Efficient and Dispersed Local *P* Element Transposition From Drosophila Females

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ABSTRACT

We have investigated how Drosophila *P* element insertions are distributed in the chromosomal region near their starting site. A single *P* element residing in the euchromatin of minichromosome *Dp1187* was mobilized following a cross to the *Δ2-3 (99B)* strain, and progeny bearing transpositions were identified with a minimum of bias by performing Southern blots on progeny. Approximately 1–2% of all progeny minichromosomes contained new insertions. Many of these "local transpositions" landed very close to or within the starting *P* element; however, nearly 1% of all progeny chromosomes contained new insertions 1–180 kb from the donor element. More local insertions were observed in the progeny of females than from male parents, and most occurred in a preferred orientation relative to the starting element. These observations suggested that donor elements are frequently excised and reinserted locally without ever dissociating from a transposition complex. The high frequency and diverse distribution of local transpositions recovered from females suggested that the efficiency of insertional mutagenesis can be significantly enhanced by using a starting *P* element(s) located near the target of interest.

P elements have been widely used to tag previously known genes subsequent to cloning (ROBERTSON *et al.* 1988) and to mutagenize genes defined only by molecular criteria (HAMILTON *et al.* 1991). The ability to use single *P* elements as a mutagen has also made it possible to systematically identify new genes by insertional mutagenesis (COOLEY, KELLEY and SPRADLING 1988). The strains produced in such experiments, containing a single, engineered *P* element disrupting a gene of interest, are particularly useful for subsequent genetic and molecular studies. However, currently used methods for insertionally mutagenizing a particular gene or chromosome region introduce multiple *P* elements in order to increase the mutation rate (ROBERTSON *et al.* 1988; BALLINGER and BENZER 19989; KAISER and GOODWIN 1990; HAMILTON *et al.* 1991).

We have undertaken a series of studies attempting to overcome this problem. In a companion study (TOWER *et al.* 1993), *P* elements located in two different chromosome regions were shown to transpose at greatly elevated rates into sites near their starting positions. This predilection was reminiscent of the well established tendency of the maize *Ac/Ds* element to integrate near its site of origin (VAN SCHAIK and BRINK 1959). The current study was designed to address a number of questions about local *P* element transposition. These included the frequency of local jumps, the distance distribution they displayed around the donor site, whether the sequence and chromosome location of the starting site influenced the mobilized *P* elements, and whether local transpositions occurred with equal frequency in the male and female germlines. Insight into the mechanism that causes the local insertion preference was also sought.

In this report we present experiments that addressed these questions. Insertions in the 1A region of the *X* chromosome were studied, a genetically well characterized section containing eight known genes (FLEMING, DESIMONE and WHITE 1989; LINDSLEY and ZIMM 1992). To facilitate the analysis, this chromosome region was present on a minichromosome, *Dp(1;f)1187* [hereafter *Dp1187*; see LINDSLEY and ZIMM (1992)]. *Dp1187* contains 290 kb from the tip of the *X* chromosome joined at the position of the $In(1)sc^8$ inversion breakpoint to 1000 kb of the *X* centromeric heterochromatin (KARPEN and SPRADLING 1990, 1992). New insertions anywhere on the minichromosome could be unambiguously identified and mapped by pulsed-field gel electrophoresis. For the first time, these studies report the frequency and distribution of insertions from a defined starting site in the absence of any selection. In conjunction with a companion study (TOWER *et al.* 1993), our results demonstrated that *P* elements located at several different sites preferentially transpose locally and that this property is likely to extend the usefulness of single *P* element insertional mutagenesis.

MATERIALS AND METHODS

Drosophila stocks: Flies were grown on standard corn meal/agar media (ASHBURNER 1990), at 22°. Strains and

mutations were described in LINDSLEY and ZIMM (1992), unless stated otherwise. However, the map of genes in the 1A region used in this paper does not incorporate the unpublished complementation data alluded to in LINDSLEY and ZIMM (1992), because we had no way to evaluate its accuracy. The *Dp1187* minichromosome and its derivative *Dp8002* which carries a nonautonomous *P[lacZ, ry$^+$]* element called *PZ* (MLODZIK and HIROMI 1992) at position −68, were described in KARPEN and SPRADLING (1990, 1992). A coordinate system for the minichromosome was used in which the left telomere is −290 kb, the *sc^8* breakpoint is 0 kb, and the heterochromatic right telomere is +1000 kb. The structure of the 120 kb of *Dp1187* distal to the *sc^8* breakpoint is very similar to the corresponding region of the normal *X* chromosome studied by CAMPUZANO *et al.* (1985) and by FLEMING, DESIMONE and WHITE (1989); however, a few small changes exist, including insertion/deletion polymorphisms (see KARPEN and SPRADLING 1990). The approximate position on the coordinate system used by CAMPUZANO *et al.* (1985) can be obtained by adding 45 kb to the absolute value of the *Dp1187* coordinate.

