Copyright © 1993 by the Genetics Society of America

Preferential Transposition of Drosophila *P* Elements to Nearby Chromosomal Sites

John Tower,[1] Gary H. Karpen,[2] Nancy Craig[3] and Allan C. Spradling

Howard Hughes Medical Institute Research Laboratories, Carnegie Institution of Washington, Baltimore, Maryland 21210

Manuscript received August 20, 1992
Accepted for publication October 12, 1992

ABSTRACT

Two different schemes were used to demonstrate that Drosophila *P* elements preferentially transpose into genomic regions close to their starting sites. A starting element with weak *rosy*$^+$ marker gene expression was mobilized from its location in the subtelomeric region of the 1,300-kb *Dp1187* minichromosome. Among progeny lines with altered *rosy*$^+$ expression, a much higher than expected frequency contained new insertions on *Dp1187*. Terminal deficiencies were also recovered frequently. In a second screen, a *rosy*$^+$-marked element causing a lethal mutation of the *cactus* gene was mobilized in male and female germlines, and viable revertant chromosomes were recovered that still contained a *rosy*$^+$ gene due to an intrachromosomal transposition. New transpositions recovered using both methods were mapped between 0 and 128 kb from the starting site. Our results suggested that some mechanism elevates the frequency 43–67-fold with which a *P* element inserts near its starting site. Local transposition is likely to be useful for enhancing the rate of insertional mutation within predetermined regions of the genome.

ONE of the best characterized forms of mobile DNA in Drosophila is the *P* family of transposable elements [reviewed by ENGELS (1989) and RIO (1990)]. Complete *P* elements are 2907 bp in length, contain short inverted terminal repeats and transpose by a nonconservative mechanism (ENGELS *et al.* 1990). The elements encode a well characterized, germline-limited transposase and regulate their own copy number and activity in wild strains. Insertional mutagenesis using *P* elements has been applied widely, and the ability to control a single transposase source has further increased its usefulness (COOLEY, BERG and SPRADLING 1988; ROBERTSON *et al.* 1988). Potential targets of *P* element insertion reside throughout euchromatin as well as in at least a few heterochromatic chromosome regions (KARPEN and SPRADLING 1992)

A highly advantageous method of insertional mutagenesis is to introduce just one genetically marked *P* element into each mutagenized strain (COOLEY, KELLEY and SPRADLING 1988). The presence of only one insertion simplifies mapping, cloning, and subsequent genetic analysis. Employing mutagenic elements bearing an appropriate *lacZ*-fusion allows enhancer trapping (O'KANE and GEHRING 1987). One problem with single *P* element mutagenesis is the low insertion rate at any single locus. It was proposed that a large library of single insert strains be established to reduce the effort required to obtain mutations in particular genes (COOLEY, KELLEY and SPRADLING 1988). Alternatively, methods have been described for generating strains containing multiple *P* elements (ROBERTSON *et al.* 1988; BALINGER and BENZER 1989; KAISER and GOODWIN 1990; HAMILTON *et al.* 1991). Although these approaches may decrease the effort required to obtain an insertion in a locus of interest, multi-insert strains lack most of the molecular-genetic advantages of single-insert strains. Therefore, methods that enhanced the rate of single-element insertion within a predetermined chromosomal region would be worthwhile.

The *Ac/Ds* element of *Zea mays* will preferentially transpose to sites on the same chromosome where it resided, and these target sites are frequently linked genetically to the starting site (VAN SCHAIK and BRINK 1959; GREENBLATT and BRINK 1962; GREENBLATT 1984; OSBORNE *et al.* 1991; WEIL *et al.* 1992; ATHMA, GROTEWOLD and PETERSON 1992; MORENO *et al.* 1992). This phenomenon, which we will refer to as "local transposition," has been demonstrated for a variety of *Ac* and *Ds* elements located at several different starting sites. It has been proposed that use of a donor element near the desired target might enhance the rate of mutagenesis by *Ac* insertion (DOONER and BELACHEW 1989; KERMICLE, ALLEMAN and DELLAPORTA 1989). *P* elements belong to the same general category of transposons as *Ac*, although the double-strand breaks generated by element excision appear to be repaired by gene conversion much more

[1] Present address: Department of Biological Sciences, University of Southern California, University Park, Los Angeles, California 90089.
[2] Present address: Molecular Biology and Virology Laboratory, The Salk Institute, 10010 North Torrey Pines Road, La Jolla, California 92037.
[3] Howard Hughes Medical Institute Research Laboratories, Department of Molecular Biology and Genetics, The Johns Hopkins University School of Medicine, 725 N. Wolf Street, Baltimore, Maryland 21205.

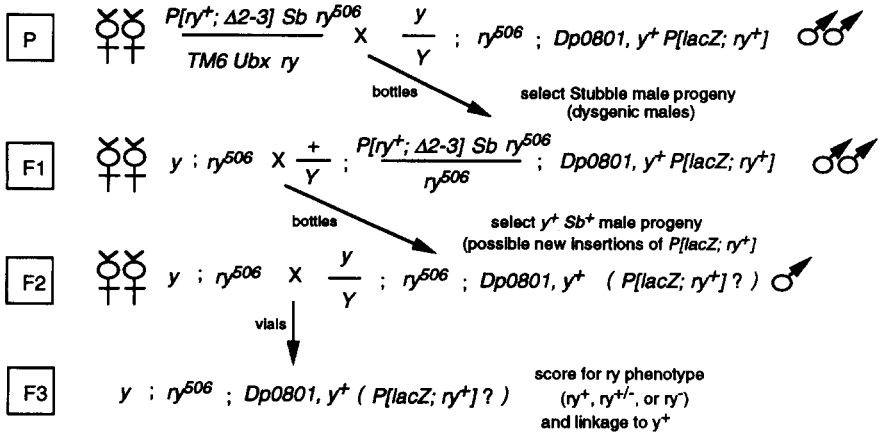

FIGURE 1.—Genetic screen used to detect local *PZ* transposition in males bearing the *Dp0801* minichromosome. For description of genetic symbols, see LINDSLEY and ZIMM (1992). In the progeny of the F_1 generation, all y^+ Sb^+ males were selected, regardless of ry^+ expression, because it was thought that desired heterochromatic inserts might not efficiently express the ry^+ gene. The progeny of the F_2 cross were scored for segregation of the y^+ and ry^+ markers. Lines where $y^+$and ry^+ segregate represent interchromosomal transpositions, lines in which the markers cosegregate represent potential intrachromosomal transpositions, or local hops. The same screen was used for the 9901 insertion. Further details are described in the text.

efficiently in Drosophila (ENGELS *et al.* 1990) than in maize (BARAN *et al.* 1992). If *P* elements were also able to transpose locally at an elevated rate, then selection of a starting element near a target of interest might enhance its mutation by single *P* element insertion.

Several previous observations suggested that *P* elements might sometimes transpose locally. Strains bearing tandem inverted repeats of two similar or identical *P* elements have been observed (ROIHA, RUBIN and O'HARE 1988; SALZ, CLINE and SCHEDL 1987). Strains containing *P* elements at the *singed* or *yellow* locus repeatedly gave rise to progeny containing a second identical element at the site in inverted orientation (HAWLEY *et al.* 1988; EGGLESTON 1990). These double elements resemble some of the derivatives subsequently recovered in maize following local transposition (DOWE, ROMAN and KLEIN 1990; ATHMA, GROTEWOLD and PETERSON 1992). However, the presence of multiple *P* elements in the progenitor strains used in these experiments obscured the actual origin of the twin *P* elements.

In this paper, we describe experiments demonstrating that *P* elements preferentially transpose to nearby sites on the starting chromosome. In combination with the accompanying study (ZHANG and SPRADLING 1993), we show that under optimal conditions this enhanced transposition is likely to be of significant utility for insertional mutagenesis of defined genomic regions.

MATERIALS AND METHODS

Drosophila stocks: Flies were grown on cornmeal/agar media (see ASHBURNER 1990) at 22°. Unless stated otherwise, strains and mutations are as described in LINDSLEY and ZIMM (1992). All strains used in the experiments with the *Dp(1;f)1187* minichromosome are described in KARPEN and SPRADLING (1992). The *P[lacZ, ry+]* element used in the *Dp1187* experiments is hereafter referred to as *PZ* (MLODZIK and HIROMI 1992). Lines that were derived from the *Dp1187* derivatives, *Dp0801* and *Dp9901*, contain the prefix 8- and 9-, respectively. *cactus*^{255} is a lethal *cactus* (*cact*) allele caused by the insertion of the *P[lacZ, ry+]* element *FZ* (MLODZIK and HIROMI 1992), and was provided by YASUSHI HIROMI. Lines derived from reversion of *cact*^{255} were named with the number of the bottle from which they were obtained, preceded by the letter "X" if derived from the female germline. *cact*^{AA5} is an EMS-induced lethal allele of *cactus* provided by SIEGFREID ROTH (ROTH *et al.* 1991).

