I-*Sce*I Endonuclease, a New Tool for Studying DNA Double-Strand Break Repair Mechanisms in Drosophila

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ABSTRACT

As a step toward the development of a homologous recombination system in Drosophila, we have developed a methodology to target double-strand breaks (DSBs) to a specific position in the Drosophila genome. This method uses the mitochondrial endonuclease I-*Sce*I that recognizes and cuts an 18-bp restriction site. We find that >6% of the progeny derived from males that carry a marker gene bordered by two I-*Sce*I sites and that express I-*Sce*I in their germ line lose the marker gene. Southern blot analysis and sequencing of the regions surrounding the I-*Sce*I sites revealed that in the majority of the cases, the introduction of DSBs at the I-*Sce*I sites resulted in the complete deletion of the marker gene; the other events were associated with partial deletion of the marker gene. We discuss a number of applications for this novel technique, in particular its use to study DSB repair mechanisms.

IONIZING radiation and radiomimetic drugs induce DNA double-strand breaks (DSBs) at random positions in the genome. During mating-type switching in yeast, transposition of *P* elements in Drosophila, or rearrangements of immunoglobulin genes in vertebrates, DSBs are introduced at specific positions within the genome (Klar 1989; Jackson and Jeggo 1995; Weaver 1995). During evolution, to maintain genome integrity, a number of genetic pathways have been deployed to repair DSBs (reviewed in Haber 1995).

The mechanisms underlying DSB repair have been studied in Drosophila using the *P*-element transposase as a means to generate the chromosomal breaks (Engels *et al.* 1990; Kaufman and Rio 1992). These studies have revealed that DSBs, induced by excision of *P* elements, can be repaired by a conservative mechanism during which the genetic information near a DSB site is copied from a homologous region in the genome (Formosa and Alberts 1986; Engels *et al.* 1990, 1994; Gloor *et al.* 1991; Nassif *et al.* 1994; Mueller *et al.* 1996; Keeler and Gloor 1997). The synthesis-dependent strand annealing (SDSA) model has been put forward to describe the mechanisms underlying the repair of those conservative events. This model proposes that the broken ends of DNA invade and displace independently a local loop of homologous regions of DNA during the repair process (Formosa and Alberts 1986; Nassif *et al.* 1994; Mueller *et al.* 1996).

Although studies of the repair mechanism of DSBs induced by *P*-element excision have been very successful, the use of the *P* element to induce DSBs is technically limiting. *P* elements are used as transformation vectors; therefore, most of the studies can only analyze DSB events introduced at the extremity of a given transgene. Further, it is possible that there is a bias in the repair process of *P*-element-induced DSBs caused by the inverted repeat binding protein (IRBP), a homologue of the Ku70 protein that plays a central role in the repair process (Rio and Rubin 1988; Beall *et al.* 1994; Beall and Rio 1996; Dynan and Yoo 1998). *P*-element termini are bound by IRBP, and the *P*-element transposase cuts the *P*-element termini directly adjacent to the IRBP binding site (Beall and Rio 1996; Beall and Rio 1997). Thus, it is possible that the binding of IRBP to the *P* element prior to the cut might affect the kinetics of the repair process, and in this way affect the outcome of the repair mechanism, such as the ratio between conservative and nonconservative repair (Staveley *et al.* 1995; Beall and Rio 1996). Further, it has been proposed that the ratio of imprecise *vs.* precise repair following *P*-element-induced DSBs is biased toward imprecise repair because of the unusual 17-bp overhang that is left after cleavage by the *P*-element transposase (O'Brochta *et al.* 1991; Engels *et al.* 1994; Beall and Rio 1997).

The availability of a technique that is different from the use of *P* elements to induce DSBs in Drosophila would allow the analysis of the SDSA model in a more general manner. A number of recent studies have shown that the yeast I-*Sce*I homing endonuclease can introduce DSBs in the genome of mouse cells or Xenopus oocytes (for review see Jasin 1996). Such studies have confirmed some aspects of DSB repair mechanisms previously ana-

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lyzed by transfection or injection of linear DNA molecules. I-*Sce*I is encoded by an intron of the large mitochondrial rRNA (Dujon 1988). Biochemical studies have shown that this restriction enzyme has an 18-bp specificity and leaves a 4-bp 5′ overhang after the cleavage (Colleaux *et al.* 1988). In this article, we show that expression of the yeast I-*Sce*I endonuclease in Drosophila can be used as a general method to induce DSBs at I-*Sce*I target sites in the Drosophila genome. We discuss the use of this novel technique to study SDSA and to analyze the functions of *mutagen-sensitive* (*mus*) mutations (Dusenbery and Smith 1996) that have been implicated in DNA repair mechanisms.

