Homology Requirements for Targeting Heterologous Sequences During *P*-Induced Gap Repair in *Drosophila melanogaster*[1]

Tammy Dray and Gregory B. Gloor

Department of Biochemistry, University of Western Ontario, London, Ontario, Canada

Manuscript received January 23, 1997
Accepted for publication June 26, 1997

ABSTRACT

The effect of homology on gene targeting was studied in the context of *P*-element-induced double-strand breaks at the *white* locus of *Drosophila melanogaster*. Double-strand breaks were made by excision of *P-w*^{hd}, a *P*-element insertion in the *white* gene. A nested set of repair templates was generated that contained the 8 kilobase (kb) *yellow* gene embedded within varying amounts of *white* gene sequence. Repair with unlimited homology was also analyzed. Flies were scored phenotypically for conversion of the *yellow* gene to the *white* locus. Targeting of the *yellow* gene was abolished when all of the 3′ homology was removed. Increases in template homology up to 51 base pairs (bp) did not significantly promote targeting. Maximum conversion was observed with a construct containing 493 bp of homology, without a significant increase in frequency when homology extended to the tips of the chromosome. These results demonstrate that the homology requirements for targeting a large heterologous insertion are quite different than those for a point mutation. Furthermore, heterologous insertions strongly affect the homology requirements for the conversion of distal point mutations. Several aberrant conversion tracts, which arose from templates that contained reduced homology, also were examined and characterized.

DOUBLE-STRAND breaks arise in the genome as a direct result of ionizing radiation, transposon excision or site-specific nucleases, and indirectly through DNA processing following other types of DNA damage (FREIDBERG *et al.* 1995). Scientific interest in double-strand breaks has intensified since they were implicated as the initiating event in recombination (RESNICK 1976; SZOSTAK *et al.* 1983). Findings from many laboratories have confirmed that double-strand breaks are recombinogenic and that they coincide with meiotic initiation sites in *Saccharomyces cerevisiae* (NICOLAS *et al.* 1989; SUN *et al.* 1989; STAHL 1996). Furthermore, repair and recombination share many protein components in common (WEST 1992; KOWALCZYKOWSKI *et al.* 1994; HABER 1995; JACKSON and JEGGO 1995). Taken together, this evidence shows that double-strand breaks are not merely DNA damage that needs to be repaired, but important initiating events in the process of recombination and other genome rearrangements.

The double-strand-break repair model proposed over a decade ago (RESNICK 1976; SZOSTAK *et al.* 1983), postulated that the recombination pathway begins with a double-strand break in the genome, which is enlarged by exonucleases leaving 3′ single-stranded overhanging ends. These free ends are sequentially elongated by copying from a homologous template, repairing the gap in the process. Whether this produces a crossover or noncrossover event depends upon the resolution of the Holiday junctions on either side of the newly synthesized DNA (SZOSTAK *et al.* 1983). However, other possible models for double-strand break repair have also emerged (LIN *et al.* 1984; HASTINGS 1988; ROSENBERG and HASTINGS 1991; BELMAAZA and CHARTRAND 1994; NASSIF *et al.* 1994) to explain recombinational outcomes that deviated from those predicted by the double-strand break repair model.

P elements are transposons in Drosophila that leave behind a double-strand break in the genome upon their excision (ENGELS *et al.* 1990; GLOOR *et al.* 1991; KAUFMAN and RIO 1992). The ensuing repair process has been rigorously studied and proceeds by gene conversion in which sequence from a homologous template is copied into the break site (ENGELS *et al.* 1990; GLOOR *et al.* 1991; JOHNSON-SCHLITZ and ENGELS 1993; NASSIF and ENGELS 1993; ENGELS *et al.* 1994; NASSIF *et al.* 1994). The small amount of crossing over that is associated with *P*-element excision is mainly due to a mechanism that is independent of DNA repair (SVED *et al.* 1995; SVOBODA *et al.* 1995; GRAY *et al.* 1996; PRESTON and ENGELS 1996; PRESTON *et al.* 1996). This is distinct from double-strand break repair in yeast, where both gene conversion and crossing over are observed when a double-strand break is repaired (STAHL 1996), except for conversion at the mating type locus (STRATHERN 1988). The homologous template for double-strand break repair in Drosophila can be located anywhere in the genome, with the highest repair frequencies being achieved when it is located on the homologue at the

Corresponding author: Gregory B. Gloor, Department of Biochemistry, University of Western Ontario, London, Ontario, Canada, NGA 5C1. E-mail: ggloor@julian.uwo.ca

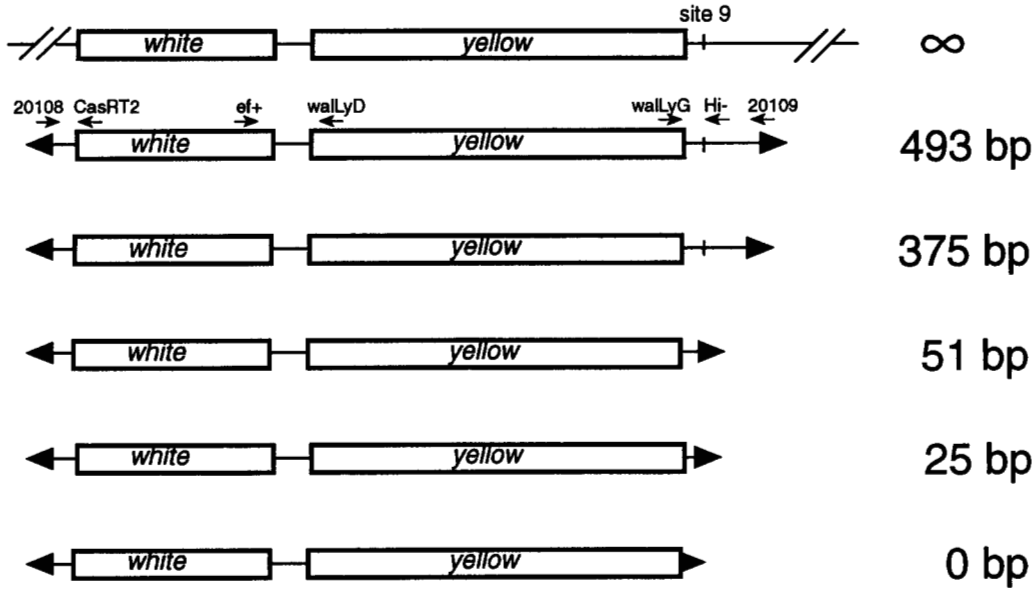

FIGURE 1.—Repair templates contain *white* gene sequence derived from P{*w*alter}. The *white* gene is flanked by *P*-element ends (large arrowheads) and contains up to 12 single bp substitutions depending on the construct. These substitutions act as markers for a conversion event. The heterologous *yellow* gene sequence was cloned in just upstream of the *white* gene 3′ untranslated region. Alterations to the length of *white* gene sequence were made in the 3′ untranslated region using restriction enzymes. A chromosome with unlimited homology (∞) on either side of the heterologous insert was produced by *P*-element-mediated gene targeting. The locations of primers used in PCR amplification are shown using the Δ493 template for reference (see text for details). The arrows indicate the direction of primer elongation during PCR. In this and subsequent figures, the *white* and *yellow* genes are shown as open boxes, the untranslated and untranscribed regions are shown as thin lines.

same relative position of the break site (ENGELS *et al.* 1990; GLOOR *et al.* 1991; NASSIF and ENGELS 1993; ENGELS *et al.* 1994). Using this system, heterologous insertions have been targeted efficiently to a double-strand break in the genome (JOHNSON-SCHLITZ and ENGELS 1993; NASSIF *et al.* 1994; KEELER *et al.* 1996).

Homology recognition during recombination, which is catalyzed by RecA and its respective homologues (HEYER 1994), is important for selection of a proper exchange partner. Many studies have been carried out in a variety of organisms to determine the homology requirements for a particular recombination system. Experiments in *Escherichia coli* (WATT *et al.* 1985; SHEN and HUANG 1986), bacteriophage T4 (SINGER *et al.* 1982), mammalian cells (RUBNITZ and SUBRAMANI 1984; LISKAY *et al.* 1987; BAKER *et al.* 1996), plants (BAUR *et al.* 1990), and yeast (JINKS-ROBERTSON *et al.* 1993) have determined homology requirement ranges for their respective recombination systems.