Restriction mapping using pulsed-field and conventional gel electrophoresis: Lines containing potential intrachromosomal *Dp1187* transpositions were identified genetically as those displaying a heritable increase in ry^+ expression that was linked to *Dp1187* [see KARPEN and SPRADLING (1992) for a description of the linkage analysis]. Genomic DNAs from such candidate lines [isolated from adult flies (BENDER, SPIERER and HOGNESS 1983)] were digested with *Eco*RI and subjected to conventional gel electrophoresis. Southern blots were hybridized separately with probes homologous to the 5′ end of the transposon (*pBS5′ PHR 0.55;* see Figure 2A), the 3′ end of the transposon (*pBS 5′ ryHR3.2;*), and the 1.8-kb tandemly repeated element that flanks the starting insertions [= C in Figure 2A; see KARPEN and SPRADLING (1992) for a description of the probes], according to the methods described in KARPEN and SPRADLING (1990). Some of the lines that contained altered or additional *Eco*RI flanking fragments were subjected to pulsed-field Southern analysis to determine the location of the *PZ* insertions within *Dp1187*. The *Not*I and *Sfi*I restriction map of *Dp1187* (see Figure 2A) and the presence of sites for these enzymes within the *PZ* transposon were used to localize the insertions, as described in KARPEN and SPRADLING (1992). The conventional DNA blot hybridization analyses of the *cact* transpositions (see Figure 7) were performed according to standard protocols (SAMBROOK, FRITSCH and MANIATIS 1989).

Plasmid rescue: Sequences flanking the 5′ end of *FZ* insertions were rescued by first preparing DNA from 10 adult males as described by BENDER, SPIERER and HOGNESS (1983). Following digestion with *Xba*I, or *Xba*I and *Nhe*I, the reaction was diluted, ligated overnight at 15° and used to transform competent *Escherichia coli* DH5 cells to *kanamycin* resistance, essentially as described previously (PIROTTA 1986; COOLEY, KELLY and SPADLING 1988). Approximately one colony was obtained for each fly equivalent of DNA used for transformation.

Polymerase chain reaction (PCR): Sequences flanking the 3′ end of the *FZ* element inserts were amplified from genomic DNA by inverse PCR. Briefly, DNA was isolated from adult flies as described (BENDER, SPIERER and HOGNESS 1983), and one fly equivalent was digested with *Taq*I. The DNA was then ligated with 2 units of T4 ligase in a total volume of 100 μl for 16 hr at 15°, precipitated with ethanol,

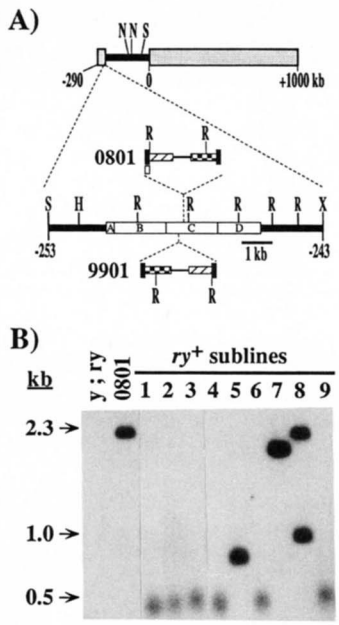

FIGURE 2.—A, Structure of starting *Dp1187 PZ* chromosomes. The overall structure of the *Dp1187* minichromosome is shown above. Values indicate distance in kilobases from the sc^8 breakpoint (= 0) into the euchromatin (solid line, − values) and the centromeric heterochromatin (stippled bar, + values). N = *Not*I and S = *Sfi*I sites. Stippled area between −240 and −290 corresponds to a region of subtelomeric heterochromatin. See KARPEN and SPRADLING (1992) for details of *Dp1187* molecular structure and the generation and analysis of *Dp1187 PZ* lines. The structure of the subtelomeric *P* element insertion "hotspot" is shown below, displaying the location and orientation of *PZ* elements in lines 0801 and 9901. Black boxes = *P* ends; box with diagonal lines = 5′ *P-lacZ* fusion; solid line = the *kanamycin*^{R} gene and the bacterial *origin of replication*; box with checkerboard is the *rosy*$^+$ gene (*ry*$^+$); open box = 5′ *P* end probe. S, *Spe*I; H, *Hin*dIII; R, *Eco*RI; X, *Xba* I. Boxed areas labeled A, B, C, and D refer to the 1.8-kb tandem repeats present in the subtelomeric *P* insertion hotspot (KARPEN and SPRADLING 1992). B, Conventional Southern analysis to identify lines with intrachromosomal transpositions. Adult genomic DNA was digested with *Eco*RI, electrophoresed in a 0.7% agarose gel, and transferred to membrane (see MATERIALS AND METHODS). Sample autoradiogram is shown, after hybridization with the 5′ *P* end probe. The genotypes of the lines are *y ; ry*^{506} (= *y ; ry*), *y ; ry*^{506}; *Dp0801*, *y*$^+$ *ry*$^{\pm}$ (= 0801) and *y ; ry*^{506}; *Dp* (with *PZ* insertion), *y*$^+$ *ry*$^+$ (= ***ry***$^+$ sublines). *ry*$^+$ sublines are transposase-generated *Dp0801* derivatives that exhibit full expression of *ry*$^+$; this includes terminal deficiencies (lines 1–4, 6 and 9), derivatives that contain both the old 0801 insertion plus a new insertion linked to *Dp1187* (line 8), and lines in which the original 0801 insertion has been altered or moved to a new site (lines 5 and 7). Fragment sizes (in kilobases) are shown to the left.

suspended in TE and digested with *Pst*I. The 0.5 fly equivalent was then used in a standard 50-μl PCR reaction containing: 0.3 mM dATP, dGTP, dCTP and dTTP; 2 mM $MgCl_2$; 5 mM KCl; 100 μg/ml gelatin; 5 mM Tris-HCl, pH 8.4; and 0.2 μg each of primers "5′ rosy" and "IR." The mixture was boiled for 5 min, 10 units of *Taq*I DNA polymerase were added, and the reaction was subjected to 40 cycles of: 94°, 1 min; 58°, 2 min; 72°, 3.5 min; in a Perkin-Elmer thermal cycler. The reaction products were subsequently purified by agarose gel electrophoresis. The sequence of the "5′ rosy" oligo is 5′-CATCCGCAGGTATCAATGGTCCGTCAGGAG-3′. The sequence of the "IR" oligo is 5′-CGATCGGGACCACCTTATGTTATTTCATCAT-3′.

***cactus* region genomic walk:** Overlapping λ phage encompassing the *cact* gene were generously provided by DAVID MARCEY (unpublished data), and restriction map data was generously provided by ROBERT GEISLER and CHRISTIANNE NÜSSLEIN-VOLHARD (R. GEISLER, A. BERGMANN, Y. HIROMI and C. NÜSSLEIN-VOLHARD, in preparation). Cosmid clones overlapping the ends of the λ phage portion of the walk were cloned from a genomic library, as previously described (KARPEN and SPRADLING 1990), and this genomic walk will be described in detail elsewhere (G. LANDIS and J. TOWER, unpublished data).

Estimation of insertion rates: Insertion rates in this study are based on the total number of target chromosomes that were recovered in the appropriate genotype for balancing and further analysis. For example, in the experiment described in Figure 1, the presence or absence of insertions in female flies, or in flies that lacked a minichromosome or retained the Δ2-3 chromosome, was not scored. When comparing transposition rates onto different chromosomes, corrections may be necessary for chromosomes that were not scored. Further details are given in the footnotes to Tables 1 and 3. The *PZ* insertion rate onto the minichromosome from a distant site calculated in a comparable manner was not directly determined in the experiments of KARPEN and SPRADLING (1992). However, it could be estimated since 12.5% of the 100 average F_2 progeny in the 22,000 crosses would be minichromosome-containing males that lacked Δ2-3. Forty-two of these contained new insertions, yielding a rate of 42/275,000 = 0.015%.

The total number of local insertions derived from *cactus*^{255} (Table 3) was calculated as follows. The plasmid rescue experiments detected 1 local transposition among the 30 of 31 lines tested that were recovered from the female germline. Three local jumps were detected among the 30 of 109 lines tested bearing insertions recovered in males. This method would therefore have detected a total of about 11 local jumps (109 × 3/30) in males, and 1 (31 × 1/30) in females. Among the 98 lines derived from males and 30 lines derived from females that lacked an insertion detected by the plasmid rescue method, *in situ* hybridization detected 2 additional local insertions at the cytogenetic locus of *cactus* from a sample of 13 lines analyzed. Therefore a total of 15 additional local insertions were estimated to be present in the remaining lines derived from males (98 × 2/13) and 5 additional local insertions (30 × 2/13) in lines derived from females. Summing the lines detected by plasmid rescue and *in situ* hybridization yielded 11 + 15 = 26 lines derived from males, and 1 + 5 = 6 lines derived from females.