MATERIALS AND METHODS

Plasmid constructions: *P{FRT-I-SceI-y$^+$-Δ(w)::XEN-I-SceI-FRT}*: Two direct flip recombinase target (FRT) repeats of the J32 vector (Struhl and Basler 1993) were PCR amplified using the oligonucleotides 5′-GCCTAACTGCAGGGTACC CAGCTTCAAAAGCGCTCT and 5′-AGTGAATTCGAGCTCG GTACCCGGG, and they were cloned at the *Sac*I and *Pst*I site of a P-CarY vector (Patton *et al.* 1992) in which the *Not*I site has been deleted by blunt-end ligation. Two I-*Sce*I sites were then added in direct orientation by subcloning two double-strand oligonucleotides, 5′-CTAGCTAGGGATAACAGGGTA ATG/3′-GATCCCTATTGTCCCATTACAGCT and 5′-TCGA CGCGGCCGCTAGGGATAACAGGGTAATG/3′-GCGCCGGC GATCCCTATTGTCCCATTACCTAG, at the *Nhe*I and *Bam*HI sites, creating the *P{2XFRT-I-SceI}* vector. The two double-strand oligonucleotides also contain a *Sal*I site, as well as a *Not*I site between two I-*Sce*I sites. The 5.2-kb *Sal*I fragment of the *yellow* gene from the Y.E.S. vector (Patton *et al.* 1992) was then cloned into the *Sal*I site of the *P{2XFRT-I-SceI}* vector, creating the *P{FRT-I-SceI-y$^+$-FRT-I-SceI}* vector. A 9-kb *Hin*dIII fragment from the *white* gene containing two-thirds of the first exon and 5 kb of 3′ untranslated region (O'Hare *et al.* 1984) was modified by insertion of the double-strand oligonucleotide 5′-CCGGATAGCTCGAGAATAAATCGCGATGAATTCGT/3′-C CGGACGAATTCATCGCGATTTATTCTCGAGCTAT at the *Bsp*M I (11063) site (O'Hare *et al.* 1984). The insertion introduces a frameshift and three new restriction sites for *Eco*RI, *Nru*I, and *Xho*I in the *white* sequence. This fragment was then flanked by *Not*I linkers and cloned at the *Not*I site of the *P{FRT-I-SceI-y$^+$-FRT-I-SceI}* vector. The resulting plasmid was named *P{FRT-I-SceI-y$^+$-Δ(w)::XEN-I-SceI-FRT}*.

P{β2-tubulin-3nls-I-SceI}: The *Bam*HI/*Hin*dIII fragment, which contains a DNA sequence from −511 to +156 of the *β2-tubulin* promoter from the PWMelPvu vector (Michiels *et al.* 1993), was cloned between the *Bam*HI and *Eco*RI sites of the pPGK3Xnls-I-*Sce*I vector (Donoho 1996). This vector contains the 3nls-I-*Sce*I sequence cloned between the *Eco*RI and *Sal*I sites of pBluescriptKS+ vector (Stratagene, La Jolla, CA). The *Not*I, *Sal*I fragment, which contains the promoter and 3nlsI-*Sce*I, was then cloned with a *Sal*I, *Not*I fragment from the 3′ sequence of the *hsp70* gene from the pCasperHsp70 vector at the *Not*I site of the pDM30 transformation vector. The pDM30 transformation vector contains the *ry*$^+$ gene.

Molecular methods: Genomic DNA was prepared as described in Ashburner (1989), and Southern blot analysis was conducted as described in Sambrook *et al.* (1989). PCR amplifications of genomic fragments were carried out with the Ready-To-Go kit (Pharmacia, Piscataway, NJ) using the following primers: TCTCACGGCGGACTTATTAAGC or ATATGC GTAATTAGCGTTCG for the 3′ end of *P* element, and CACG TTTGCTTGTTGAGAGG or AAAGCTTGTCGGCGTCAT for the 5′ end of *P* element. PCR products were subcloned into a Promega pGEM-T vector and sequenced using the Sequenase2 kit (United States Biochemical/Amersham).

Flies were grown on standard cornmeal media. Mutations and chromosome aberrations not described in the text can be found in Lindsley and Zimm (1992). *P*-element transformation was performed using either the *yw*; Delta 2–3, *Sb*/*TM6* stock, or the pπ25.1 helper plasmid (Ashburner 1989).

RESULTS

As part of an attempt to develop a general technique to induce gene knockouts in Drosophila, we have developed a new system to induce DSBs. We first describe the gene knockout strategy to provide the background for which the new technique to induce DSBs was developed.

Gene targeting in Drosophila: One of the technical limitations of Drosophila as a system to study specific gene functions is the absence of a general gene-targeting system to allow systematic, reverse genetic studies. Such an approach to analyze gene functions is greatly needed, especially in light of the growing amount of information generated by the Drosophila Genome Project. A technique to target specific gene conversion events close to a preexisting *P*-element insertion site using *P*-element-induced DSBs has been developed (Gloor *et al.* 1991). However, this system does not allow a systematic analysis of every gene in the genome since *P*-element insertions are not evenly distributed throughout the genome. To circumvent this problem, we have attempted to develop a homologous recombination system for Drosophila genes. One of the critical steps of this system involves the linearization of a circular plasmid in the male germ line. To achieve this, we have used the yeast mitochondrial I-*Sce*I enzyme to cut a circular piece of DNA generated by the FLP-out event (Figure 1). The I-*Sce*I endonuclease recognizes a specific 18-bp sequence and leaves a 4-bp 5′ overhang following the cut (Colleaux *et al.* 1988). We expected that few, if any, I-*Sce*I sites would be present in the Drosophila genome since, theoretically, a single I-*Sce*I site should be found in a genome equivalent to 350 Drosophila genomes (Ashburner 1989). A number of studies have shown that I-*Sce*I efficiently cleaves genomic DNA or extrachromosomal DNA in plants and mammalian cell lines (reviewed in Jasin 1996). However, no studies have assessed the activity of a rare cutting endonuclease in a whole organism. Thus, we decided to conduct a detailed analysis of the activity of I-*Sce*I in Drosophila.