Transposition of the *P* element on *Dp8002*: The schemes used to generate local transpositions of the *PZ* element present on *Dp8002* in males and females are described in the text (see Figure 1) and were similar to previous single element mutation screens (COOLEY, KELLEY and SPRADLING 1988; BERG and SPRADLING 1991; KARPEN and SPRADLING 1992). To recover transpositions in males, single *y/Y ; Dp8002 ; Sb Δ2-3 ry/ry^{506}* males were crossed to *y ; ry^{506}* females in vials, and 1–8 sublines established from the progeny of each vial. Sublines established from the same male parent that contained identical minichromosome derivatives were assumed to represent a single event produced by a premeiotic cluster. To recover transpositions in females, *y ; Dp8002 ; Sb Δ2-3 ry/TM6,ry* females were crossed to *y/Y ; ry^{506}* males in bottles, and multiple sublines established from individual male progeny. While this procedure did not rigorously eliminate premeiotic clusters, any derivatives with identical structure that derived from the same bottle were treated as a single event.

DNA probes: Six genomic DNA fragments used to map derivative chromosomes are shown schematically in Figure 2 [see also KARPEN and SPRADLING (1990, 1992)]. Probe 1 = pBS6.1RH2.5, the 2.5-kb *Eco*RI/*Hin*dIII fragment from cosmid p6.1 (map position −125 to −122.5); probe 2 = pBS6.1HR1.75, the 1.75-kb *Hin*dIII/*Eco*RI fragment from cosmid p6.1 (map position −104 to −102); probe 3 = pBS12.1RH9.0, the 9.0-kb *Eco*RI/*Hin*dIII fragment from cosmid 12.1 (map position −81 to −72); probe 4 = pBSTG3R3.5, the 3.5-kb *Eco*RI fragment from TG-3 (FLEMING, DESIMONE and WHITE 1989) (map position −57 to −54); probe 5 = pBSTG1BH2.4, the 2.4-kb *Bam*HI/*Hin*dIII fragment from TG-1 (FLEMING, DESIMONE and WHITE 1989) (map position −38 to −36); probe 6 = pBSsc101RX4.0, the 4.0-kb *Eco*RI/*Xba*I fragment from λsc^{101} (CAMPUZANO *et al.* 1985) (map position −8 to −4). To detect the *PZ* transposon, the 0.55-kb *Eco*RI/*Hin*dIII fragment from the 5′ end of the Drosophila *P* element (pBS5′PHR0.55), and the 3.2-kb *Eco*RI/*Hin*dIII fragment from the 3′ half of the rosy gene (pBS5′ryHR3.2) were used as probes (called "probe *P*" and "probe *ry*," respectively). The DNA fragments were purified from agarose gels using Geneclean (Bio 101) and labeled by the random primer method [see SAMBROOK, FRITSCH and MANIATIS (1989)].

Physical mapping of local transpositions: The minichromosome *Dp(1;f)1187* contains only two sites for the restriction enzyme *Not*I and one site for *Sfi*I both of which recognize eight base pair sequences. Since the *PZ* element itself contains two *Not*I and one *Sfi*I sites (see Figure 2), these sites could be used to position new insertions. To map new insertions, ovaries were dissected from adults that had been fed with wet yeast for 36 hr. High molecular weight DNA was then prepared in agarose inserts and digested with restriction enzymes (KARPEN and SPRADLING 1990, 1992). Digested DNA corresponding to about ten ovaries per lane were separated in 0.8% agarose gels and 0.5 × TBE buffer, by pulsed-field gel electrophoresis (SCHWARTZ and CANTOR 1984; CHU, VOLLRATH and DAVIS 1986). The molecular sizes of the detected bands were estimated from the sizes of concatenated lambda phage and yeast chromosomal DNAs.