RESULTS

Generating *Dp1187* derivatives that alter *ry*$^+$ expression: The generation of single *PZ* insertions on the *Dp1187* minichromosome has recently been described (KARPEN and SPRADLING 1992). Transposition from the *X* chromosome to *Dp1187* occured at a low frequency: only about 0.015% of the progeny minichromosomes contained an insertion. In order to try to more efficiently generate new insertions on *Dp1187*, we tested whether *P* elements preferentially transpose intrachromosomally to nearby sites. Our strategy to identify new insertions exploited the observation that insertions within a 4.7-kb subtelomeric

TABLE 1

***PZ* transpositions from 0801 or 9901 in the male germline**

Starting element	Chromosomes tested[a]	*rosy*$^+$ sublines[b]	*rosy*$^+$ increase[c]	*rosy*$^+$ decrease[d]	Local insertion[e]	Terminal deletion[f]	Other events[g]	Transposition[h]
0801	1900	263	82	50	19 (1%)	68 (3%)	8	65 (3%)
9901	1700	258	114	63	17 (1%)	60 (3%)	26	55 (3%)

[a] The estimated number of y$^+$ Sb$^+$ male F_2 progeny from the scheme presented in Figure 1, extrapolated from counts of 30% of the total progeny.
[b] The number of F_2 crosses in which at least some ry$^+$ F_3 progeny were observed.
[c,d] The number of F_2 crosses in which *ry*$^+$ expression appeared to have increased or decreased in the F_3 progeny.
[e] Based on DNA analysis of all lines with altered *ry*$^+$ expression.
[f] Based on DNA analysis of all lines with altered *ry*$^+$ expression, and the estimated number among the remaining lines extrapolated from a subset subjected to DNA analysis.
[g] Includes internal deletions, chromosomal translocations, and putative gap repair events that will be reported elsewhere (G. H. KARPEN and A. C. SPRADLING, in preparation).
[h] Corrected for 25% of the F_2 crosses in which transpositions unlinked to the minichromosome were accidentally not scored. In columns 6, 7 and 9, the % shown in parentheses = % of F_2 progeny that could have contained a new transposition (Sb$^+$ progeny).

P insertion "hotspot" expressed the *rosy*$^+$ (*ry*$^+$) gene on the *P* element very weakly, due to a position effect that could be overcome by the presence of an extra *Y* chromosome (KARPEN and SPRADLING 1992). Transpositions to other sites on the minichromosome might restore normal eye color, either by relieving the position effect (LEVIS, HAZELRIGG and RUBIN 1985) or by the additive effects of a second gene copy.

The crossing scheme used in these experiments is diagrammed in Figure 1. Two different insertions, 0801 and 9901, that were located at similar sites but in opposite orientation (Figure 2A), were mobilized in male germ lines using Δ2-3 (99B) as a transposase source. The results of this experiment are summarized in Table 1. Approximately 3,600 F_2 male progeny that contained the minichromosome (*i.e.*, were yellow$^+$) and lacked Δ2-3 (99B) (*i.e.*, were Stubble$^+$) were analyzed by test crossing in the F_3 generation, and progeny scored for the segregation of the *ry*$^+$ and *yellow* (*y*$^+$) markers. One hundred and twenty lines had undergone an interchromosomal transposition since they contained y$^-$ ry$^+$ F_3 progeny. In 521 lines, *ry*$^+$ still segregated with *y*$^+$, but in 309 of these, the eye color of F_3 flies suggested that *rosy*$^+$ expression had increased or decreased compared to the parental flies.

Changes in DNA structure accompanied altered *rosy*$^+$ expression: A molecular analysis was used to identify alterations in minichromosome structure, including new transposon insertions, that might be responsible for the observed changes in *rosy*$^+$ expression. DNA isolated from adult flies of each line showing altered expression was digested with *Eco*RI for conventional DNA blot analysis. Probes specific for the *P* element 5′ end, for *rosy*$^+$ sequences present at the 3′ end, and for the 1.8-kb repeat sequences flanking both the 0801 and 9901 insertions, were used. An example of these experiments using the 5′ *P* element-specific probe to analyze lines with increased *rosy*$^+$ expression are shown in Figure 2B. The sequences flanking the 5′ end of the element had changed in most of the ry$^+$ lines; one line (subline 8) retained the parental 5′ fragment, but also contained a new one, indicating the likely presence of a second *P* element insertion.

Representative lines were analyzed in greater detail using pulsed-field gel electrophoresis. DNA was prepared in agarose inserts, digested with *Not*I or *Sfi*I, separated on a pulsed-field gel, and filters hybridized using probes to the *P* element ends, and also to minichromosome sequences at two positions (see MATERIALS AND METHODS). These studies showed that the most common events causing increased rosy expression were terminal deletions of most or all of the minichromosome distal to the 0801 and 9901 insertions. Terminal deletions could be recognized by the altered size and heterogeneous length of the DNA fragment distal to the insertion (Figure 2B, sublines 1–4, 6 and 9). Similar behavior of terminally deleted chromosomes has been described previously (BIESSMANN and MASON 1988; LEVIS 1989; KARPEN and SPRADLING 1992). In addition, the distal end of the fuzzy band was observed to be at the same location on the minichromosome using several different restriction enzymes (data not shown), indicating that this position was in fact the end of the chromosome. Usually the new chromosome end was located close to the distal end of the *P* element; however, some lines still retained several hundred base pairs of DNA distal to the *P* element, while in a few others the chromosome terminated inside the element.

A high frequency of insertions occurred on *Dp1187*, close to the starting element: The mapping experiments revealed 36 lines that contained new *P* element insertions. Examples of this analysis are illustrated in Figure 3. The probe labeled a 280-kb *Sfi*I

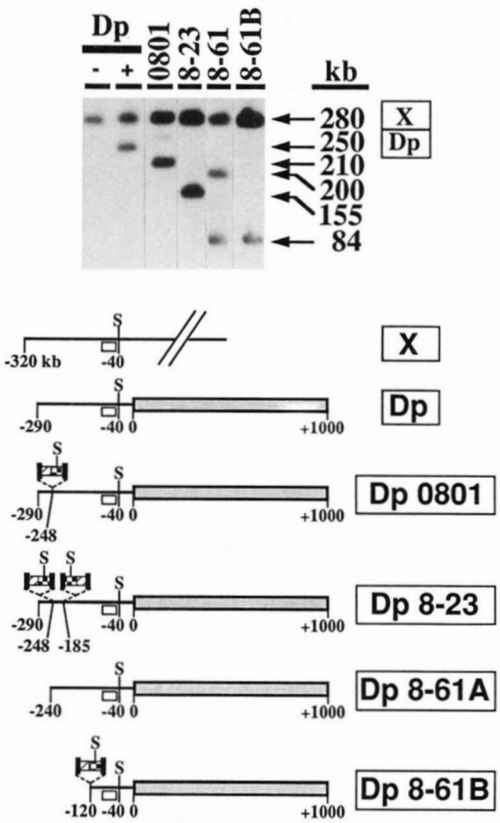

FIGURE 3.—Mapping new *PZ* insertions on *Dp1187* by pulsed-field gel electrophoresis. Pulsed-field Southern hybridization analysis of *Sfi*I-digested ovary DNA from *Dp1187* insertion lines was performed as described in MATERIALS AND METHODS, and a representative autoradiogram is shown. The genotypes of the lines are y ; ry^{506} (= Dp −), y ; ry^{506}; *Dp(1;f)1187*, y^+ (= Dp +), and y ; ry^{506}; *Dp* (with *PZ* insertion), y^+ ry^+ (= 0801, 8-23, 8-61, 8-61B). Sizes of restriction fragments are indicated to the right; X = *X* chromosome-specific fragment, Dp = *Dp1187*- specific fragment. Diagrams of the structure of this region of the *X*, *Dp1187* and *Dp0801* chromosomes are shown below, with the locations of the *Sfi*I sites indicated. Open box denotes the probe used in this experiment (*TG1BP11.5*). *Not*I digests and probe *12.1BH9* were used to analyze the structure of the derivatives proximal to −100, in a similar manner, capitalizing on the presence of two *Not*I sites in the *PZ* element. For a description of other symbols refer to the Figure 2 legend. Note that line *8-61* contains both *Dp 8-61A* and *Dp 8-61B*.

fragment derived from the *X* chromosome, representing the distance from the *Sfi*I site at −40 to the telomere. In lines containing *Dp1187*, a 250-kb band was also observed, since this chromosome contains a smaller amount of subtelomeric DNA. This *Sfi*I fragment was reduced to 210 kb in the *Dp0801* chromosome, since the inserted *P* element contains an *Sfi*I site. In two lines, 8-23 and 8-61, new insertions could be localized 63 and 128 kb from the starting site. For example, in line 8-23, an *Sfi*I fragment of only 155 kb was observed, indicating a new insertion at position −185.