Expression of I-*Sce*I in Drosophila induces DSBs: To promote nuclear localization of the I-*Sce*I enzyme, we used a fusion between the SV40 nuclear localization signal (nls) and the I-*Sce*I coding sequence: this construct is referred to as 3nlsI-*Sce*I (Donoho 1996). We generated four independent, *P*-element-transformed lines that carry the 3nlsI-*Sce*-I sequence downstream of

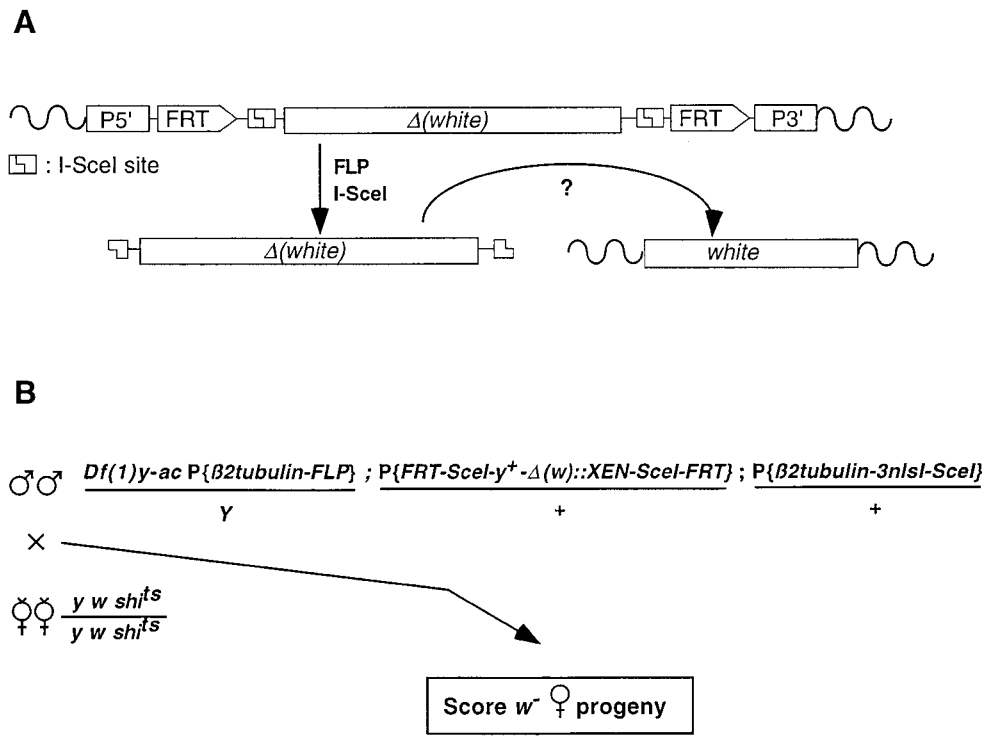


Figure 1.—Homologous recombination strategy. (A) General strategy: the general scheme for gene targeting in various organisms involves the transfection of a linear molecule of DNA containing a modified version of an endogenous gene. Following a homologous recombination event, the modified gene will replace the endogenous sequence. To adapt a similar system to Drosophila, we introduced an inactive version of the *white* gene into the fly genome via *P*-element transformation. The mutant version of the *white* gene is subsequently released and linearized in the nucleus. The excision step is achieved by FLP-mediated recombination between the two direct FRT repeats, and linearization of the circular plasmid is catalyzed by I-*Sce*I that recognizes and cuts an 18-bp restriction site. (B) Outline of the homologous recombination screen for the *white* locus. We designed a *P*-element vector in which a *yellow*$^+$ marker and an inactive version of the *white* gene are flanked by the two FRT and the two I-*Sce*I sites (see also Figure 2 for details). The *yellow*$^+$ marker is used as a *P*-element transformation marker, and it can be used to monitor the excision of the inactive *white* gene and to analyze the reintegration events. This construct is named *P{FRT-I-SceI-y*$^+$*-Δ(w)::XEN-I-SceI-FRT}*. We expressed both the FLP and I-*Sce*I enzymes under the control of the β*2 tubulin* promoter. The β*2 tubulin* promoter drives expression of reporter genes in the male germ line cells during the late stages of spermatogenesis; therefore, the progeny of a single male will derive from a number of independent, I-*Sce*I-induced excision events.