Homology is also an important parameter in optimizing gene targeting, a technique that involves the exchange of genomic information for *in vitro*-modified DNA sequence. This being the case, sections of nonhomology will be present between the two partners that will interfere with recombination. Gene targeting, in general, is extremely sensitive to the amount of available homology, and sequence divergence between the target and template, as well as insufficient amounts of flanking homology, reduces the overall conversion frequency (HASTY *et al.* 1991; DENG and CAPECCHI 1992; TE RIELE *et al.* 1992; NASSIF and ENGELS 1993; SCHEERER and ADAIR 1994). The present experiments were undertaken to determine the homology requirements for targeting an 8 kb heterologous insert to a *P*-element excision-induced gap in the Drosophila *white* (*w*) gene.

MATERIALS AND METHODS

Template constructs: All constructs were derived from pP{π*w*alL} (NASSIF *et al.* 1994). This plasmid contains a 4 kb mini *white* gene that is marked by 12 individual bp substitutions that add or remove a restriction site without affecting *white* gene function (GLOOR *et al.* 1991). A polylinker is located in the 3′ untranslated region of the *white* gene, 238 bp downstream of the relative point of *P-w*^{hd} excision (O'HARE *et al.* 1984). Sequences that are cloned into the polylinker do not affect the function of the white protein. The 8 kb *yellow* (*y*) gene, obtained on a *Sal*I fragment from a plasmid containing the *yellow* and *rosy* genes (GEYER and CORCES 1987), was cloned into the *Xho*I site of the polylinker in each of the constructs. The *white* gene sequence is flanked on both sides by *P*-element ends that enable the construct to move when supplied with a transposase source. The general structures of the repair templates are shown in Figure 1.

The 3′ untranslated region in pP{π*w*alL} corresponds to the region from nucleotide position —2233 to —3027 using the numbering scheme of O'HARE *et al.* (1984). The 763 bp of this region that are located downstream of the polylinker were chosen to investigate the effect of altering the amount of homologous sequence flanking a heterologous insert in the context of double-strand break repair.

Five repair templates were made using unique restriction enzyme cleavage sites in the 3′ untranslated region. An *Eco*RI/*Eco* 0109I double digest removed the entire untranslated region flanking the *yellow* gene to produce Δ0. *Bss*HII (−2387)

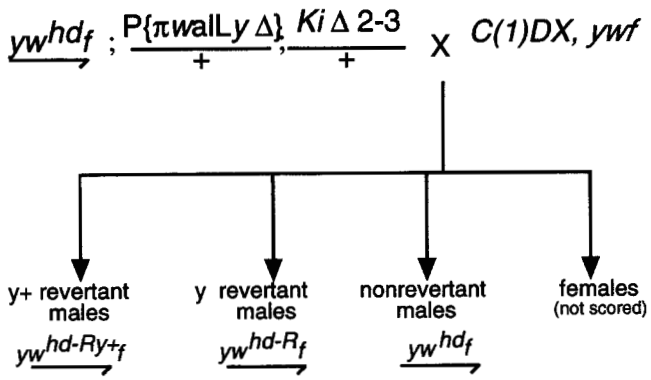

FIGURE 2.—Male flies carrying a *P*-element insertion allele of the *white* (w^{hd}), a repair template (P{π*w*alL*y*Δ}), and a transposase source (Δ2–3(99B)) linked to *Kinked* (*Ki*), were mated to attached *X* virgins. Male progeny were scored for targeting of both *white* and *yellow* gene sequences. A similar mating scheme was used for the infinite homology template, except female flies with the template on the homologous *X* chromosome and Δ2–3(99B) linked to the third chromosome marker *Drop* (*Dr*) were used. PCR was performed to confirm these results.

cleavage followed by *Bal*31 exonucleolytic degradation and *Eco* 0109I cleavage produced the Δ25 and Δ51 constructs. In the same manner, Δ375 was made, except that the *Nsi*I site (−2758) was used instead of *Bss*HII. Lastly, an *Nsi*I/*Eco* 0109I digest produced Δ493. Each construct name is derived from the amount of 3′ flanking homology that remains in the construct. All cloning steps were done as per standard protocols found in SAMBROOK *et al.* (1989).

Drosophila stocks: Drosophila were grown on a standard cornmeal-agar medium with 4% sucrose, brooded every 5–7 days and kept at 24°. Genetic symbols not otherwise explained are in LINDSLEY and ZIMM (1992).

C(1)DX, yf/ shi^{ts}; +; +: A compound X stock with M cytotype and *shibire^{ts}*, a temperature-sensitive, lethal gene. Male flies do not survive a 28° incubation, facilitating virgin collection.

yw^{hd}f; +; +: A stock that contains the *P*-element insertion allele $w^{hd80k17}$ (w^{hd}) (ENGELS *et al.* 1990). *P-w^{hd}* is a 629 bp nonautonomous *P* element inserted at position —2025 in the *white* locus (O'HARE *et al.* 1984). Insertion results in an 8 bp duplication of surrounding genomic sequence, and a bleach-white eye phenotype.

yw; +; KiΔ2–3 (99B): A stock homozygous for an immobile *P* element on the third chromosome (ROBERTSON *et al.* 1988). The Δ2–3 (99B) chromosome contains a stable transposase source, capable of mobilizing other *P* elements. It is marked by the dominant bristle phenotype, *Ki*.

Transformation: The constructs were inserted into the genome by *P*-element-mediated transformation (RUBIN and SPRADLING 1982; SPRADLING and RUBIN 1982; ROBERTSON *et al.* 1988). Transformants were stocked out with a new chromosome marked with *yw^{hd}f*. Transformed males that contained w^{hd} were mated to females that carried *Ki*Δ2–3 (99B) (ROBERTSON *et al.* 1988).

Double-strand break repair in Drosophila: Male progeny carrying *whd*, a template and a transposase source were individually mated to five compound X females (Figure 2). Progeny with phenotypes indicative of successful double-strand break repair using the ectopic template were identified on the basis of eye and body color. Reversion of the *white* gene produced a wild-type eye (white$^+$) phenotype. If the heterologous *yellow* gene sequence was also copied into the gap during the process, a wild-type body (yellow$^+$) phenotype also resulted.

To study repair under optimal conditions, an *X* chromosome with a phenotypically wild-type *white* locus marked by the 12 single bp alterations and containing the *yellow* gene insertion was used as a repair template. In effect, homology extended to the tips of the chromosome outside of the region containing the base alterations and the *yellow* gene insertion. Female flies carrying this repair template chromosome, an *X* chromosome containing the potential break site (w^{hd}) flanked by *achaete* and *split*, and Δ2–3 (99B) were mated to two *yw^{hd}f*; +; + males. Progeny were scored as outlined above.

Polymerase chain reaction: Phenotypic revertants were analyzed by PCR (SAIKI *et al.* 1988). They were screened for the presence of the original repair template using primers that amplified the *P*-element ends of the construct (20108, GAG TGTCGTATTGAGTCTGAG; CasRt-2, AAGAGATAGCGGAC GCAGCG; *w*alL*y* D, TACGCAATTTTCTTGAGCGG; 20109, GAAAGGTTGTGTGCGGACGA) (Figure 1). An 85° hot start was followed by the following cycling profile: 1 min at 95° (denaturation), 1 min at 72° (annealing) and 1 min 30 sec at 72° (elongation). Each subsequent cycle reduced the annealing temperature by 1°, until a final annealing temperature of 55° was reached. Twenty more cycles at this annealing temperature followed. PCR products were resolved on a 1.4% agarose gel. Revertants that retained a copy of the repair template were discarded.

A second PCR screen was performed to analyze the conversion tract of each revertant fly. Primers flanked the site of *P* w^{hd} excision (ef+, GGTTGTCGTACCTCTCATGG; Hi-, ACA GCGAAAGAGCAACTACG; *w*alL*y* G, TAATAGTCGAGGATC CTGC; *w*alL*y* D) (Figure 1). PCR was done as outlined above, except a final annealing temperature of 50° was chosen. PCR products were cut with three separate restriction enzymes, which correspond to the alterations made in the repair template (GLOOR *et al.* 1991; NASSIF *et al.* 1994). PCR products were run on a 3% agarose gel. Analysis of fragment lengths determined if repair of the gap involved the template, and to what extent it was copied.