Most of the new insertions mapped within the previously identified 4.7-kb subtelomeric *P* element hotspot (KARPEN and SPRADLING 1992). The locations and orientations of 19 of the new *Dp1187* inserts (including all those outside the hotspot) are summarized in Table 2 and Figure 4. The exact locations within the hotspot of the remaining insertions were not determined from the restriction analyses; they are not shown in Figure 4 or Table 2. These data allowed several conclusions to be drawn. First, the recovery of 36 new insertions from among 3,600 progeny chromosomes (1%, Table 1) represented a substantial increase in the rate of insertion onto *Dp1187* compared to our previous interchromosomal transposition experiment (0.015%, see MATERIALS AND METHODS). Most of the intrachromosomal insertions were recovered within or very close to the starting transposons, resulting in the production of "double" *P* elements. However, 4 insertions were recovered 2–3 kb away from the starting element; two were much more distant (Table 2). Thus, 17% (6/36) of the local transpositions were to sites between 2 and 128 kb from the starting element.

Another unusual feature of the insertions, besides their tendency to cluster around the starting site, was their nonrandom position and orientation on the minichromosome relative to the starting element. Most of the insertions were located within the starting *P* element, or in flanking genomic DNA on its 5′ side (15/19). All these insertions pointed in the direction opposite to the starting element. A smaller number of elements transposed to the 3′ side of the starting element (4/19), and they occupied both possible orientations.

The high frequency of transpositions onto *Dp1187* should have increased the opportunity to recover insertions within subtelomeric or centromeric heterochromatin. However, it was not clear if the presence of such insertions would be detected by expression of the $rosy^+$ marker gene. The 0801 and 9901 insertions already expressed $rosy^+$ weakly due to a heterochromatic position effect that could be alleviated by the addition of an extra *Y* chromosome. To look for new heterochromatic insertions that might have suffered strong position effects, an extra *Y* chromosome was introduced into several hundred lines that had been scored as $rosy^-$ in the F_2 and F_3 generations. Five lines were detected in which rosy expression was rescued by the presence of an extra *Y* chromosome. One of them, 8-152, contained a transposition within subtelomeric heterochromatin distal to all other insertions previously recovered on *Dp1187*. In contrast, the other *Y*-suppressed lines contained double *P* elements at the starting site (not shown in Figure 4), or lacked any detectable changes in the starting element. The reduced $rosy^+$ expression in these four lines may have resulted from changes elsewhere in the genome that enhanced the original position effect on the 0801 starting element.

TABLE 2

Local transpositions from 0801 and 9901

Strain	Position of new insertion (kb)[a]	Structure of original insert	Comments
8-18	−248	Insertion	Jump in starting element 0.4 kb from *rosy* RI
8-23	−185	No change	Jump, 63 kb right
8-61(B)	−120	Terminal deletion	Jump, 128 kb right
8-73	−248	No change	Jump, 0.2 kb left
8-103	−250	Excision	Jump, 2.1 kb left
8-152	−251	No change	Jump, 3 kb left
8-233	−248	Insertion	Jump in starting element, near 3′ end
8-265	−248	5′ deletion	Double *P*, 0 kb left
8-354	−248	No change	Jump, 0.4 kb left
8-385	−248	5′ deletion, +insertion	Jump into starting element, near middle
8-541	−250	Excision	Jump, 2 kb left
8-584	−248	Insertion	Jump into starting element, near 3′ end
9-143	−248	Insertion	Jump into starting element, near 5′ end
9-268	−248	No change	Jump, 0.2 kb right
9-286	−248	No change	Jump, 0.4 kb right
9-332	−248	Insertion	Jump into starting element, near 5′ end
9-568	−248	No change	Jump, 0.1 kb right
9-638	−248	No change	Jump, 0.2 kb left
9-658	−250	Internal deletion	Jump, 2.2 kb left

[a] Position on minichromosome coordinate system; kb = kilobases from the sc^8 breakpoint (see Figure 4). No change was detected in the structure of the new insertion in all strains tested.

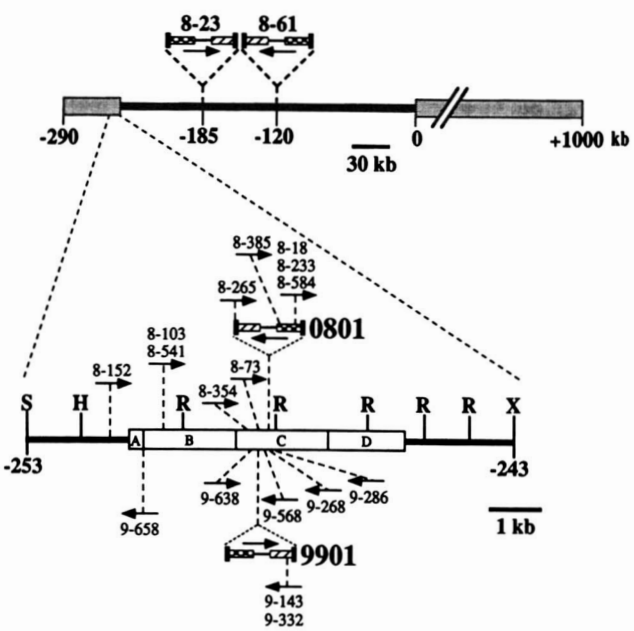

FIGURE 4.—Location of *PZ* insertions on *Dp1187*. The approximate location and orientation of the *PZ* insertions, determined by pulsed-field and conventional Southern analyses (see MATERIALS AND METHODS and Figures 2 and 3), is shown. The locations and orientations of insertions within the subtelomeric hotspot are displayed below. Numbers indicate strain name. Arrow points toward the 5′ *P* end of the *PZ* transposon. Refer to the Figure 2 legend for all other symbols.

Unusual *yellow*$^+$ variegation associated with a terminal deficiency: The original 8-61 line was unusual, in that a fraction of rosy$^+$ progeny variegated strongly for *yellow*$^+$. Two new bands of 84 and 200 kb were observed (Figure 3) when 8-61 DNA was analyzed on pulsed-field gels. Outcrossing demonstrated that these bands were present on two different minichromosomes, which were subsequently purified in separate stocks. The 200-kb band (8-61A) was associated with a terminal deficiency whose endpoint was located just proximal to the position of the starting element at coordinate −240. This chromosome, which contained no *P* element sequences or *ry*$^+$ gene, but which expressed *y*$^+$ normally, was retained only because of its presence in the same F_2 male as the *Dp8-61B* chromosome. In contrast, the other minichromosome, *Dp8-61B*, possessed a new insertion at coordinate −120, corresponding to the 84-kb *Sfi*I band, and was itself terminally deleted just distal to the new *P* element. The expression of the *y*$^+$ gene on the *Dp8-61B* chromosome strongly variegated in all individuals, possibly due to an effect of the terminal deficiency. Expression of *y*$^+$ was characterized by small patches of *y*$^+$ tissue on the abdomen, leading to the name: *yellow*^{leopard}.