Southern hybridization: After electrophoresis the DNA molecules were depurinated, denatured, neutralized and transferred to GeneScreen membranes (Dupont), according to the methods described in SAMBROOK, FRITSCH and MANIATIS (1989). Hybridization was carried out as described by CHURCH and GILBERT (1984).

RESULTS

Experimental strategy: Transpositions of specific Drosophila mobile elements have previously been detected using a variety of genetic methods. New insertions may change the expression of marker genes present on the element due to increased dosage or position effects (LEVIS, HAZELRIGG and RUBIN 1985; TOWER *et al.* 1993), cause altered segregation of transposon-associated markers relative to chromosomal markers (see COOLEY, KELLEY and SPRADLING 1988), create new phenotypes due to their site of insertion (HAWLEY *et al.* 1988) or if accompanied by excision, alter phenotypes associated with the original insertion (SPRADLING and RUBIN 1982; TOWER *et al.* 1993). All of these methods undoubtedly fail to detect some insertions, and their actual sensitivity is not generally known. In order to obtain the widest possible spectrum of events, we decided to use a physical rather than a genetic method to detect transpositions and any associated chromosomal alterations.

Two schemes were used to recover transpositions of a specific *P[lacZ, ry$^+$]* element hereafter called "*PZ*" (MLODZIK and HIROMI 1992) that was present at coordinate −68 kb in the *Dp1187* minichromosome derivative *Dp8002* (KARPEN and SPRADLING 1992) (see Figure 1). To study transpositions in the male germline, *Dp8002, PZ y$^+$; y ; ry^{506}* females were crossed to *y$^+$/Y ; Sb Δ2-3 ry/TM6* males, and 111 males of genotype *Dp8002, PZ y$^+$; y/Y; Sb Δ2-3 ry/ry^{506}* were collected and then mated singly to *y ; ry^{506}* females to generate individual lines. Sons carrying the *y$^+$* marked *Dp8002*, the *ry $^+$*-marked *PZ* element, and *ry^{506}* third chromosomes (*i.e.*, that lacked *Δ2-3 Sb*), were crossed singly to *y ; ry^{506}* females. Up to eight individual male progeny were used for each of the 111 single-mating crosses, yielding a total of 510 sublines containing potential minichromosome derivatives. Scoring the segregation of *yellow$^+$* and *rosy$^+$* in the subsequent

TABLE 1

Sublines derived from *P* element transpositions in male and female germlines

Sex	Progeny minichromosomes[a]	*rosy*$^+$ sublines analyzed	Local insertions[b]	Independent insertions[c]	Rearrangements	Transpositions
M	1900	510 (27%)	15	13 (0.7%)	0	60 (3%)
F	1150	1028 (89%)	28	21 (1.8%)	20 (1.7%)	48 (4%)

[a] The number of male progeny from the schemes shown in Figure 1 that contained a minichromosome and lacked Δ2-3 (*i.e.*, y^+ Sb^+); calculated based on sample counts.

[b] The number of lines containing a second insertion on the minichromosome; 2 lines from males and 7 lines from females were subsequently shown to have probably derived from premeiotic clusters.

[c] The number of lines containing new independent insertions on the second chromosome (see Tables 2 and 3).

generation allowed transpositions of *PZ* to other chromosomes to be recognized, and revealed that one or more *PZ* elements were still genetically linked to the minichromosome in 450 of these lines (Table 1). All these lines were further analyzed by Southern blotting (see below).

Several modifications to this scheme were made to recover transpositions from the female germ line (Figure 1B). *TM6, ry*, a third chromosome balancer, was introduced to suppresses meiotic recombination on the third chromosome and thus maintain the linkage between the *Δ2-3* element and the *Sb* marker. The *Dp8002, PZ* y^+ *; y ; Sb Δ 2-3 ry/TM6, ry* females were mass-mated to *y/Y;* ry^{506} males to generate sons carrying minichromosomes that had passed through the transposition-activated female germline. Interestingly, only about 10% of the sons of these *Dp8002, PZ* y^+*; y ; Sb Δ2-3 ry/TM6, ry* females were yellow$^+$ but rosy, indicating that the *P* element had precisely or imprecisely excised from the minichromosome. In contrast, 60–70% of the *Dp8002, PZ* y^+*; y/Y; Sb Δ23* ry/ry^{506} males in Figure 1A gave rise to yellow$^+$ but rosy sons, a significantly higher frequency of excision. A total of 1,028 *Dp8002,* y^+ *; y/Y;*ry^{506}*/TM6, ry* male progeny were used to establish temporary individual stocks by crossing them to *y ;* ry^{506} females. The inheritance of *yellow*$^+$ and *rosy*$^+$ in these lines revealed 48 transpositions to other chromosomes, as well as 980 lines in which y^+ cosegregated with ry^+, indicating that they carried minichromosomes with at least one *PZ* element (Table 1). The minichromosomes in these lines were subsequently analyzed as described below.