Several flies were collected that were predicted to contain aberrant conversion tracts based upon the products of the above PCR reactions. Convertants containing an aberrant conversion tract were analyzed by long-range PCR (BARNES 1994). The following primers were used: *white* gene primers (ef+, 2362 GCACATCGTCGAACACCACG, H-GTGTTTTATGTA CCGATAAACGAG, *w*7-GCGAGAGCGGGCCAACCAAG, *w*8-GTGCCTCTTTCATGTCTTCG, *w*9-CTTGCCGAGCATCGA GCACA, *w*10-ACCACACAAATGGCAAGTAG), *yellow* gene primers (*w*alL*y*D, *w*alL*y*G), and a *P*-element end primer (2223 CGTCCGCACCACAACCTTTCC).

Cloning and sequencing of PCR products: Several of the aberrant conversion tracts were cloned into pBluescript II SK+ for further characterization. Ten conversion tracts were amplified using the primers that flanked the region of interest. Each PCR amplimer was phosphorylated by the addition of T4 polynucleotide kinase. The vector, pBluescript II SK+, was digested with *Eco*RV and then dephosphorylated by the addition of Shrimp Alkaline Phosphatase. Each PCR amplimer was ligated into pBluescript II SK+ and transformed into *E. coli*. DNA was isolated from each clone, and dideoxy sequencing was performed to precisely characterize the conversion tracts. All steps were done following standard protocols found in SAMBROOK *et al.* (1989).

RESULTS

Constructs were prepared that contained various amounts of *white* gene sequence flanking the 8 kb *yellow*

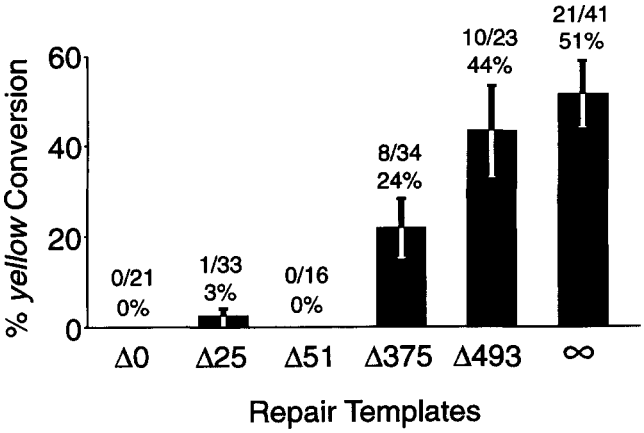

FIGURE 3.—Conversion of the *yellow* gene to the site of *P-w*^{hd} excision was measured as a percentage of total conversion events. Conversion was determined using PCR amplification in conjunction with restriction enzyme analysis that identified template-specific *white* gene sequence. The error bars shown on this and later figures show the standard error of the binomial.

gene to determine the homology requirements for targeting a large heterologous insertion to the *white* locus (Figure 1). Flies that carried *w*^{hd}, a transposase source and a repair template were mated, and their progeny were inspected for evidence of double-strand break repair and *yellow* gene conversion (Figure 2). The repair templates contained *white* gene sequence, marked by up to 12 individual bp substitutions, and the *yellow* gene inserted just downstream of the relative break site. The amount of *white* sequence on the 3′ flanking side of *yellow* varied from no homology to unlimited homology. All white$^+$ revertants were tested, using PCR, for conversion of the template sequence into the *P*-element-induced double-strand break. Flies that were phenotypically yellow$^+$ were analyzed by PCR to ensure that the *yellow* gene had been targeted to the *white* locus.

Conversion of the *yellow* gene: Flies that had undergone *P*-element-induced double-strand break repair were characterized according to the conversion of a series of base pair substitutions and the nonhomologous insert into the break site. Targeting of the *yellow* gene was determined as a percentage of total conversion events, as opposed to a percentage of total flies scored, because the repair frequency is subject to the position of the template within the genome (ENGELS *et al.* 1990; GLOOR *et al.* 1991; ENGELS *et al.* 1994; NASSIF *et al.* 1994). Therefore, an analysis of conversion events in relation to total flies scored would have reflected the template position-dependence of repair, as opposed to the effect of homology. Figure 3 shows the relative proportion of independent *yellow* gene targeting events as a percentage of the total number independent conversion events for each template, which are given in Table 1.

A set of constructs, each with a differing amount of flanking *white* gene homology, were tested. When the entire 3′ flanking homology was deleted, conversion of the *yellow* gene was 0% (0/21). At 25 bp of homology, one targeting event was noted (1/33), however, this was not significantly different from the Δ0 data. There was no *yellow* conversion observed when Δ51 was supplied as a template (0/16).

TABLE 1

Reversion and conversion summary

Template	Males	Revertants tested[b]	Convertants[a]		
			Total	yellow$^+$	Site 9+
Δ0	22,263	90	21	0	NP[c]
Δ25	47,899	120	33	1	NP
Δ51	24,932	28	16	0	NP
Δ375	37,811	64	34	8	6
Δ493	23,229	67	23	10	9
Infinite	3,956	61	41	21	17

[a] Independent events.
[b] Some of these events were not independent.
[c] NP, Site 9 not present in the template.

The *yellow* gene was not targeted at a high frequency until 375 bp of 3′ flanking sequence was provided. In this instance, the conversion frequency was 24% (8/34). The addition of another 118 bp of homology almost doubled the percentage of *yellow* gene convertants, as seen by the number of targeting events using the Δ493 repair template (10/23). The *yellow* conversion frequency was elevated further when homology extended to the tips of the chromosome (21/41, 51%), although it was not significantly different from that seen with the Δ493 targeting template.

Conversion of the *white* gene: Single base pair substitutions in the template *white* gene served as markers to follow the double-strand break repair process. Previous work (GLOOR *et al.* 1991; NASSIF and ENGELS 1993; NASSIF *et al.* 1994) demonstrated that the conversion of individual sites characteristically decreases with increasing distance from the relative point of *P-w*^{hd} excision, irrespective of template. For example, site 6, which precisely corresponds to the position of *P-w*^{hd}, is copied into the gap more frequently than site 4, which is 135 bp upstream.

Site 9, in which an *Rsa*I site is introduced into the template *white* gene, is common to Δ375, Δ493 and the infinite homology repair templates, but deleted in the others. Table 1 and Figure 4 show that this site was copied into the *P*-element-induced gap less than half as efficiently with Δ375 (6/34) than with the other two (9/23 for Δ493 and 17/41 for infinite homology). When Δ375 was present in the genome, 18% of all conversion events included site 9. Increasing the amount of flanking homology to 493 bp increased the proportion of convertants with site 9 to 39%, with little increase in the value when homology extended to the end of the chromosome (42%). Site 9, as seen in Figure

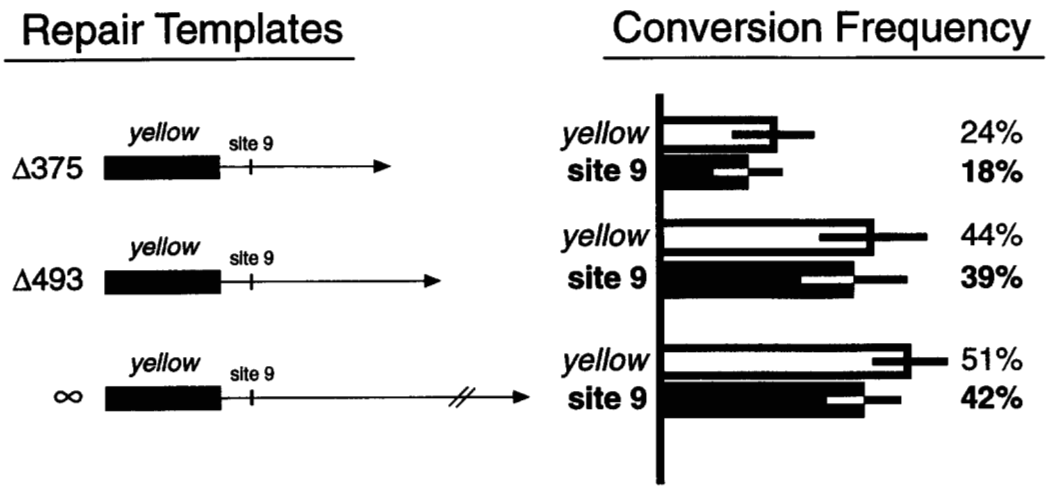

FIGURE 4.—The conversion frequencies for site 9, a point mutation, were calculated as a proportion of total conversion events for Δ375, Δ493 and the infinite homology templates. Conversion frequencies for the *yellow* gene are shown for reference.