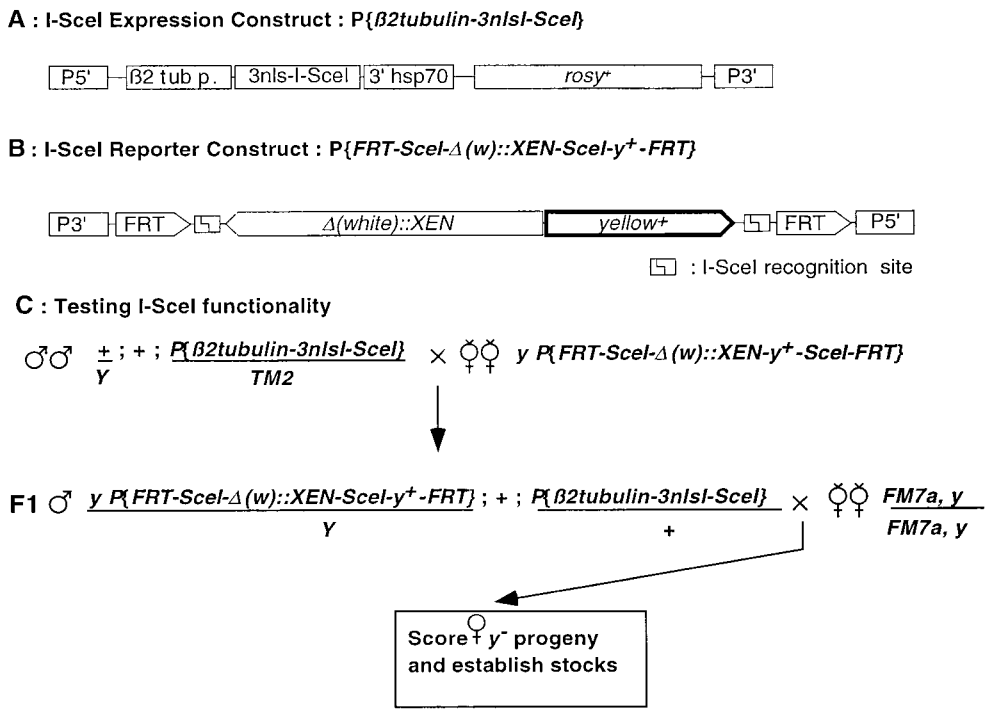
the β*2-tubulin* promoter. This promoter drives expression in postmitotic spermatids (Fuller 1995) such that progeny from a single male should be derived from a number of independent repair events. None of the transgenic insertions affected male fertility since stocks that are either homozygous or heterozygous for these insertions can be maintained.

To determine whether the I-*Sce*I enzyme, which is expressed under the control of the β*2-tubulin* promoter, could induce DSBs, we constructed a reporter designated *P{FRT-I-SceI-y*$^+$*-Δ(w)::XEN-I-SceI-FRT}* (Figure 2B). In this construct, the *yellow*$^+$ gene is flanked by two I-*Sce*I sites and, therefore, introduction of DSBs at the I-*Sce*I sites should result in the loss of the *yellow*$^+$ marker. To use the same construct for our gene-targeting experiment, two FRT sites and an inactive version of the *white* gene are also present in the reporter construct (Figures 1B and 2B). These additional sequences should not affect our assay, which is based on the loss of the *yellow*$^+$ marker that is flanked by the two I-*Sce*I sites. We obtained three independent *P*-element-transformed fly lines of this reporter construct.

Since I-*Sce*I was expressed under the control of the male-specific β*2-tubulin* promoter, we scored the progeny of males containing the 3nls-I-*Sce*I-expressing construct and one copy of *P{FRT-I-SceI-y*$^+$*-Δ(w)::XEN-I-SceI-FRT}* crossed with homozygous *yellow* females (Figure 2C). In this cross, we found that 4–10% of the progeny had lost the *yellow*$^+$ marker (Figure 2D). In addition, we observed that the occurrence of phenotypically yellow mutant flies in the progeny is strictly dependent on the presence of the *P{*β*2-tubulin-3nls-I-SceI}* transgene. Similar results were obtained using different combinations of *P{*β*2-tubulin-3nls-I-SceI}* and *P{FRT-I-SceI-y*$^+$*-Δ(w)::XEN-I-SceI-FRT}* transgenes. From these results, we conclude that the 3nls-I-*Sce*I enzyme is functional and able to induce DSBs in spermatids.

Anatomy of the repaired DNA: To characterize in more detail the molecular events following DSBs, we randomly selected 25 independent, phenotypically *yellow* lines generated in the previous experiments and analyzed them by Southern analysis. These lines fall into 2 major classes on the basis of analysis of the sequences present after induction of DSBs at the I-*Sce*I sites (Figure 3). In class 1 events, which represent 22 cases, a complete deletion of the sequences encoding both the *yellow* and *white* sequences was observed. In class 2 events, which represent the other 3 cases, only partial deletion of the *yellow* gene was detected.