Identification of changes in *P* elements residing on the minichromosome: An initial test was done to identify new transpositions on *Dp8002*, and to determine if the junctions of the starting *P* element were altered. A map showing relevant restriction sites and probes used in these experiments is shown in Figure 2. Genomic DNA was isolated from adult males and digested with *Eco*RI (male germline screen) or *Eco*RI and *Xba*I (female germline screen). Southern blots of these DNA samples were labeled with probes specific for the 5′ and 3′ ends of the *PZ* element (see MATERIALS AND METHODS). Among 510 sublines derived from male germlines, 15 contained new bands using both 5′ and 3′ probes (Table 2). All of these lines retained one or both of the bands that flank the *Dp8002* insertion. Three of the sublines with new bands (*90-1, 90-2* and *90-3*) derived from a single vial and shared bands of identical size. These insertions were subsequently mapped to the same site and are hereafter considered as a single event. The 15 lines with new insertions were retained for further study, while the remaining stocks were discarded.

A similar initial study of the 1,028 sublines derived from female germlines showed that 28 contained new insertions on the minichromosome (Table 3). As in the case of the male transpositions, these minichromosomes also retained the initial *PZ* element. In several cases, insertions appeared to have an identical structure, suggesting that they arose as premeiotic clusters. In addition to lines with new insertions, 20 lines containing changes in restriction fragments flanking the starting *P* element were also retained for further study (Table 4). The remaining temporary lines were not analyzed further.

Structure and location of new insertions on *Dp1187*: The new insertions, as well as any alterations in transposon or minichromosome structure, were analyzed in detail by a series of mapping experiments. The location of new insertions could usually be mapped within a few kilobases using the restriction enzymes *Sfi*I and *Not*I. These enzymes recognize eight base pair sequences that are present only once or twice, respectively, on *Dp1187*. Since the *PZ* element also contains one *Sfi*I site and two *Not*I sites (Figure 2), each insertion alters the restriction map in a manner that can be recognized by probing digests with probes derived from various regions of the minichromosome, or derived from the *PZ* element (KARPEN and SPRADLING 1992). The exact probes used are described in Figure 2 and in MATERIALS AND METHODS. For routine mapping within euchromatin, the adult ovary provided a convenient source of DNA. No reductions in the relative representation of restriction fragments derived from the minichromosome compared to those on the *X* chromosome were observed when DNA regions within the 290-kb euchro-