1, is located downstream of the heterologous insertion. The conversion frequencies of the other marker sites, which are located on the 5′ side of the *yellow* gene, were similar to those published previously (GLOOR *et al.* 1991; NASSIF and ENGELS 1993; NASSIF *et al.* 1994) (data not shown).

Aberrant events: In addition to the conversion tracts characterized in the previous sections, two classes of aberrant events were recovered. The classes are divided on the basis of phenotype. The first class consisted of 28 revertant flies with a yellow phenotype in which no PCR product was observed upon PCR amplification with ef^+ and Hi^- (Figure 1). These events were obtained from all five reduced homology constructs. Eight events, chosen at random, were examined by PCR and all were found to contain some *yellow* sequence inserted into the *white* gene. Furthermore, in all eight events, the sequence 5′ to the *yellow* gene insertion originated from the P{*w*alLy} template, and the sequence 3′ to the *yellow* gene originated from the genomic *white* gene. Long range PCR analysis (BARNES 1994) indicated that 5′ portions of the *yellow* gene had been copied into the gap in each case.

Six of these events were cloned and characterized by DNA sequencing (Figure 5, A–F). There were two types of structures. One type had a deletion of some *yellow* gene and the adjacent *white* gene sequence (Figure 5, A–D). The junction between the *yellow* and *white* sequence always had a few nucleotides of sequence that was apparently unrelated to either the *yellow* or *white* sequence. In general, the junctions were fairly A/T rich and somewhat repetitive. These junction sequences and the flanking *yellow* and *white* gene sequences are given in Table 2.

The second type of structure contained a portion of the 5′ *yellow* gene and a duplication of the *white* sequence (Figure 5, E, and F). The junction between the *yellow* and *white* sequences contained short segments of A/T rich sequence, followed by a stretch of nucleotides derived from the *P*-element terminal repeat (Table 2). A portion of the junction of Δ493–42 was strikingly similar to *yellow* sequence immediately downstream of the *yellow* gene sequence in the conversion tract. There are four base pair differences between this junction region shown in square brackets in Table 2 and the canonical *yellow* sequence. This may be indicative of mismatch repair between two nonhomologous ends.

Another class of aberrant events was $yellow^+$ and contained the 5′ *P*-element end. Three flies with this general structure were collected from experiments that used either Δ0 or Δ25 as repair templates. Their structures, determined by PCR and DNA sequencing of cloned PCR products, are shown in Figure 5, G–I. The presence of a *yellow* gene in the genome located at cytological position 3*C* was confirmed by *in situ* hybridization to polytene chromosomes (ENGELS *et al.* 1986). Along with the expected *white* gene and *yellow* gene sequence found in other $yellow^+$ conversion events, the 5′ *P*-element end is found directly downstream of the template 3′ untranslated region. In one of these three events, Δ0–53, there was ~1000 bp of sequence of unknown origin adjacent to this *P*-element end (Figure 5, G). An oligonucleotide complementary to this sequence was prepared and used for PCR amplification. A productive PCR amplification resulted when this primer was used to amplify DNA prepared from the Δ0–53 repair event, but not when DNA prepared from the Δ0 P{*w*alLy} transformant line was used (data not shown). This indicates that the unknown flanking sequence did not originate from the region flanking the template insertion site. The other two events, Δ0–101 and Δ25–67, also had the template P end inserted at the $P\text{-}w^{hd}$ excision site. Sequencing adjacent to the *P*-element end indicated that there was *white* gene sequence originating from the 3′ region that abuts the $P\text{-}w^{hd}$ excision site (Figure 5, H, and I). However, PCR amplimers of this region, in both Δ0–101 and Δ25–67, were larger than expected (data not shown), indicating the insertion of sequences of unknown origin within the 3′ untranslated region of the *white* gene.

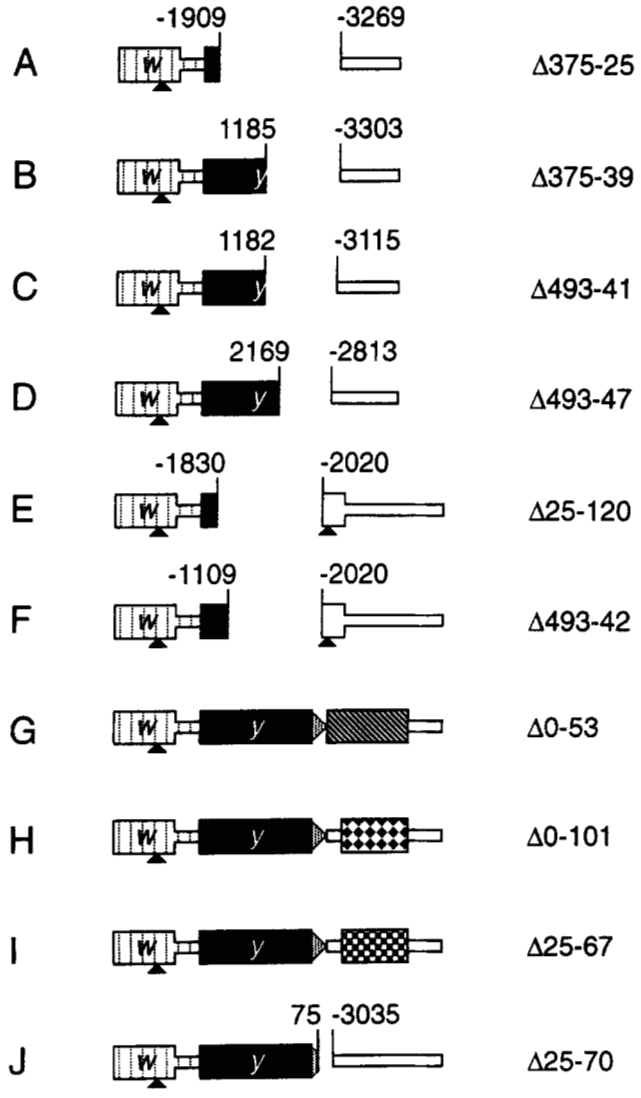

FIGURE 5.—Twelve aberrant events were characterized molecularly to determine their conversion tracts. Six of these conversion events had varying-sized deletions of the 3′ end of the *yellow* gene (A–F). Four others carried complete copies of the *yellow* gene and also contained a 5′ *P*-element end (G–I), with three of these containing sequence of unknown origin. The size of the unknown sequence is ~1000 bp in Δ0–53, between 1300 and 2300 bp in Δ0–101 and between 4300 and 5300 bp in Δ25–67. In this figure the *P*-element ends are shown as gray arrows; unconverted *white* gene sequence is represented as an open box, converted *white* gene sequence is shown as a vertical-line filled box; *yellow* gene sequence is the black-filled box; and large segments of unknown sequence are represented as hatched or diamond filled boxes. Wider areas within the *white* gene correspond to the location of exons. The numbers in A–F and J represent the last nucleotide in either the *yellow*, *white* or *P*-element sequences remaining at the junction. The sequence coordinates for the *yellow* gene are those of CHIA *et al.* (1986), for the *white* gene are those of O'HARE *et al.* (1984), and for the *P*-element sequence are those of O'HARE and RUBIN (1983). The sequences of the junction regions that join the two ends from A–F are given in Table 2.