Local transposition from the *cactus* locus-"reversion-jumping": Both the enhanced rate and clustered target sites of the transpositions seen in the previous experiments might have been influenced by the location of the starting elements in a *P* element "hotspot." To examine whether an elevated level of "local" transpositions could be recovered at a more typical euchromatic location, we analyzed the 35F region on chromosome *2L* that includes the genes *cactus* (*cact*), *cornichon* (*cni*) and *chiffon* (*chif*) (SCHÜPBACH and

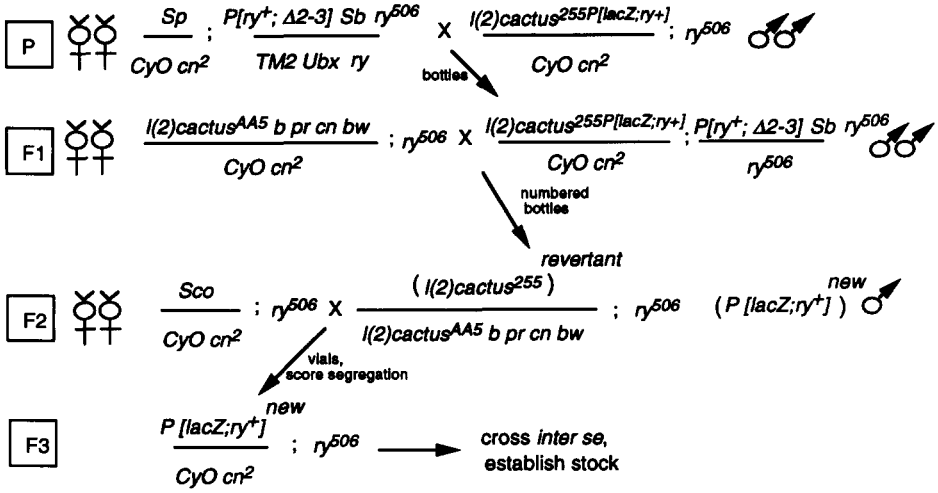

FIGURE 5.—Genetic screen used to detect local *FZ* transposition on chromosome *2* in the male germline by "reversion jumping." For description of genetic symbols, see LINDSLEY and ZIMM (1992). In the progeny of the F_1 cross, potential local hops were also recovered in females. These females were mated to *Sco/CyO; ry*^{506} males, and individual *P[lacZ,ry*$^+$*]/CyO; ry*^{506} male progeny were crossed to *Sco/CyO; ry*^{506} virgins, as in the F_2 cross. Further details are described in the text.

WEISCHAUS 1989; ASHBURNER *et al.* 1990). Since an insertion causing a weak eye color phenotype was not available, the strategy employed previously for recognizing potential transpositions could not be used.

We therefore devised an alternative approach termed "reversion-jumping" (Figure 5). A lethal *FZ* insertion in the *cactus* gene, *cact*^{255}, was mobilized by crossing to the Δ2-3(99B) transposase source. Males containing both Δ2-3 and *cact*^{255} were crossed to females containing an EMS-induced lethal cactus mutation, *cactus*^{AA5}, that is not marked with *ry*$^+$ (cross F_1). Flies lacking a second chromosome balancer in the next generation (F_2) must have reverted the *cact*^{255} mutation, and therefore are likely to be ry$^+$ only if they contain a new *FZ* insertion. Those lines whose new elements segregated with the second chromosome were identified by scoring the progeny of the F_2 cross, and were retained for further study. A similar reversion-jumping experiment was performed to determine whether local jumping would occur in the female germline (Figure 6). Revertants containing new *FZ* inserts were identified by scoring the segregation of *ry*$^+$ with the second chromosome in the progeny of the F_2 cross.

The results of these experiments are summarized in Table 3. The observed *cact*^{255} reversion frequency was 15% in the male germline and 23% in the female germline. Two hundred and fifty-one F_2 revertants recovered from the male germline experiment were rosy$^+$, and thus were likely to contain a new insertion (Figure 5). In the female germline experiment, all 350 F_2 revertants were initially ry$^+$, due to the *ry*$^+$ *TM2 Ubx* balancer chromosome, so the presence of transpositions could not be scored (Figure 6). However, marker segregation in the next generation indicated that 109 of the presumptive insertions recovered from

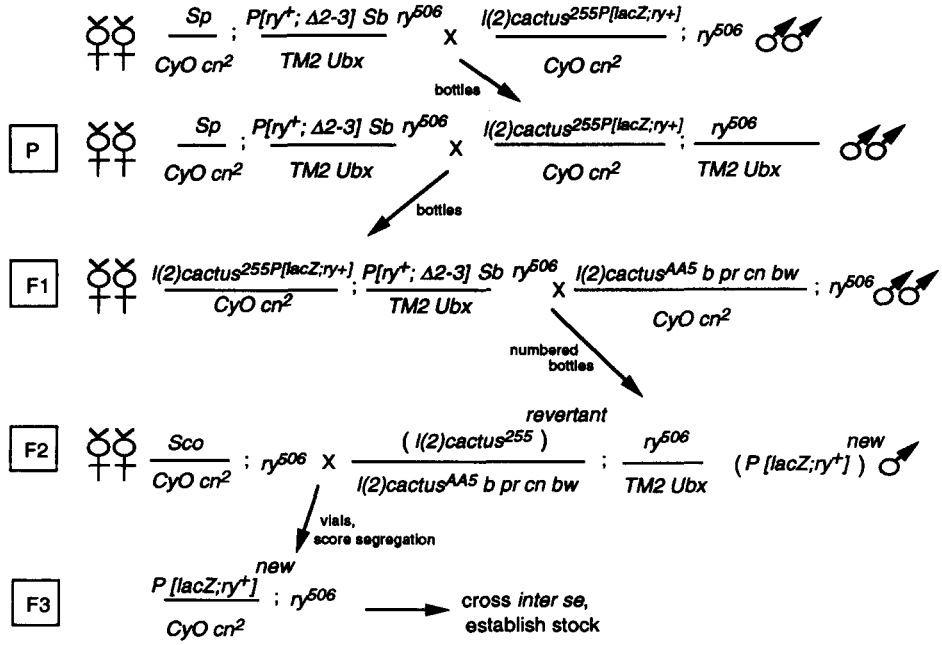

FIGURE 6.—Genetic screen used to detect local *FZ* transposition on chromosome *2* in the female germline by "reversion jumping." For this experiment, we decided to recover transpositions only from F_2 males. For description of genetic symbols, see LINDSLEY and ZIMM (1992). Further details are described in the text.

TABLE 3

***FZ* transpositions from *cact*^{255} in male and female germlines**

Sex	Chromosomes tested[a]	*cact*^{225} revertant[b]	*rosy*$^{+}$ sublines[c]	Insertions on II[d]	Local insertions in sample[e]	Total local insertions[f]
Male	13,800	2,030 (15%)	251	109	4	26 (0.2%)
Female	1,500	350 (23%)	ND	31	2	6 (0.3%)

[a] The total number of second chromosomes that would have been recovered over *cact*^{AA5} in F_2 flies lacking Δ2-3 if all the *cact*^{225} chromosomes had reverted; calculated as ½ of the F_2 progeny heterozygous for CyO and lacking Δ2-3, based on counts of 10 of 217 bottles (male experiment), or 9 of 75 bottles (female experiment). In the female experiment, only chromosomes recovered in males were analyzed.

[b] The number of *cact*^{225} chromosomes that were recovered over *cact*^{AA5} in F_2 flies lacking Δ2-3, based on counts of 10 of 217 bottles (male experiment), or 9 of 75 bottles (female experiment); percentages are of total second chromosomes analyzed (second column).

[c] The number of revertant chromosomes recovered in rosy^{+} flies, indicating a possible new transposition; in the female experiment all revertants were rosy^{+} because of the presence of the TM2 balancer chromosome; ND = not determined.

[d] The number of revertant chromosomes containing a rosy^{+} insertion on chromosome II based on F_3 segregation analysis.

[e] The number of local insertions (*i.e.*, within 50 kb of the *cact*^{225} insertion site or in band 35F) recovered among the subset analyzed: 60 lines studied by marker rescue and Southern hybridization (30 from male germline experiment and 30 from female germline experiment), and 13 lines (containing 15 total insertions) examined by *in situ* hybridization to polytene chromosomes (9 from male germline experiment and 4 from female germline experiment, see Table 4).

[f] The total number of revertant chromosomes containing local insertions as extrapolated from the sample group (see MATERIALS AND METHODS); percentages are of total second chromosomes analyzed (second column).

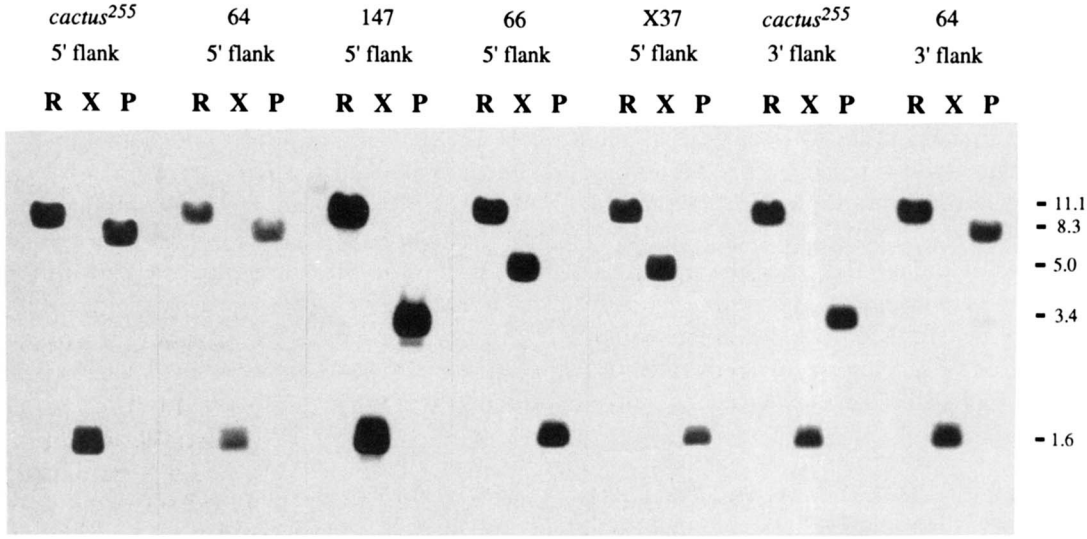

FIGURE 7.—Mapping the local *FZ* insertions within the region surrounding *cactus*. Cosmid cc10-1 DNA was digested with the indicated restriction enzyme, separated by electrophoresis and transferred to filters. Hybridization was with probes complementary to 5′ or 3′ genomic sequences flanking the new *FZ* inserts in the indicated strains. R, *Eco*RI; X, *Xba*I; *P*, *Pst*I. Fragment sizes (in kilobases) are shown to the right.

males and 31 likely new insertions recovered from females mapped to the second chromosome. The F_3 segregation analysis also mapped insertions on the third or *X* chromosome in about the proportions expected from the crossing schemes (data not shown).