We analyzed class 1 lines in more detail, and found that both the 3′ and 5′ *P*-element termini were present. These results suggest that the cut was not followed by



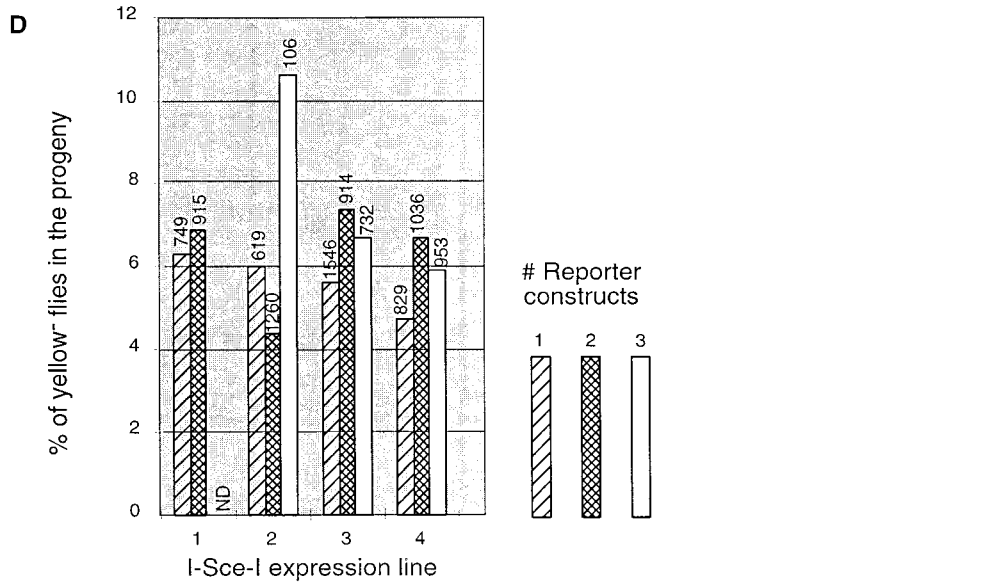


Figure 2.—Test of the activity of the I-*Sce*I endonuclease in the male germ line. (A) The *P{β2tubulin-3nls-I-SceI}* expression construct: the I-*Sce*I sequence was fused with the SV40 nuclear localization signal (3nls-I-*Sce*I) to promote localization of the enzyme into the nucleus. The signal 3nls-I-*Sce*I was cloned downstream of the β*2-tubulin* promoter in a *P*-element vector marked with the *rosy*$^+$ gene. Four independent *P{β2tubulin-3nls-I-SceI}* insertions were recovered on the third chromosome. Two of these, insertions 1 and 2 are associated with zygotic lethality, while insertions 3 and 4 are homozygous viable insertions. (B) The *P{FRT-I-SceI-y*$^+$*-Δ(w)::XEN-I-SceI-FRT}* reporter construct: this construct contains a *yellow*$^+$ gene placed between two recognition sites for *I-SceI*. Three independent *P{FRT-I-SceI-y*$^+$*-Δ(w)::XEN-I-SceI-FRT}* insertions were recovered, one on the first chromosome and two on the second chromosome. (C) To show that the *I-SceI* can induce DSBs in the male germ line, *y, P{FRT-I-SceI-y*$^+$*-Δ(w)::XEN-I-SceI-FRT}/Y; +/+; P{β2tubulin-3nls-I-SceI}* males were crossed with *FM7a, y* homozygous virgin females, and their progeny were scored for *yellow* females. To demonstrate that the loss of the *yellow*$^+$ marker was dependent on the expression of the *I-SceI* enzyme, we scored the progeny of *y, P{FRT-I-SceI-y*$^+$*-Δ(w)::XEN-I-SceI-FRT}/Y; +/+; TM2/+* males crossed with *FM7a, y* virgin females. We also determined the occurrence of DSB events using a reporter construct located on the second chromosome. In this experiment, progeny of single *y; P{FRT-I-SceI-y*$^+$*-Δ(w)::XEN-I-SceI-FRT}/CyO; P{β2tubulin-3nls-ISce-I}* males crossed to *y w; CyO/Sco* virgin females were analyzed and scored for *CyO* or *Sco yellow* progeny. (D) Percentage of *yellow*$^-$ progeny obtained in various experiments. Numbers on top of the bars indicate the number of flies scored in each cross. The results are given for different combinations of reporter and expression transgenes. A total of 4–10% of *yellow*$^-$ progeny (mean value, 6.47%) were recovered. In the absence of the *P{β2tubulin-3nls-I-SceI}* expression transgene, no phenotypically yellow flies were recovered (1900 progeny scored in six independent experiments). ND, not determined.