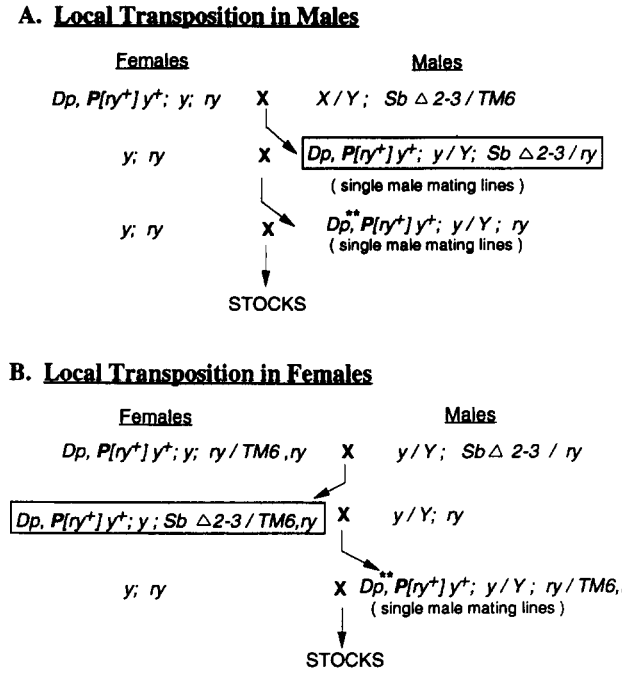

FIGURE 1.—Genetic screens to detect local *P[ry⁺]* (= *PZ*) transposition in males and females bearing the *Dp8002* minichromosome. The crosses used to generate strains that may have undergone new transpositions on the *Dp8002* minichromosome are shown. Genetic symbols are described in LINDSLEY and ZIMM (1992). The genotypes of the male and female flies in which *PZ* transposition was activated by the transposase-producing element *P[ry⁺ Δ2-3](99B)* (abbreviated *Δ2-3*.) are boxed. *Dp*** represents *Dp8002* minichromosomes that may have undergone transposition events.

matic region were studied using genomic probes. However, restriction fragments from heterochromatic regions are strongly underrepresented in Southern blots using DNA from predominantly endopolyploid tissues such as the ovary (KARPEN and SPRADLING 1990; R. GLASER, G. KARPEN and A. SPRADLING, unpublished results). Consequently, DNA from imaginal disks or adults was sometimes used to analyze minichromosome regions proximal to the *sc*[8] junction.

Detailed restriction analysis using pulsed-field and conventional gels was carried out on most of the lines. Figure 3 presents an example of these experiments for one line and two enzymes. The initial experiments had indicated that *Dp733* contained the original and one new *PZ* insertion. The position of the new insertion within the distal portion of the minichromosome was revealed by digesting with *Sfi*I and probing with genomic probe 1. In the absence of any insertion (*Dp1187*), the probe labeled a 250-kb fragment extending from the telomere to the genomic *Sfi*I site at position −40. This fragment was truncated to about 230 kb by the insertion at −68 in *Dp8002*. In *Dp733* the fragment was further reduced to about 120 kb, suggesting an insert at about position −170. The new insertion was oppositely oriented relative to the original element, since "probe *P*" hybridized only to a

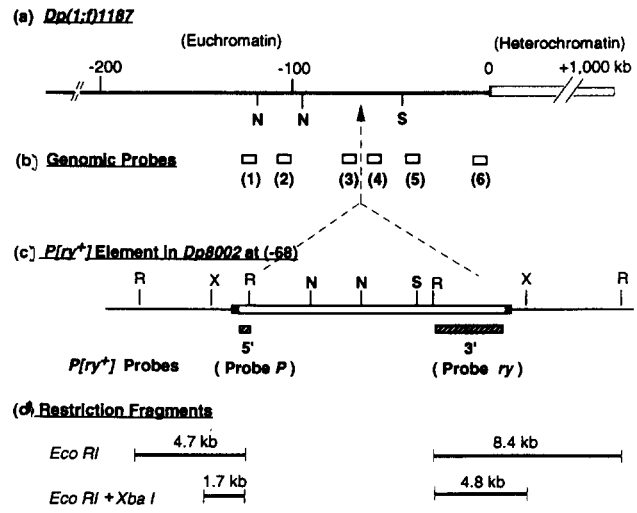

FIGURE 2.—Structure of the *Dp8002* minichromosome. A *Not*I and *Sfi*I restriction map and the coordinate system of the *Dp8002* minichromosome is shown in (a). The location of genomic and transposon DNA fragments (see MATERIALS AND METHODS) used to map new *PZ* insertions are indicated below (b, c). The structure of the *PZ* element on *Dp8002* and its flanking genomic sequences, and the position of key restriction sites used in mapping are indicated (c). The *Dp8002* restriction fragments detected by the 5′ and 3′ *P* element probes used for initial screening are diagrammed (d). The open bar in (c) represents the *PZ* element. Two filled blocks represents the *P* element termini. The arrow indicates the position of the *PZ* insertion on *Dp8002* at −68. N, *Not*I; R, *Eco*RI; S, *Sfi*I; X, *Xba*I.

TABLE 2

Local transpositions from male germlines

Strain	Position[a]	Orientation	Structure[b]	Original insert
7-3	−62	←	5′ end deletion	No change
11-1	se	→	nc	Rearrangement
33-3	se	→	nc	No change
36-3	se	→	nc	No change
58-2	se	→	nc	No change
61-3	se	→	nc	No change
63-1	−246	→	nc	Internal deletion
70-1	−68[c]	←	nc	No change
90-1,-2,-3	−247	→	3′ end deletion	5′ end deletion
94-5	se	→	nc	No change
95-4	se	→	nc	No change
104-4	se	→	Internal deletion	No change
111-1	−246	→	nc	No change

[a] The location of the new insertion according to the minichromosome coordinate system (kb); se = insertion in the starting *PZ* element at coordinate −68.