Molecular analysis of revertant chromosomes: Lines containing new second chromosome insertions located near the *cact*^{255} starting site were identified using a direct hybridization test. Phage clones (provided by DAVID MARCEY) and newly selected cosmid clones (data not shown) defined approximately 100 kb of DNA surrounding the *cact*^{255} insertion. *P* element internal cloning sequences were used to marker rescue genomic DNA flanking the new insertions from 60 of the 140 candidate lines. 3′ Flanking sequences were also recovered from the *cactus*^{255} and #64 lines, by inverse PCR amplification. Labeled flanking DNA was then hybridized to slot-blots containing DNA from the genomic walk (data not shown).

Among 60 lines whose 5′ flanking DNA was recovered and tested, four hybridized to the test panel of DNA, all within approximately 10 kb of the starting site. These inserts were subsequently mapped in greater detail by hybridizing the same probes to blots of digested cosmid cc10-1 DNA, which contains the *cactus* gene and flanking regions (Figure 7). The sizes of the rescued 5′ flanking fragments indicated the distance from the 5′ end of the *P* element to the nearest *Xba*I site, and the comparison of these data allowed the location and orientation of the 5′ ends of

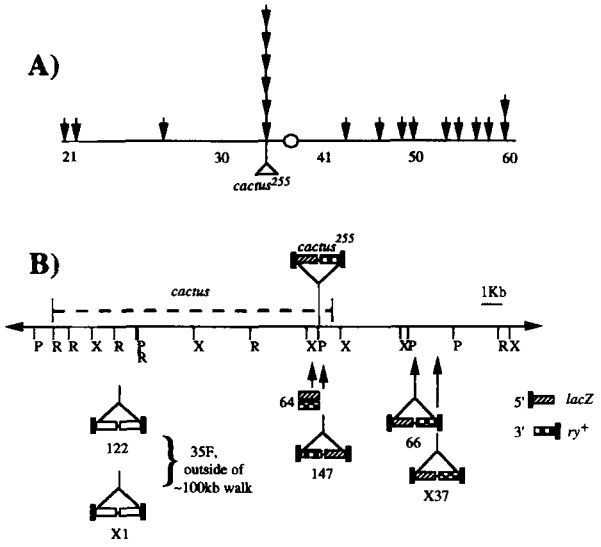

FIGURE 8.—Summary map of transpositions recovered following the mobilization of *cact*^{255}. A, Sites of insertion mapped by *in situ* hybridization. The entire second chromosome comprising polytene regions 21–60 is shown. The location of the starting element in *cact*^{255} is indicated by a triangle. Downward pointing arrows indicate the location of insertions within lines selected as described in the text. B, Molecular mapping of insertions near *cactus*. A restriction map of the genomic DNA surrounding the *cact*^{255} insertion (from cosmid cc10-1) is indicated at the top. The centromere is to the right. The approximate location of the *cactus* gene is indicated by a dashed line. The triangles indicate the location of new insertions mapped within the indicated strains. Two lines, 122 and X1, mapped cytologically to the polytene band containing *cactus* (35F), but were located outside the cloned region (orientation not determined). The orientation and structure of the *P* elements is diagrammed as described in Figure 2. P, *Pst*I; R, *Eco*RI; X, *Xba*I.

the new insertions to be determined. These assignments were then confirmed by hybridizing a *P* element 5′ end specific probe to a blot of *Eco*RI- and *Pst*I-digested genomic DNA from each line, which indicated the distance from the *P* element 5′ end to the nearest *Pst*I and *Eco*RI sites. The position of the inserts is summarized in Figure 8B and Table 4).

The line 64 chromosome was found to have undergone both insertion and rearrangement. Single 5′ and 3′ *P* element ends were detected near the starting site (Figure 7). However, the DNA structure surrounding the insertion(s) was altered (data not shown). Examination of polytene chromosomes indicated that line 64 contained a large chromosomal deletion extending from the 35F/36A border to 36D. The 64 chromosome failed to complement *chi* and *l(2)35Fe*, as well as the more proximal mutations *dachsund* and *Df(2L)H20*. Preliminary molecular analysis of the 64 line with probes from the *cactus* region genomic walk indicated that the breakpoint at 35F/36A was ≥50 kb proximal to *cact* (G. LANDIS and J. TOWER, unpublished data). The deficiency may have resulted from a second local transposition event to the 35F/36A border, followed by a deletion that removed the element.

***In situ* hybridization mapping:** The plasmid rescue hybridization analysis showed that 56 of 60 lines tested did not contain insertions within the 100 kb walk. However, localization of the insertions in 13 of these lines by *in situ* hybridization to polytene chromosome squashes (Table 4 and Figure 8A) revealed that two of them, lines 122 and X1, contained insertions at 35F, the cytogenetic locus of *cactus*. Because the walk extends ~50 kb in either direction from the *cact* gene, these inserts must be located ≥50 kb distant from the site of the original *cact*^{255} insert. The other insertions were distributed along the second chromosome (Figure 8A), with no apparent preference for regions close to 35F.

These data allowed the frequency of local jumping in these experiments to be estimated (Table 3). Extrapolating from the lines analyzed by plasmid rescue and/or *in situ* hybridization (see MATERIALS AND METHODS), an estimated 26 local insertions occurred in the male germline, while about 6 took place in the female germline. The rates of local transposition recovered by reversion jumping were therefore lower than the rates detected previously by eye color changes, 0.2–0.3% compared to about 1%. However the fraction of second chromosome transpositions that were local was substantial, 32/140 = 23%.

DISCUSSION

***P* elements transposed at elevated frequencies near their starting sites:** *P* elements mobilized at both the minichromosome tip and at *cactus*, like the maize *Ac* element, transposed more frequently to nearby chromosomal sites than was expected from previous analyses of intrachromosomal transpositions. Following activation of the 0801 or 9901 *P* elements, approximately 1% of tested chromosomes contained new insertions located within 128 kb of the starting site. This frequency was about 67-fold higher than expected, since only about 0.015% of the progeny minichromosomes received a transposition from the *X* chromosome in a similar experiment (KARPEN and SPRADLING 1992) (see MATERIALS AND METHODS). Another way to measure the enhancement yielded similar results. Only 42 of 7,825 lines containing transpositions derived from the *X* chromosome harbored new minichromosome insertions (KARPEN and SPRADLING 1992), compared to 36 of 156 new insertion lines derived from 0801 or 9901 (Table 1), an enhancement of (36/156) × (7,825/42) = 43-fold. The local transposition rate was also increased in the 35F region following mobilization of *cact*^{255}. Extrapolation from the test samples suggested that 23% (32/140) of new second chromosome insertions were located in 35F. This is 55-fold higher than the percentage of chromosome two that is represented by 35F (1/240 letter divisions = 0.42%). There is no evidence that the 35F

TABLE 4

Transpositions from *cact*[255]

Strain[a]	Cytogenetic position[b]	Orientation[c]	Comments
X1	35F	ND	Jump >50 kb from starting element
X37	35F	←	Jump 3-4 kb 3′ to starting element
64	35F	ND	Jump near 5′ end of starting element
66	35F	←	Jump 3 kb 3′ to starting element
122	35F	ND	Jump >50 kb from starting insert
147	35F	→	5′ head-to-head double *P* element
74-A	21A	ND	
74-B	22B	ND	
X8	26A	ND	
85	43E	ND	
X7	45C	ND	
44-A	47B	ND	
38	48A	ND	
X50	53D	ND	
15	54F	ND	
40	56E	ND	
44-B	57F	ND	
79	58E	ND	
141	58E	ND	

[a] -A and -B denote different insertion sites present on a single second chromosome.
[b] Cytogenetic location of the *FZ* insertion on the polytene chromosome map (see Figure 8A).
[c] Orientation of the transposed FZ element near *cactus* on chromosomes *2L*: the arrowhead indicates the 5′ *P-lacZ* end of the element, while the chromosome *2L* centromere is assumed to lie to the right, and the telomere to the left (see Figure 8B).

region is a hotspot for *P* element insertions from distant sites based on the positions of insertions previously mapped by *in situ* hybridization (unpublished data).