extensive degradation of the broken DNA since the *P*-element termini are located within 100 bp from the cleavage site. To confirm these results, the genomic DNA located between the two *P*-element termini was cloned from nine independent, randomly selected lines. Interestingly, sequence analysis of these DNAs revealed that the repair of the DSBs had proceeded in different ways (Figure 4A). In six out of the nine cases, we could identify partial sequences from the FRT and/or I-*Sce*I sites, suggesting that following the cut, the gap was enlarged and repaired by direct end joining. One of the nine lines was found to be associated with a reconstitution of a perfect I-*Sce*I site. This event can be explained either by repair through direct ligation of the two DNA strands following cuts at the two I-*Sce*I sites or by a single cut at one of the I-*Sce*I sites followed by single-strand

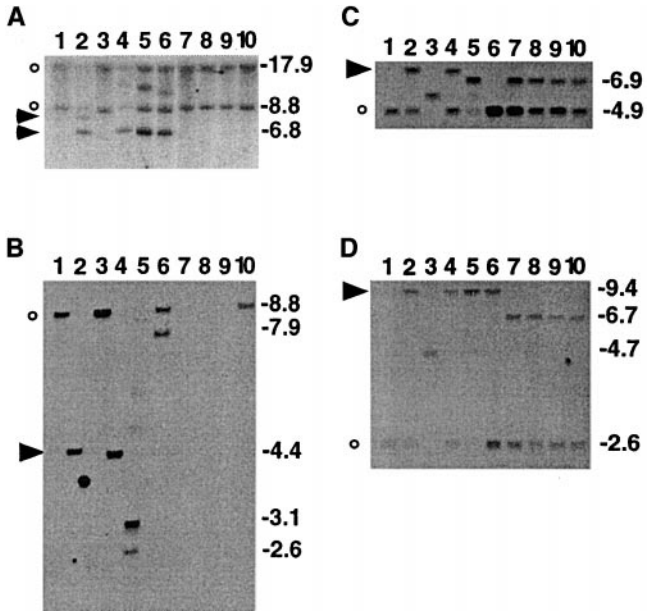


Figure 3.—Southern blot analysis of the *yellow*$^-$ lines. (A) The genomic DNA was cut by *Xba*I and hybridized with the 9-kB Δ(*white*)::XEN fragment. (B) The genomic DNA was cut by *Hin*dIII and hybridized with the *Sal*I *yellow* fragment from the Y.E.S. vector. In A and B, the circles indicate the position of the fragments associated with the endogenous *white* and *yellow* genes, respectively. (C and D) The genomic DNA was cut by *Xho*I and probed with either the 5′ end of the *P* element (C, using a 500-bp fragment obtained by PCR; see materials and methods for details) or with the 3′ end of the *P* element (D, using a 300-bp fragment). Lane 1, *FM7a* stock; lane 2, reporter construct alone; lane 3, expression construct alone; lanes 4–6, *yellow*$^-$ lines with partial deletions of different sizes; lanes 7–10, four examples of complete deletion of the *yellow* and *white* sequences. In B, the 1.4- and 0.9-kb fragments of the *yellow* gene are not shown. C and D show the existence of another *P* element (circle on the left of the blot) in the *FM7a, y* and in the *y, P{FRT-I-SceI-y*$^+$*-Δ(w)::XEN-I-SceI-FRT}* parental stocks. The nature of this *P* element is unknown, but this *P* element is clearly nonfunctional since we did not recover any *yellow* progeny in the control crosses: *y, P{FRT-I-SceI-y*$^+$*-Δ(w)::XEN-I-SceI-FRT}/Y*; +/+; *TM2*/+ males crossed with *FM7a, y* virgin females. The arrowheads indicate the positions of bands associated with the original reporter construct. The approximate calculated sizes of the restriction DNA fragments are shown in kilobases on the right side of each blot.

annealing (SSA) repair. Finally, sequence analysis of the remaining two DSB events revealed the presence of a unique FRT site. These two events can either be the result of two independent cuts at each I-*Sce*I site or of a single cut at one of the I-*Sce*I sites. In both cases, the repair would have then proceeded via SSA using the direct repeat from the FRT sites. The length of the FRT repeats is compatible with the length necessary for homologous pairing (Haber 1995; Keeler and Gloor 1997).

Southern blot analysis of class 2 events revealed that a partial deletion of the *yellow* sequence had occurred following the cut by I-*Sce*I. In every case, consistent with the yellow phenotype, the coding sequence of the *yellow* gene was altered (Figure 4B). Since the *white* sequence appears intact, we interpret these events as the result of a single I-*Sce*I cut at the I-*Sce*I site located close to the 5′*P*-element end. In two cases, the DSB was slightly enlarged and appeared to leave the *P*-element terminus intact. In a final case, the 5′ *P*-element end appeared to have been deleted. This suggests that after the cut at one I-*Sce*I site, the cut was enlarged and the *yellow* sequence was altered. We could not determine whether a part of the *yellow* sequence was then copied from the other intact chromatid. We suspect that a number of similar events should have taken place at the I-*Sce*I site located close to the 3′ *P*-element end; however, such events would not have been recovered since the selection was based on the loss of the *yellow* marker.