Finally, one event, Δ25–70, had a structure similar to those described above, but did not contain any unknown flanking sequence (Figure 5, J). This conversion tract had a deletion of approximately 75 bp from the 3′ side of the *P*-element end. The 3′ flanking *white* gene sequence begins at position −3035 (O'HARE *et al.* 1984), which corresponds to a location in the 3′ untranslated region, 1010 bp downstream of the *P-w*^{hd} excision site.

Together, these results indicate that conversion of an 8 kb insertion into the *white* locus depends upon the amount of *white* gene homology available in the template. The presence of the large heterology within the template can disrupt the conversion of point mutations located within the 3′ flanking *white* sequence. In addition, reductions in the amount of homology can lead to the partial copying of both *white* and *yellow* gene sequence into the double-strand break.

DISCUSSION

Gene targeting in *Drosophila melanogaster:* The positive association between the nature and extent of homology and the frequency of meiotic and mitotic recombination has been well documented in a variety of organisms (SINGER *et al.* 1982; RUBNITZ and SUBRAMANI 1984; WATT *et al.* 1985; SHEN and HUANG 1986; LISKAY *et al.* 1987; BAUR *et al.* 1990; JINKS-ROBERTSON *et al.* 1993; BAKER *et al.* 1996). One of the main interests has been determining the homology requirements for a particular system, with the impetus being a desire to specifically incorporate recombinant DNA into the genome, or what is now known as gene targeting.

Research in *Drosophila melanogaster* has shown that *P*-element-induced breaks can be repaired from a template located on the homologue, ectopically or on a plasmid (ENGELS *et al.* 1990; GLOOR *et al.* 1991; JOHNSON-SCHLITZ and ENGELS 1993; NASSIF and ENGELS 1993; ENGELS *et al.* 1994; NASSIF *et al.* 1994; KEELER *et al.* 1996). Furthermore, large heterologous inserts located on ectopic templates can also be copied efficiently into the gap. In fact, it was shown that the 8 kb *yellow* gene, when embedded within *white* sequence, could be targeted at frequencies comparable to a point mutation (NASSIF *et al.* 1994).

Homology saturation: This targeting study investigated the effects of altering the amount of homology adjacent to the *yellow* gene. A repair template containing 493 bp of 3′ flanking homology showed a *yellow* gene conversion frequency of 44%. A template containing homology that extended to the tips of the chromosome had a comparable conversion frequency (51%), indicating that further extensions in homology past 493 bp did not significantly increase the conversion frequency. This suggests that there is an upper limit for template homology requirements, above which, no further increase in conversion efficiency occurs. Re-

TABLE 2

Sequences of the aberrant junctions in the *yellow* gene deletions

Name	*yellow* gene sequence	Intervening sequence[a,b]	*white* gene sequence[c]
Δ375-25	ATAATGTCGAATGTT	ATTTATGTTACATGTTACATTACATTAC ATTATTTACA	GCATGTGTGTGAGTG
Δ375-39	GCAATGAAACTAAAA	TC	GGAATCGCAGAAAA
Δ493-41	AAAGCAATGAAACTA	TTATTAAACTTATTTCATGAAATAA	GCGAAAGGCAAGAGA
Δ493-47	AAAAAATATCTGA	ATAATAGTTATT	CTATAATAATAAGAA
Δ25-120	TTGACCACTTAGCGC	ATATAATAATTT(CATCATG)	GTGGGTCTGGCCATT
Δ493-42	GTTTATTATGCGTGT	[GGCTGGTTGTATTACTTTACTTAACTT ATTT]CATGTTAATTATAACATAATGTT ATTACTTA(ATGTTATTTCATCATG)	GTGGGTCTGGCCATT

[a] *P*-element terminal inverted repeat sequence shown in parentheses.
[b] Sequence that closely resembles *yellow* gene sequence shown in square brackets.
[c] Sequence from the eight base target site duplication in the *white* gene is underlined.

search on gene targeting using replacement and insertion vectors in mammalian cells has indicated that these systems can be saturated with respect to homology as well (HASTY *et al.* 1991; DENG and CAPECCHI 1992), although the amount of homology required for saturation is ~20-fold greater than is required in the experiments we report here.

Heterologous inserts: The conversion frequency of the *yellow* gene is lowered by ~50% when the amount of flanking homology is decreased to 375 bp from 493 bp. Therefore, we conclude that the boundary for optimal targeting lies somewhere between the values of 375 and 493 bp. The question is whether this boundary is static, or whether it moves depending on the type of heterology in the template.

Results of earlier work (NASSIF and ENGELS 1993) indicated that <115 bp of flanking homology were required for maximum conversion from a template containing 12 engineered bp alterations. Subsequent studies with 763 bp of flanking homology indicated that the *yellow* gene behaved comparably to a point mutation with respect to conversion (NASSIF *et al.* 1994). This observation suggested that <115 bp of homology might be sufficient for *yellow* gene conversion. However, a study done using human nuclear extracts found that larger inserts required longer stretches of flanking DNA for efficient recombination (LOPEZ *et al.* 1992). Our results are consistent with this study, and indicate that the size of heterologous sequence does affect targeting efficiency in Drosophila, since greater than 375 bp is required for high levels of *yellow* conversion at the *white* locus.

Therefore, the two key parameters in this experimental system are the amount of available homology and the size of the heterologous sequence. We have noted that when homology is limiting, differences in conversion efficiencies between a large (8 kb) and a small (point mutation) heterology are observed. When the template was located on the homologue at the same relative position of transposon excision, the reversion frequency using a template with 12 bp alterations was between 5 and 7.5% (NASSIF and ENGELS 1993). In the same context, our experiments show that a template with a large 8 kb insertion had a reversion frequency of 1.5 ± 0.2% (61 events of 3956 progeny scored, Table 1). This is a threefold reduction, at least, in reversion efficiency, indicating that, even when homology is unlimited, small and large heterologies are not equivalent.

The analysis of our data using a model for double-strand break repair, which assumes a constant probability of gap enlargement (GLOOR *et al.* 1991; NASSIF and ENGELS 1993), indicates that the reduction in *yellow* gene conversion is due to the decrease in flanking homology, and is not a function of gap widening. This is consistent with the idea that the homology requirements for a heterologous insertion and a point mutation are significantly different.

Resetting the homology requirements: Within the full-length repair template, there are 12 single bp alterations, three of which are located within the 3′ flanking sequence. Site 9 is located 70 bp downstream of the *yellow* insertion, and is found in all repair templates that contain >70 bp of adjacent sequence. Conversion of this point mutant into the double-strand break site depended on available homology, as seen in Figure 5. When Δ375 was provided, the conversion frequency was less than half those seen when constructs containing ≥493 bp were used for repair.

This observation is interesting when put in the context of the data obtained from the conversion of point mutations. It was proposed that <115 bp of homology was required to convert a base substitution at maximum efficiency (NASSIF and ENGELS 1993). However, the present data suggests that this homology requirement is "reset" when a large heterologous insert is located within close proximity. As seen with the *yellow* gene, a conversion frequency plateau for site 9 was not observed until the Δ493 template was provided. With this

amount of homology, conversion frequencies were comparable to those seen with infinite homology, indicating that an upper limit had been reached. Since site 9 is located 70 bp downstream of *yellow*, Δ493 actually contains 423 bp of *white* sequence adjacent to this marker. This is more than three times the amount of flanking sequence required with a template containing only the 12 bp alterations. This means that the presence of a large heterology affects the ability of a smaller one positioned nearby to be converted during double-strand break repair. In effect, the heterologous sequence alters the homology requirements for the conversion of the point mutation.

The system analyzed in this experiment has properties that are quite different from those observed in yeast. Gene conversion in yeast cells is associated with reciprocal exchange, but there is not an association in Drosophila mitotic cells. Furthermore, in *Saccharomyces cerevisiae*, the presence of one or more small heterozygous sites between the break site and another heterozygosity significantly reduce the conversion frequency of the distal site (BORTS and HABER 1987, 1989). This is different from similar experiments in Drosophila, in which small heterozygosities have little effect on the conversion of distal sites (PRESTON *et al.* 1996). Our results show that a large heterozygosity is required to affect the conversion of distal sites. Interestingly, the presence of multiple heterozygosities, including the presence of large insertions, have no effect on the frequency of meiotic recombination in Drosophila (HILLIKER *et al.* 1988, 1991). These observations demonstrate the differences between double-strand break repair in yeast and Drosophila, and between mitotic double-strand break repair and meiotic recombination in Drosophila (HILLIKER *et al.* 1994).