In the reversion jumping experiments, the rate of local transposition in males per chromosome tested (0.2%, Table 3) was about 5-fold lower than was observed by analyzing eye color changes (1%). This lower rate was expected because only a fraction of the local insertions are likely to be recovered by the reversion jumping method. New insertions in lines where the starting element was retained, and local insertions within essential *cactus* sequences, would not have been detected because of the requirement for reversion of the *cactus* mutation. In the minichromosome experiments reported here and in a companion study (ZHANG and SPRADLING 1993), the great majority (>95%) of local insertions were recovered on chromosomes that retained the starting *P* element. The larger fraction of insertions associated with reversion of the starting element at *cactus* probably reflected a greater accessibility of a wild-type homolog than was available to the minichromosome (ENGELS *et al.* 1990).

Mechanism of local transposition: Relatively little is known about the molecular mechanism of *P* element transposition (RIO 1990). Any proposed mechanism must account for the observations reported here that a substantial fraction of transpositions (10–25%) traverse only a small distance from their starting site, and are constrained in their orientation and direction of movement along the chromosome. It could be argued that the direction and orientation effects observed were caused at least in part by some property of the tandemly repeated 1.8-kb sequence surrounding the 0801 and 9901 starting elements. However, insertions recovered in the hotspot that originated at a distant site occurred with equal frequency in both orientations (KARPEN and SPRADLING 1992). Furthermore, additional evidence for local transpositions with nonrandom properties is presented in a companion study (ZHANG and SPRADLING 1993).

Terminal deficiencies: Other types of events in addition to new transpositions were recovered from the genetic screens. Mobilization of the 0801 or 9901 insertions produced a very high frequency of terminal deletions, that were recovered by virtue of the increased expression of the *rosy*$^+$ gene that accompanied the deletion of the distal, subtelomeric DNA. Approximately 3% of all progeny contained terminal deletions near the distal end of the starting *P* element (the actual endpoint varied in a fraction of the lines). Comparison of the results with 0801 and 9901 suggested that even this high rate may underestimate the frequency of these events. Although the starting elements were oppositely oriented, a similar frequency of deletions was recovered at their distal ends (5′ for 0801, 3′ for 9901). If terminal deletions can occur equally well at both ends of the starting element, then half the time the *Dp1187* terminus and the entire *P* element would be removed, preventing their detec-

tion based on *rosy*$^+$ expression. Indeed, this is just the structure found on the fortuitously recovered *Dp8-61A* chromosome. Thus, the actual frequency of terminal deletions may have been twice as high as the 3% that were detected.

Two models were considered to explain the structure and high frequency of the observed terminal deletions. The first model assumes that terminal deletions result from some special interaction between two closely linked *P* elements. Germline cells that sustained local transposition events that created double *P* elements might frequently undergo a secondary, transposase-catalyzed reaction creating a deleted chromosome. One test of the model would be to remobilize an existing double *P* element (for example, lines 8-73 or 9-268) and look for an elevated rate of terminal deletions. The second model postulates that terminal deletions arise when the gap generated by element excision (ENGELS *et al.* 1990) is incompletely repaired from the sister strand or homolog. If only the proximal free end successfully invaded, deletions would be produced that end at some point within or beyond the element (as observed) depending on how long the conversion tract progressed. If only the distal free end successfully invaded, the deletions would include the entire *P* element and whatever proximal sequences were removed by exonuclease prior to stabilization of the free end. As mentioned above, this class of deletions would not have been recovered in our scheme, but in principle is experimentally accessible.

The *Dp8-61B* terminal deletion was unusual because it was associated with a novel position effect on the *yellow*$^+$ locus. The small spots of yellow$^+$ cuticle that were characteristic of this allele were likely to be caused by a variegating position effect (rather than mitotic chromosome loss) since pigmentation responded as expected to the addition or removal of a *Y* chromosome (data not shown). The breakpoint at position −120 was located nearly 100 kb distal to y^+, and several genes reside in the intervening region. No other alterations in the chromosome were detected on pulsed-field gels. Thus, strong position-effect variegation appeared to result not from juxtaposition with heterochromatin, but from the removal of the normal telomeric and subtelomeric sequences of the chromosome. The telomeric sequences appeared to suppress variegation associated with the euchromatin-heterochromatin junction at position 0 on the minichromosome, 20 kb from the y^+ gene (see KARPEN and SPRADLING 1990). Although it could be argued that the increased variegation resulted from some change in the centromeric region that accompanied the terminal deletion, and that was not detected on the pulsed-field gels, this interpretation is ruled out by the independent recovery of terminal deletions with similar phenotypes as reported in the accompanying paper (ZHANG and SPRADLING 1993).

Local transposition is likely to be useful to enhance mutagenesis rates in selected genomic regions: The rate of insertional mutagenesis increased 43–67-fold in the region surrounding the starting elements in both the *cactus* and *Dp1187* local jumping experiments. The low single-locus mutation rates currently achieved using interchromosomal single *P* element insertional mutagenesis can therefore be enhanced by selecting a starting element located within a short distance (100 kb?) of the region to be targeted. This will be true whether the target of interest is defined genetically by a point mutation, or only by its expression pattern or molecular structure.

Identifying new insertions in the possible presence of the starting element, is unlikely to prove a major obstacle. Scoring for increased expression of a marker gene, as in the experiments with 0801 and 9901, is likely to be simple and efficient whenever a starting element with appropriate properties is available in the region of interest. While *rosy*$^+$ transposons rarely are subject to the strong position effect seen in the 0801 and 9901 lines, transposons containing the mini-*white* gene often provide only a weak white+ phenotype that is sensitive to additional copies elsewhere in the genome (for example, see GOLIC and LINDQUIST 1989). It may also be possible to directly select for increased *rosy*$^+$ expression by adding an appropriate amount of purine to the medium (GELBART, MCCARRON and CHOVNICK 1976). Alternatively, molecular screening methods, such as the PCR-based methods reported by BALINGER and BENZER (1989) and KAISER and GOODWIN (1990), could be used to screen directly for insertions within adjacent cloned regions.

The "reversion jumping" method provides another approach with some distinct advantages. First, the new insertions will more frequently be recovered free of the starting element than using the other methods (which may require retention of the starting element in order to increase marker gene dosage). Although both singly and doubly inserted chromosomes can be useful, the original element may have to be removed before the enhancer trap pattern of the new insertion can be interpreted, and before it can be used to generate additional alleles.

Second, although the rate of local insertions recovered by reversion jumping was lower, for many applications a larger fraction are likely to be useful. Four of six characterized events were greater than 2 kb from the starting site (*vs.* 6 of 36 in the case of 0801 and 9901). In fact, 33% (2/6) of the events generated by reversion jumping were located >50 kb from the starting element, compared to only 6% (2/36) isolated in the *Dp1187* experiments. In the minichromosome experiments, many insertions resided close to or within the original element; these events

probably would not revert a lethal phenotype associated with the starting element and were presumably selected against by the reversion jumping scheme.

Third, the materials needed to apply this method are more widely available than insertions with weak marker gene expression. The reversion jumping scheme should be applicable to all *P* element insertions which cause a lethal or visible phenotype, and which are capable of reversion and transposition. A large number of lethal insertions on the autosomes have been recovered (KARPEN and SPRADLING 1992). The other reagent that is required for the reversion jumping procedure is an allele of the starting gene which is not marked with the *P* element marker gene. If necessary, this can be generated from the marked *P* element allele, by imprecise excision.

In conclusion, both of the local transposition methods reported in this paper are likely to be of use in targeting *P* element insertions to specific regions of the genome. The choice of method will depend upon the reagents available for that region. In the accompanying paper, ZHANG and SPRADLING (1993) describe the generation of a large number of *Dp1187* local transpositions in the absence of any selection. This data set provides additional information about the distribution and mechanism of local transposition.

