as depicted in Figure 1B. Standard deviations were calculated for each cross that was performed at least two times.

Female fertility and MMS assays

To test the transformant strains for complementation of the heteroallelic *mus309* mutant phenotypes, two or three independent X or second chromosome-located transgenic lines were crossed to w^{1118} ; TM3/*CxD* to establish w^{1118} ; P[w⁺, IRBP]; TM3/*CxD* stocks. These lines were crossed to w^{1118} ; *mus309*^{D3} *ry*/TM3 and w⁺, *Sb* progeny selected and crossed to establish w^{1118} ; P[w⁺, IRBP]; *mus309*^{D3} *ry*/TM3 balanced stocks.

To test for rescue of female fertility, w^{1118} ; P[w⁺, IRBP]; *mus309*^{D3} *ry*/TM3 males were crossed to w^{1118} ; *st mus309*^{D2}/TM3 females, and w⁺, *Sb*⁺ females crossed, individually, to three w^{1118} males. For each transformant tested, 100 crosses were performed and fertility scored by the presence of larvae in the vial. Isogenic control crosses with strains lacking an ectopic copy of IRBP were also performed.

To test for rescue of MMS sensitivity, 15 w^{1118} ; P[w⁺, IRBP]; *mus309*^{D3} *ry*/TM3 males were crossed to 15 w^{1118} ; *st mus309*^{D2}/TM3 aged females and the flies allowed to lay for 48 hr before being removed to a fresh vial. After 69–72 hr, mutagen was applied to the food and the progeny allowed to develop at 25°C. All classes of progeny were scored until 17 days after the cross was initiated. Siblings lacking an IRBP transgene were counted as the control class. Percent survival was determined as the number of heteroallelic *mus309* mutant progeny either with or without an IRBP-containing transgene relative to their respective *mus309*^{D2} or ^{D3}/TM3 siblings $\times$ 100.

P-element excision assay

The plasmids pISP-2/Km (0.5 mg/ml), pBSKS(+) PAC KP-S Δ 2-3 (0.25 mg/ml), and pBSKS(+) PAC IRBP (0.25 mg/ml) were injected either alone or in combination into preblastoderm embryos. The injected embryos were incubated at 18°C in oxygen-saturated oil for 20–22 hr. Embryos were washed with heptane to remove the oil and transferred to 1.5-ml Eppendorf tubes. Plasmid DNA was recovered as described (Rio et al. 1986; O'Brochta and Handler 1988) and resuspended in 10 mM Tris-

HCl, 1 mM EDTA (pH 7.5). Thirty-five embryo equivalents were used to transform 60 μ l of the RecA⁻ *Escherichia coli* strain AG1574 (Kaufman and Rio 1992) by electroporation with a Bio-Rad Gene Pulser as described by the manufacturer. Cells were plated onto Luria broth (LB) plates containing 100 μ g/ml of ampicillin or LB plates containing 100 μ g/ml of ampicillin and 50 μ g/ml of kanamycin. Colonies were allowed to develop for 12–16 hr at 37°C. Plasmid DNA was isolated from single Amp^R Kan^R colonies and analyzed by restriction endonuclease cleavage and chain-termination sequencing with Sequenase 2.0 (U.S. Biochemical) according to the manufacturer's instructions. The primer used for sequencing was 5'-CCGGCTCGTATGTTGTGTGG-3'.

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Drosophila IRBP/Ku p70 corresponds to the mutagen-sensitive mus309 gene and is involved in P-element excision in vivo.

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