Aberrant events: There were 10 aberrant conversion tracts that were characterized (Figure 4, A–J). The model for *P*-element-induced double-strand break repair outlines a process whereby the free ends search the genome for a homologous template, invade it, and initiate DNA synthesis (NASSIF *et al.* 1994). The *yellow* deletion events could be explained if copying initiated at the left end, and extended into the heterologous *yellow* sequence before DNA replication ceased. If a construct with a deletion in the 3′ untranslated region was provided, we suggest that the right end might not find a suitable template, and may simply undergo exonucleolytic degradation. The deletions in the flanking *white* gene sequence seen in the conversion tracts from Δ375–25, Δ375–39, Δ493–41 and Δ493–47 indicate that it has been degraded prior to joining with the other free end. The conversion tracts from Δ25–120 and Δ493–42, which include *P*-element inverted repeat terminus sequence, imply that the right end has undergone very little, if any, degradation. In this model, the amount of correctly targeted sequence would only depend upon the extent of left end elongation. At the resolution step, the two ends would be joined by a nonhomologous pathway (HABER 1995). Similar types of events have been seen in other systems where they were explained by a model in which only one of the ends invaded the template (BELMAAZA and CHARTRAND 1994; JASIN 1996).

The observation that such *yellow* deletions were not found using the infinite homology template, and that the side of decreased homology in the template corresponds to the side where the deletion occurs, suggests that the formation of deletions is promoted on the side of decreased homology. However, we cannot rule out that a bias in our selection scheme prevented us from recovering deletions on the other side of the double-strand break.

The junctions between deleted *yellow* sequence and the 3′ flanking sequence (either *white* gene sequence or *P*-element terminal repeat sequence) varied in size from 2 to 38 bp and were notably A/T rich and somewhat repetitive. These types of junctions have been seen often in studies that characterized imprecise *P*-element excision events (O'HARE and RUBIN 1983; TAKASU-ISHIKAWA *et al.* 1992; JOHNSON-SCHLITZ and ENGELS 1993; STAVELY *et al.* 1995). A few models have emerged to explain these junction sequences, including a hairpin model (TAKASU-ISHIKAWA *et al.* 1992) and replication slippage (O'HARE and RUBIN 1983; STAVELY *et al.* 1995). However, both of these models were based upon *P*-element sequence at the termini of the free ends. In four of the six characterized events, *P*-element sequence is not present at the ends, and yet the junction sequence is still very similar to the two conversion events that do have *P*-element sequence at one terminus. Another possibility is that the right end may invade and copy some information from a nonspecific template to produce the junction region. A previous study has suggested that junction sequence could be derived from distant sequences (TAKASU-ISHIKAWA *et al.* 1992).

The four aberrant yellow$^+$ conversion events that contain a *P*-element end can also be explained by a one-end invasion model (Figure 4, G–J). For this, we assume the left end invades the homologous template, copies through the complete *yellow* gene sequence and into the adjacent sequence including the template *P*-element end. Once again, the right end may not find a suitable template and the free ends could be ligated together at regions of short homology. In Δ25–70, a stretch of 3 A/T bp, coincident to both the *P*-element end and the *white* gene, seems to have been sufficient for recognition between the two ends. The other three events contain alterations within the 3′ untranslated region of the *white* gene, suggesting that the right end may have invaded an inappropriate duplex, or that some other repair mechanism has occurred.

Homology requirements: The model for the repair of *P*-element-induced chromosome breaks requires that the free ends be able to recognize the template. In

this instance, as proposed with most other forms of recombination, recognition is homology dependent and probably reliant upon direct interactions between DNA molecules (HEYER 1994). Results from this experiment corroborate these findings. Removal of all of the *white* gene sequence flanking one side of the insert abolishes *yellow* gene targeting, as no yellow$^+$ events are recovered with the Δ0 construct. One event was recovered when there is an adjacent 25 bp of *white* sequence, but this is not significantly different from the results obtained with the Δ0 and Δ51 templates.

No changes to the amount of 5′ flanking homology were made in any of the constructs, which meant that there was 2456 bp of available template homology to the left of the break site in each template. Even with this constant amount of *white* gene sequence present, *yellow* conversion frequencies were decreased for Δ0, Δ25, Δ51 and Δ375, indicating a need for homologous sequences on the 3′ side of the insertion. This has been found to be true in other cases where a homology interruption located close to the break site in the target caused a decrease in recombination rates (STRUHL 1987; LOPEZ *et al.* 1992), and also when the amount of homology flanking one side of a mutation in a targeting vector was reduced below a critical threshold (THOMAS *et al.* 1992).

Studies on transposon replacement at the *white* locus have found that 31 bp of homology at the searching ends is sufficient for targeting 15 kb of nonhomologous sequence (KEELER and GLOOR 1997). This indicated that very small stretches of DNA can be used to conduct the homology search. Such short stretches of homology did not suffice in this experiment; almost double this amount of homology in the template (51 bp of homology in Δ51) did not produce any conversion events in this series of experiments.

This discrepancy may reflect varying homology requirements for different types of searching ends. The investigation into the role of the searching ends involved the precise replacement of *P-w^{hd}* with the template transposon by a gene conversion process. The searching ends in this circumstance included sequence derived from the *P*-element ends (O'BROCHTA *et al.* 1991; JOHNSON-SCHLITZ and ENGELS 1993; STAVELY *et al.* 1995; KEELER and GLOOR 1997), which are known to bind specific proteins that facilitate transposition. For example, inverted repeat binding protein (IRBP), a host-encoded *trans*-acting factor and recently discovered to be the Drosophila homologue of the Ku autoantigen, binds to the inverted repeat ends of the transposon (RIO and RUBIN 1988; BEALL *et al.* 1994). While Ku has been found to bind to free ends indiscriminately, it is yet to be determined whether IRBP binds to the *P* element previous to, or following excision. If the former, then it would be conceivable that the *P*-element ends, unlike other free DNA ends, would be less predisposed to exonuclease degradation (BEALL *et al.* 1994; STAVELY *et al.* 1995), giving them more time to conduct the homology search. Indeed, it has been noted that most double-strand breaks made by *P*-element excision are repaired without gap widening (JOHNSON-SCHLITZ and ENGELS 1993).

In contrast, free ends that do not contain *P*-element end sequence, as a result of exonucleolytic degradation of a double-strand break, might be expected to have a more dynamic nature because IRBP may not bind immediately, or as tightly. Consequently, the ends could be undergoing exonucleolytic degradation while trying to find a template for repair. As a result, more homology may be required in the template when *P*-element end sequence is not contained within the searching end.

At least two pathways have been proposed in yeast for eukaryotic double-strand break repair: one that is RAD52 and homology dependent, and another that is Ku dependent and requires little or no sequence homology (JACKSON and JEGGO 1995; JEGGO *et al.* 1995). If this holds true in flies, then the above differences could be attributed to the presence or absence of IRBP in the repair process. Unlike a previous study (KEELER and GLOOR 1997), none of the events reported here involve the transposon ends in the homology search. The effect of the *P*-element ends on this double-strand break repair system need to be examined in future studies. We cannot rule out the possibility that the observed differences also may be attributed to the particular sequence of nucleotides in the DNA itself.

Summary: Double-strand breaks have been widely used to induce high frequencies of gene targeting in Drosophila at the *white* locus (ENGELS *et al.* 1990; GLOOR *et al.* 1991; JOHNSON-SCHLITZ and ENGELS 1993; NASSIF and ENGELS 1993; ENGELS *et al.* 1994; NASSIF *et al.* 1994; KEELER *et al.* 1996; KEELER and GLOOR 1997), at other loci in Drosophila (LANKENAU *et al.* 1996; MCCALL and BENDER 1996; MERLI *et al.* 1996; WILLIAMS and O'HARE 1996), in nematodes (PLASTERK and GROENEN 1992) and in mammalian cells (ROUET *et al.* 1994; CHOULIKA *et al.* 1995; JASIN 1996). The findings in this article will serve to enhance the efficiency of these eukaryotic targeting systems, as sufficient homology is required to induce a high number of correct targeting events. As well, the characterization of the aberrant events will help to define possible outcomes when homology is limiting, and further explain the process of *P*-element-induced gene conversion.