We thank YASUSHI HIROMI and SIEGFREID ROTH for strains, DAVID MARCEY for *cactus* region DNAs, and DAVID MARCEY, ROBERT GEISLER, A. BERGMANN, YASUSHI HIROMI and CHRISTIANNE NÜSSLEIN-VOLHARD for unpublished information. We thank D. STERN and D. THOMPSON-STEWART for expert technical assistance. This work was supported in part by U.S. Public Health Service grant GM27875. G.K. was supported by a National Research Service Award of the U.S. Public Health Service and by the Howard Hughes Medical Institute. J.T. was supported by the Helen Hay Whitney Memorial Foundation and by the Howard Hughes Medical Institute. N.C. was supported by a Scholar Grant from the American Cancer Society and was on sabbatical leave from the Department of Microbiology and Immunology, the George W. Hooper Foundation, University of California, San Francisco.

LITERATURE CITED

ASHBURNER, M., 1990 *Drosophila: A Laboratory Handbook*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

ASHBURNER, M., P. THOMPSON, J. ROOTE, P. F. LASKO, Y. GRAU, M. EL MESSEL, S. ROTH and P. SIMPSON, 1990 The genetics of a small autosomal region of *Drosophila melanogaster* containing the structural gene for alcohol dehydrogenase. VII. Characterization of the region around the *snail* and *cactus* loci. Genetics **126:** 679–694.

ATHMA, P., E. GROTEWOLD and T. PETERSON, 1992 Insertional mutagenesis of the maize P gene by intragenic transposition of *Ac*. Genetics **131:** 199–209.

BALINGER, D., and S. BENZER, 1989 Targeted gene mutations in Drosophila. Proc. Natl. Acad. Sci. USA **86:** 9402–9406.

BARAN, G., C. ECHT, T. BUREAU and S. WESSLER, 1992 Molecular analysis of the maize *wx-B3* allele indicates that precise excision of the transposable Ac element is rare. Genetics **130:** 377–384.

BENDER, W., P. SPIERER and D. S. HOGNESS, 1983 Chromosome walking and jumping to isolate DNA from the *Ace* and *rosy* loci and the *bithorax* complex in *Drosophila melanogaster*. J. Mol. Biol. **168:** 17–33.

BIESSMANN, H., and J. M. MASON, 1988 Progressive loss of DNA sequences from terminal chromosome deficiencies in *Drosophila melanogaster*. EMBO J. **7:** 1081–1086.

COOLEY, L., C. BERG and A. SPRADLING, 1988 Controlling *P* element insertional mutagenesis. Trends Genet. **4:** 254–258.

COOLEY, L., R. KELLEY and A. SPRADLING, 1988 Insertional mutagenesis of the Drosophila genome with single *P* elements. Science **239:** 1121–1128.

DOONER, H. K., and A. BELACHEW, 1989 Transposition pattern of the maize element Ac from the bz-m2(Ac) allele. Genetics **122:** 447–457.

DOWE, M. F., G. W. ROMAN and A. S. KLEIN, 1990 Excision and transposition of two *Ds* transposons from the *bronze* mutable 4 derivative 6856 allele of *Zea mays* L. Mol. Gen. Genet. **221:** 475–485.

EGGLESTON, W.B., 1990 P element transposition and excision in Drosophila: interactions between elements. Ph.D. Thesis, University of Wisconsin, Madison.

ENGELS, W., 1989 *P* element in *Drosophila melanogaster*, pp. 437–484 in *Mobile DNA*, edited by D. BERG and M. HOWE. American Society for Microbiology, Washington, D.C.

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

GELBART, W., M. MCCARRONN and A. CHOVNICK, 1976 Extension of the limits of the XDH structural element in *Drosophila melanogaster*. Genetics **84:** 211–232.

GOLIC, K. G., and S. LINDQUIST, 1989 The FLP recombinase of yeast catalyzes site-specific recombination in the Drosophila genome. Cell **59:** 499–509.

GREENBLATT, I. M., 1984 A chromosome replication pattern deduced from pericarp phenotypes resulting from movements of the transposable element, modulator, in maize. Genetics **108:** 471–485.

GREENBLATT, I. M., and R. A. BRINK, 1962 Twin mutations in medium variegated pericarp maize. Genetics **47:** 489–501.

HAMILTON, B. A., M. J. PALAZZOLO, J. H. CHANG, K. VUAYRAGHAVAN, C. A. MAYEDA, M. A. WHITNEY and E. M. MEYEROWITZ, 1991 Large scale screen for transposon insertion into cloned genes. Proc. Natl. Acad. Sci. USA **88:** 2731–2735.

HAWLEY, R. S., R. A. STEUBER, C. H. MARCUS, R. SOHN, D. M. BARONAS, M. L. CAMERON, A. E. ZITRON and J. W. CHASE, 1988 Molecular analysis of an unstable *P* element insertion at the singed locus of *Drosophila melanogaster*: evidence for intracistronic transposition of a *P* element. Genetics **119:** 85–94.

KAISER, K., and S. F. GOODWIN, 1990 "Site-directed" transposon mutagenesis of Drosophila. Proc. Natl. Acad. Sci. USA **87:** 1686–1690.

KARPEN, G. H., and A. C. SPRADLING, 1990 Reduced DNA polytenization of a minichromosome region undergoing position-effect variegation in Drosophila. Cell **63:** 97–107.

KARPEN, G. H., and A. C. SPRADLING, 1992 Analysis of subtelomeric heterochromatin in a Drosophila minichromosome *Dp1187* by single *P* element insertional mutagenesis. Genetics **132:** 737–753.

KERMICLE, J. L., M. ALLEMAN and S. L. DELLAPORTA, 1989 Sequential mutagenesis of a maize gene, using the transposable element *Dissociation*. Genome **31:** 712–716.

LEVIS, R., 1989 Viable deletions of a telomere from a Drosophila chromosome. Cell **58:** 791–801.

LEVIS, R., T. HAZELRIGG and G. M. RUBIN, 1985 Effects of genome position on the expression of transduced copies of the *white* gene of Drosophila. Science **229:** 558–561.

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

MORENO, M. A., J. CHEN, I. GREENBLATT and S. L. DELLAPORTA, 1992 Reconstitutional mutagenesis of the maize P gene by short-range *Ac* transpositions. Genetics **131:** 939–956.

MLODZIK, M., and Y. HIROMI, 1992 The enhancer trap method in Drosophila: its application to neurobiology, pp. 397–414 in *Gene Expression in Neural Tissues* (Methods in Neuroscience 9), edited by P. M. CANN. Academic Press, Orlando, Fla.

O'KANE, C. J., and W. J. GEHRING, 1987 Detection *in situ* of genomic regulatory elements in Drosophila. Proc. Natl. Acad. Sci. USA **84:** 9123–9127.

OSBORNE, B. I., C. A. CORR, J. P. PRINCE, R. HEHL, S. D. TANKSLEY, S. MCCORMACK and B. BAKER, 1991 *Ac* transposition from a T-DNA can generate linked and unlinked clusters of insertions in the tomato genome. Genetics **129:** 833–844.

PIROTTA, V., 1986 Cloning Drosophila genes, pp. 83–110 in *Drosophila: A Practical Approach*, edited by D. B. ROBERTS. IRL Press, Oxford.

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

RIO, D. C., 1990 Molecular mechanisms regulating Drosophila *P* element transposition. Annu. Rev. Genet. **24:** 543–578.

ROIHA, H., G. M. RUBIN and K. O'HARE, 1988 *P* element insertions and rearrangements at the singed locus of *Drosophila melanogaster*. Genetics **119:** 75–83.

ROTH, S., Y. HIROMI, D. GODT and C. NÜSSLEIN-VOLHARD, 1991 *cactus*, a maternal gene required for proper formation of the dorsoventral morphogen gradient in *Drosophila* embryos. Development **112:** 371–388.

SAMBROOK, J., E. F. FRITSCH and T. MANIATIS, 1989 *Molecular Cloning: A Laboratory Manual*, Ed. 2. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

SALZ, H. K., T. W. CLINE and P. SCHEDL, 1987 Functional changes associated with structural alternations induced by mobilization of a *P* element inserted in the sexlethal gene of Drosophila. Genetics **117:** 221–231.

SCHÜPBACH, T., and E. WIESCHAUS, 1989 Female sterile mutations on the second chromosome of *Drosophila melanogaster*. I. Maternal effect mutations. Genetics **121:** 101–117.

VAN SHAIK, N. W., and R. A. BRINK, 1959 Transposition of modulator, a component of the variegated pericarp allele in maize. Genetics **44:** 725–738.

WEIL C. F., S. MARILLONNET, B. BURR and S. R. WESSLER, 1992 Changes in state of the *Wx-m5* allele of maize are due to intragenic transposition of *Ds*. Genetics **130:** 175–185.

ZHANG, P., and A. C. SPRADLING, 1993 Efficient and dispersed local P element transposition from Drosophila females. Genetics **133:** 361–373.

Communicating editor: T. SCHÜPBACH