DISCUSSION

I-*Sce*I can induce DSBs at a specific position in the Drosophila genome: We have demonstrated that the I-*Sce*I endonuclease from yeast is able to induce DSBs in the Drosophila genome at positions that contain the 18-bp recognition site for this endonuclease. We found that ~6% of the progeny of males expressing I-*Sce*I in their germ line lose a *yellow* gene that is flanked by two I-*Sce*I sites. Most of the events resulted in complete deletion of the sequences located between the two I-*Sce*I sites. These events are either the result of two independent cuts occurring at both I-*Sce*I sites or a single cut at one of the I-*Sce*I sites. Following the cut by I-*Sce*I, the DSB was enlarged and then repaired by direct end joining or SSA. An enlargement of the DSB has also been proposed to occur for *P*-element-induced DSBs (reviewed in Keeler and Gloor 1997).

Development of a targeting system: We developed the I-*Sce*I system as a means to linearize a circular piece of DNA *in vivo*. This method represents one of the steps in a protocol to develop a general gene-targeting system in Drosophila (Figure 1). To test for homologous recombination events, we generated a line containing two *P*-element constructs expressing FLP-recombinase and I-*Sce*I enzyme under the control of the β*2-tubulin* promoter, and we conducted the screen described in Figure 1. Although we demonstrate that the 3nlsI-*Sce*I enzyme, expressed under the control of the β*2-tubulin* promoter, is able to induce DSBs, our attempts to recover homologous recombination events failed; *i.e.*, analysis of >250,000 female progeny derived from *Df(1)y-ac, P{*β*2-tubulin-FLP}; P{FRT-I-Sce-I-y*$^+$*-Δ(w)::XEN-I-Sce-I-FRT}; P{*β*2-tubulin-3nls-I-SceI}* males (see Figure 1B) failed to produce any *white* mutant females, indicating that no homologous recombination events had occurred.

Two recently published studies may provide an explanation for our failure to induce homologous recombination using a single linear piece of DNA provided by excision. Leung *et al.* (1997) and Negritto *et al.* (1997) have designed a similar system in yeast. This system is

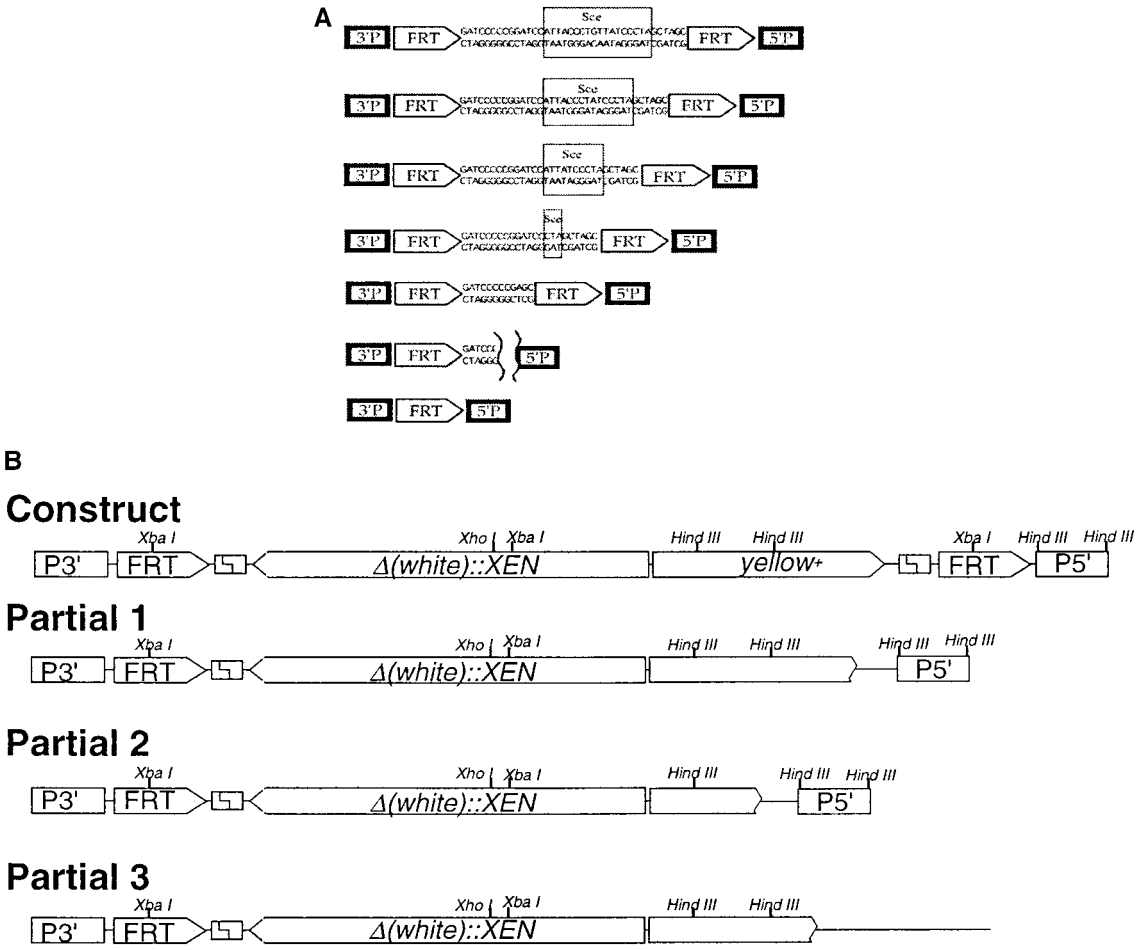


Figure 4.—Characterization of the events. (A) Sequences of the lines that were associated with complete deletions of both the *yellow* and *white* reporter sequences. The first sequence represents a perfect restoration of the I-*Sce*I recognition site. The two last cases were recovered twice. (B) Interpretation of the partial deletions based on Figure 3 genomic Southern blots. The lengths of the deletions are approximate and are based on the size of the fragments detected by Southern analysis.