The authors thank FAYE MALES and COLIN COROS for excellent technical assistance. We thank K. KEELER, G. DOSSANTOS, N. NASSIF and J. MURRAY for providing helpful comments on the manuscript. T.D. was supported by an Ontario Graduate Scholarship and by a scholarship from the National Science and Engineering Research Council of Canada. This work was supported by a research operating grant to G.G. from the Medical Research Council of Canada.

LITERATURE CITED

BAKER, M. D., L. R. READ, B. G. BEATTY and P. NG, 1996 Requirements for ectopic homologous recombination in mammalian somatic cells. Mol. Cell. Biol. **16:** 7122–7132.

BARNES, W. M., 1994 PCR amplification of up to 35-kb DNA with high fidelity and high yield from λ bacteriophage templates. Proc. Natl. Acad. Sci. USA **91:** 2216–2220.

BAUR, M., I. POTRYKUS and J. PASZKIOWSKI, 1990 Intermolecular homologous recombination in plants. Mol. Cell. Biol. **10:** 492–500.

BEALL, E. L., A. ADMON and D. C. RIO, 1994 A Drosophila protein homologous to the human p70 Ku autoimmune antigen interacts with the P transposable element inverted repeats. Proc. Natl. Acad. Sci. USA **91:** 12681–12685.

BELMAAZA, A., and P. CHARTRAND, 1994 One-sided invasion events in homologous recombination at double-strand breaks. Mutat. Res. **314:** 199–208.

BORTS, R. H., and J. E. HABER, 1987 Meiotic recombination in yeast: alteration by multiple heterozygosities. Science **237:** 1459–1465.

BORTS, R. H., and J. E. HABER, 1989 Length and distribution of meiotic gene conversion tracts and crossovers in *Saccharomyces cerevisiae.* Genetics **123:** 69–80.

CHIA, W., G. HOWES, M. MARTIN, Y. MENG, K. MOSES *et al.*, 1986 Molecular analysis of the *yellow* locus in Drosophila. EMBO J. **13:** 3597–3605.

CHOULIKA, A., A. PERRIN, B. DUJON and J. F. NICOLAS, 1995 Induction of homologous recombination in mammalian chromosomes by using the I-*Sce*I system of *Saccharomyces cerevisiae.* Mol. Cell. Biol. **15:** 1968–1973.

DENG, C., and M. R. CAPECCHI, 1992 Reexamination of gene targeting frequency as a function of the extent of homology between the targeting vector and the target locus. Mol. Cell. Biol. **12:** 3365–3371.

ENGELS, W. R., C. R. PRESTON, P. THOMPSON and W. B. EGGLESTON, 1986 In situ hybridization to Drosophila salivary chromosomes with biotinylated probes and alkaline phosphatase. BRL Focus **8:** 6–8.

ENGELS, W. R., D. M. JOHNSON-SCHLITZ, W. B. EGGLESTON and J. S VED, 1990 High-frequency P element loss in Drosophila is homolog-dependent. Cell **62:** 515–525.

ENGELS, W. R., C. R. PRESTON and D. M. JOHNSON-SCHLITZ, 1994 Long-range cis preference in DNA homology search over the length of a Drosophila chromosome. Science **263:** 1623–1625.

FREIDBERG, E. C., G. C. WALKER and W. SIEDE, 1995 *DNA Repair and Mutagenesis.* ASM Press, Washington, D.C.

GEYER, P. K., and V. G. CORCES, 1987 Separate regulatory elements are responsible for the complex pattern of tissue-specific and developmental transcription of the *yellow* locus in *Drosophila melanogaster.* Genes Dev. **1:** 996–1004.

GLOOR, G. B., N. A. NASSIF, D. M. JOHNSON-SCHLITZ, C. R. PRESTON and W. R. ENGELS, 1991 Targeted gene replacement in Drosophila via P element-induced gap repair. Science **253:** 1110–1117.

GRAY, Y. H. M., M. M. TANAKA and J. A. SVED, 1996 P-element-induced recombination in *Drosophila melanogaster:* hybrid element insertion. Genetics **144:** 1601–1610.

HABER, J. E., 1995 In vivo biochemistry: physical monitoring of recombination induced by site-specific endonucleases. BioEssays **17:** 609–620.

HASTINGS, P. J., 1988 Recombination in the eukaryotic nucleus. BioEssays **9:** 61–64.

HASTY, P., J. RIVERA-PEREZ and A. BRADLEY, 1991 The length of homology required for gene targeting in embryonic stem cells. Mol. Cell. Biol. **11:** 5586–5591.

HEYER, W. D., 1994 The search for the right partner: homologous pairing and DNA strand exchange proteins in eukaryotes. Experimentia **50:** 223–233.

HILLIKER, A. J., S. H. CLARK and A. CHOVNICK, 1988 Genetic analysis of intragenic recombination in Drosophila, pp. 506 in *The Recombination of Genetic Material,* edited by K. LOW. Academic Press, San Diego.

HILLIKER, A. J., S. H. CLARK and A. CHOVNICK, 1991 The effect of DNA sequence polymorphisms in intragenic recombination in the *rosy* locus of *Drosophila melanogaster.* Genetics **129:** 779–781.

HILLIKER, A. J., G. HARAUZ, A. G. REAUME, M. GRAY, S. H. CLARK *et al.*, 1994 Meiotic gene conversion tract length distribution within the rosy locus of *Drosophila melanogaster.* Genetics **137:** 1019–1026.

JACKSON, S. P., and P. A. JEGGO, 1995 DNA double-strand break repair and V(D)J recombination: involvement of DNA-PK. Trends Biochem. Sci. **20:** 412–415.

JASIN, M., 1996 Genetic manipulation of genomes with rare-cutting endonucleases. Trends Genet. **12:** 224–228.

JEGGO, P. A., G. E. TACCIOLI and S. P. JACKSON, 1995 Menage a trois: double strand break repair, V(D)J recombination and DNA-PK. BioEssays **17:** 949–957.

JINKS-ROBERTSON, S., M. MICHELITCH and S. RAMCHARAN, 1993 Substrate length requirements for efficient mitotic recombination in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **13:** 3937–3950.

JOHNSON-SCHLITZ, D. M., and W. R. ENGELS, 1993 P element-induced interallelic gene conversion of insertions and deletions in Drosophila. Mol. Cell. Biol. **13:** 7006–7018.

KAUFMAN, P. D., and D. C. RIO, 1992 P element transposition *in vitro* proceeds by a cut-and-paste mechanism and uses GTP as a cofactor. Cell **69:** 27–39.

KEELER, K. J., and G. B. GLOOR, 1997 Efficient gap repair in *Drosophila melanogaster* requires a maximum of 31 nucleotides of sequence homology at the searching ends. Mol. Cell. Biol. **17:** 627–634.

KEELER, K. J., T. DRAY, J. E. PENNEY and G. B. GLOOR, 1996 Gene targeting of a plasmid-borne sequence to a double-strand DNA break in *Drosophila melanogaster.* Mol. Cell. Biol. **16:** 522–528.

KOWALCZYKOWSKI, S. C., D. A. DIXON, A. K. EGGLESTON, S. D. LAUDER and W. M. REHRAUER, 1994 Biochemistry of homologous recombination in *Escherichia coli.* Microbiol. Rev. **58:** 401–465.

LANKENAU, D. H., V. G. CORCES and W. R. ENGELS, 1996 Comparison of targeted-gene replacement frequencies in *Drosophila melanogaster* at the *forked* and *white* loci. Mol. Cell. Biol. **16:** 3535–3544.

LIN, F., K. SPERLE and N. STERNBERG, 1984 Model for homologous recombination during transfer of DNA into mouse L cells: role for DNA ends in the recombination process. Mol. Cell. Biol. **4:** 1020–1034.

LINDSLEY, D. L., and G. G. ZIMM, 1992 *The Genome of Drosophila melanogaster.* Academic Press, San Diego.

LISKAY, R. M., A. LETSOU and J. L. STACHELEK, 1987 Homology requirement for efficient gene conversion between duplicated chromosomal sequences in mammalian cells. Genetics **115:** 161–167.

LOPEZ, B. S., E. CORTEGGIANI, P. BERTRAND-MERCAT and J. COPPEY, 1992 Directional recombination is initiated at a double strand break in human nuclear extracts. Nucleic Acids Res. **20:** 501–506.

MCCALL, K., and W. BENDER, 1996 Probes of chromatin accessibility in the Drosophila *bithorax* complex respond differently to Polycomb-mediated repression. EMBO J. **15:** 569–580.

MERLI, C., D. E. BERGSTROM, J. A. CYGAN and R. K. BLACKMAN, 1996 Promoter specificity mediates the independent regulation of neighboring genes. Genes Dev. **10:** 1260–1270.

NASSIF, N. A., and W. R. ENGELS, 1993 DNA homology requirements for mitotic gap repair in Drosophila. Proc. Natl. Acad. Sci. USA **90:** 1262–1266.

NASSIF, N., J. PENNEY, S. PAL, W. R. ENGELS and G. B. GLOOR, 1994 Efficient copying of nonhomologous sequences from ectopic sites via P element-induced gap repair. Mol. Cell. Biol. **14:** 1613–1625.

NICOLAS, A., D. TRECO, N. P. SCHULTES and J. W. SZOSTAK, 1989 An initiation site for meiotic gene conversion in the yeast *Saccharomyces cerevisiae.* Nature **338:** 35–39.

O'BROCHTA, D. A., S. P. GOMEZ and A. M. HANDLER, 1991 P element excision in *Drosophila melanogaster* and related drosophilids. Mol. Gen. Genet. **225:** 387–394.

O'HARE, K., and G. M. RUBIN, 1983 Structure of P transposable elements and their sites of insertion and excision in the *Drosophila melanogaster* genome. Cell **34:** 25–35.

O'HARE, K., C. MURPHY, R. LEVIS and G. RUBIN, 1984 DNA sequence of the *white* locus of *Drosophila melanogaster.* J. Mol. Biol. **180:** 437–455.

PLASTERK, R. H., and J. T. GROENEN, 1992 Targeted alterations of the *Caenorhabditis elegans* genome by transgene instructed DNA double strand break repair following Tc1 excision. EMBO J. **11:** 287–290.

PRESTON, C. R., and W. R. ENGELS, 1996 *P*-element-induced male recombination and gene conversion in Drosophila. Genetics **144:** 1611–1622.

PRESTON, C. R., J. A. SVED and W. R. ENGELS, 1996 Flanking duplica-

tions and deletions associated with *P*-induced male recombination in Drosophila. Genetics **144:** 1623–1638.

RESNICK, M. A., 1976 The repair of double-strand breaks in DNA: a model involving recombination. J. Theor. Biol. **59:** 97–106.

RIO, D. C., and G. M. RUBIN, 1988 Identification and purification of a Drosophila protein that binds to the terminal 31-base-pair repeats of the P transposable element. Proc. Natl. Acad. Sci. USA **85:** 8929–8933.

ROBERTSON, H. M., C. R. PRESTON, R. W. PHILLIS, D. JOHNSON-SCHLITZ, W. K. BENZ *et al.*, 1988 A stable genomic source of P element transposase in *Drosophila melanogaster.* Genetics **118:** 461–470.

ROSENBERG, S. M., and P. J. HASTINGS, 1991 The split end model for homologous recombination at double strand breaks and at Chi. Biochimie **73:** 385–397.

ROUET, P., F. SMIH and M. JASIN, 1994 Introduction of double-strand breaks into the genome of mouse cells by expression of a rare-cutting endonuclease. Mol. Cell. Biol. **14:** 8096–8106.

RUBIN, G. M., and A. C. SPRADLING, 1982 Genetic transformation of Drosophila with transposable element vectors. Science **218:** 348–353.

RUBNITZ, J., and S. SUBRAMANI, 1984 The minimum amount of homology required for homologous recombination in mammalian cells. Mol. Cell. Biol. **4:** 2253–2258.

SAIKI, R. K., D. H. GELFAND, S. STOFFEL, S. J. SCHARF, R. G. HIGUCHI *et al.*, 1988 Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science **239:** 487–491.

SAMBROOK, J., E. F. FRITSCH and T. MANIATIS, 1989 *Molecular Cloning: A Laboratory Manual,* 2nd ed. Cold Spring Harbor Press, Cold Spring Harbor, New York.

SCHEERER, J. B., and G. M. ADAIR, 1994 Homology dependence of targeted recombination at the Chinese Hamster APRT locus. Mol. Cell. Biol. **14:** 6663–6673.

SHEN, P., and H. V. HUANG, 1986 Homologous recombination in *Escherichia coli:* dependence on substrate length and homology. Genetics **112:** 441–457.

SINGER, B. S., L. GOLD, P. GAUSS and D. H. DOHERTY, 1982 Determination of the amount of homology required for recombination in bacteriophage T4. Cell **31:** 25–33.

SPRADLING, A. C., and G. M. RUBIN, 1982 Transposition of cloned P elements into Drosophila germ line chromosomes. Science **218:** 341–347.

STAHL, F., 1996 Meiotic recombination in yeast: coronation of the double-strand-break repair model. Cell **87:** 965–968.

STAVELY, B. E., T. R. HESLIP, R. B. HODGETTS and J. B. BELL, 1995 Protected *P*-element termini suggest a role for inverted-repeat-binding protein in transposase-induced gap repair in *Drosophila melanogaster.* Genetics **139:** 1321–1329.

STRATHERN, J. N., 1988 Control and execution of homothallic switching in *Saccharomyces cerevisia,* pp. 445–464 in *Genetic Recombination,* edited by R. KUCHERLAPATI and G. R. SMITH. American Society of Microbiology, Washington, D.C.

STRUHL, K., 1987 Effect of deletion and insertion on double-strand-break-repair in *Saccharomyces cerevisia.* Mol. Cell. Biol. **7:** 1300–1303.

SUN, H., D. TRECO, N. P. SCHULTES and J. W. SZOSTAK, 1989 Double-strand breaks at an initiation site for meiotic gene conversion. Nature **338:** 87–90.

SVED, J. A., L. M. BLACKMAN, Y. SVOBODA and R. COLLESS, 1995 Male recombination with single and homologous P elements in *Drosophila melanogaster.* Mol. Gen. Genet. **246:** 381–386.

SVOBODA, Y. H., M. K. ROBSON and J. A. SVED, 1995 *P*-element-induced male recombination can be produced in *Drosophila melanogaster* by combining end-deficient elements in trans. Genetics **139:** 1601–1610.

SZOSTAK, J. W., T. L. ORR-WEAVER, R. J. ROTHSTEIN and F. W. STAHL, 1983 The double-strand-break repair model for recombination. Cell **33:** 25–35.

TAKASU-ISHIKAWA, E., M. YOSHIHARA and Y. HOTTA, 1992 Extra sequences found at P element excision sites in *Drosophila melanogaster.* Mol. Gen. Genet. **232:** 17–23.

TE RIELE, H., E. R. MAANDAG and A. BERNS, 1992 Highly efficient gene targeting in embryonic stem cells through homologous recombination with isogenic DNA constructs. Proc. Natl. Acad. Sci. USA **89:** 5128–5132.

THOMAS, K. R., C. DENG and M. R. CAPECCHI, 1992 High-fidelity gene targeting in embryonic stem cells by using sequence replacement vectors. Mol. Cell. Biol. **12:** 2919–2923.

WATT, V. M., C. J. INGLES, M. S. URDEA and W. J. RUTTER, 1985 Homology requirements for recombination in *Escherichia coli.* Proc. Natl. Acad. Sci. USA **82:** 4768–4772.

WEST, S. C., 1992 Enzymes and molecular mechanisms of genetic recombination. Annu. Rev. Biochem. **61:** 603–640.

WILLIAMS, C. J., and K. O'HARE, 1996 Elimination of introns at the Drosophila *suppressor-of-forked* locus by *P*-element-mediated gene conversion shows that an RNA lacking a stop codon is dispensable. Genetics **143:** 345–351.

Communicating editor: R. S. HAWLEY