based on the release of a linear piece of DNA for the *Leu2* or *SAM2* genes using the HO endonuclease. The two studies show that the efficiency of homologous recombination is low in these systems and, at most, occurs once for every 20,000 excision events. Leung *et al.* (1997) proposed that this effect is due to the preferential correction of a DNA nick with the intact chromosomal DNA. This explanation has been suggested in a number of other assays as well (reviewed in Leung *et al.* 1997). Interestingly, mutations in the mismatch repair genes *PMS1* or *Msh2* have been found to improve by 20- and 40-fold, respectively, the efficiency of homologous recombination. It will be interesting to repeat our homologous recombination screen in a *PSM1* or *Mhs2* mutant background when mutations in these genes become available.

Generalization of the SDSA model using the I-*Sce*I enzyme: Studies of DSBs in Drosophila have been carried out using *P* elements as a means to introduce DSBs at specific locations in the genome (Kaufman and Rio 1992). These studies have led to the SDSA model to explain the repair mechanism following *P*-element excisions (Formosa and Alberts 1986; Nassif *et al.* 1994; Mueller *et al.* 1996; Keeler and Gloor 1997). The most comprehensive study of DSB repair has been conducted at the *white* locus. In addition, a number of studies have been conducted at both the *vestigial* and the *Broad Complex* loci (reviewed in Keeler and Gloor 1997).

The generalization of the SDSA model to other induced DSB events represents an important step toward the understanding of DSB repair mechanisms. We believe that I-*Sce*I can be used as a tool to further analyze the parameters of the SDSA model. For example, it will allow the determination of whether the binding of the IRBP protein on *P*-element termini influences the repair mechanism (see Introduction), as well as the testing of whether the high rate of imprecise *P*-element excisions is caused by the unusual 17-bp overhang left by the *P*-element transposase (Engels *et al.* 1990; O'Brochta *et al.* 1991, see Introduction; Beall and Rio 1997).

Further applications of I-*Sce*I to dissect genetic path-

ways involved in the DSB repair: The development of the I-*Sce*I system will allow the characterization of molecules involved in DSB repair. In Drosophila, 15 loci associated with *mus* or *mei* repair defects have been isolated on the basis of their sensitivity to specific mutagenic agents (Dusenbery and Smith 1996). Importantly, different studies have shown that this group of genes is involved in the DSB repair process (Sekelsky *et al.* 1995; Araj and Smith 1996; Beall and Rio 1996). To date, however, no methodology is available to classify these genes in epistatic or genetic groups. Studies in yeast have characterized in more details the function of radiation-sensitive genes (*RAD*) by comparing their effects on the repair of a DSB induced within a direct DNA repeat to a DSB induced outside of a direct repeat. For example, several studies have shown that *RAD51*, *RAD54*, *RAD55*, and *RAD57* are required for mating-type switching, but not for the SSA repair of a DSB introduced between two direct repeats. These results have led to the proposal that these genes are required for gaining access to an intact, constrained region of the chromatid to facilitate the copying of information (Sugawara *et al.* 1995). In addition, mutations in *RAD1* and *RAD10* have a slight effect on DSB gap repair at the mating-type locus (*MAT*). However, in *RAD1* and *RAD10* mutants, SSA between two direct repeats is completely blocked (Fishman-Lobell and Haber 1992). These results suggest that Rad1 is part of a complex that is necessary for removing nonhomologous sequences before SSA. It has been subsequently demonstrated that Rad1 and Rad10 form a complex that has a single-strand endonuclease activity (Bardwell *et al.* 1994). Our study shows that the I-*Sce*I system allows the analysis of DSB repair between two direct repeats, an event that cannot be generated using *P* elements. Because these events occur at relatively high frequency, it should allow experiments similar to those conducted in yeast and permit the assignment of different *mus* Drosophila genes into epistatic groups.

Finally, we envisage that the I-*Sce*I system could be used to engineer specific changes in the fly genome. For example, one can envisage using this system as a general means to induce a series of deficiencies. Following local duplication of a *P* element containing a single I-*Sce*I site, the expression of the I-*Sce*I enzyme should generate deletions between the two *P* elements. In contrast to other systems, such as the FRT/FLP system (Golic 1994; Golic and Golic 1996), the recovery of deletions between the two distant *P* elements should not be influenced by the distance between the insertion sites.

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