[b] Structure of the inserted element as determined by DNA analysis; nc = no change detected in mapping experiments using *PZ* 5′ and 3′ probes.

[c] The new insertion is located 200 bp or less outside of the starting element insertion site.

single 120-kb fragment generated by the *Sfi*I sites within the two elements, and labeled it with twice the intensity as the 230-kb band in *Dp8002*. All the other digests shown in Figure 3 provided further confir-

TABLE 3

Local transpositions from female germlines

Strain	Position[a]	Orientation	Structure[b]	Original insert
14	se	→	nc	No change
{122, 161, 168, 833, 917}[d]	−68[c]	→	nc	No change
219	−68[c]	←	nc	No change
229	−67	←	nc	No change
{237,431}[d]	−35	←	nc	No change
278	−252	→	nc	No change
282	−231	→	nc	No change
453	−110	→	nc	No change
482	se	→	nc	No change
545	−68[c]	←	nc	No change
555	se	→	nc	Rearrangement
563	se	→	5′ end deletion	No change
598	se	→	nc	No change
617	−250	←	nc	No change
655	−19	←	nc	Internal deletion
{671, 850}[d]	se	←	nc	No change
728	−10	←	nc	Terminal deletion
733	−170	→	nc	No change
{770, 843}[d]	−70	→	nc	No change
863	se	→	nc	No change
885	−78	→	nc	No change

[a] The location of the new insertion according to the minichromosome coordinate system (kb); se = insertion in the starting *PZ* element at coordinate —68.

[b] Structure of the inserted element as determined by DNA analysis; nc = no change detected in mapping experiments using *PZ* 5′ and 3′ probes.

[c] The new insertion is located 200 bp or less outside of the starting element insertion site.

[d] Lines in parentheses were identical in structure, and are presumed to derive from single premeiotic events.

TABLE 4

Chromosome rearrangements

Strain	Structure[a]
155	Terminal deletion within 5′ end of starting element
194	Terminal deletion within 5′ end of starting element
216	~200-bp insertion at 5′ end of starting element
{231, 243}[b]	Internal deletion: −68 to −120
{322, 915, 790}[b]	Internal deletion: −30 to −68
365	Terminal deletion within 5′ end of starting element
400	Terminal deletion outside 5′ end: −71
429	Terminal deletion within 5′ end of starting element
467	Terminal deletion within 5′ end of starting element
537	Internal deletion: −68 to −110
600	Terminal deletion outside 5′ end: −69
704[c]	Terminal deletion outside 5′ end: −73
728	Terminal deletion within 5′ end of starting element, and new *PZ* insertion at −10
815	Terminal deletion within 5′ end of starting element
844	Terminal deletion outside 5′ end: −68
880	Terminal deletion within 5′ end of starting element
911[c]	Terminal deletion outside 5′ end: −75

[a] At the time DNA was analyzed; lines containing terminal deletions may have subsequently lost additional terminal DNA as described by LEVIS (1989).

[b] Lines in parentheses were identical in structure, and are presumed to derive from single premeiotic events.

[c] No changes in the initial *PZ* flanking restriction analysis were present; these lines were isolated and analyzed because of their variegated *yellow*$^+$ expression.

mation of the location and orientation of the *Dp733* element. For example, a 130-kb *Sfi*I band specific to *Dp733* was labeled with "probe *ry*," which represents the expected distance from the *Sfi*I site within the *P* element at −170 to the distal telomere. Note that several digests failed to reveal minichromosome-specific bands containing the proximal heterochromatin. These bands were too large to be resolved under the conditions of electrophoresis used, and are underrepresented in Southern blots of ovary DNA (R. GLASER, G. KARPEN, and A. SPRADLING, unpublished data). However, fragments of the expected sizes containing the proximal heterochromatin were routinely visualized when adult DNA was resolved on gels run with appropriate pulsing frequencies.

These studies allowed the position and orientation of insertions on most of the new minichromosome derivatives to be determined. The 20 sublines derived from the female screen that appeared to have undergone chromosomal alterations were also subjected to a series of similar studies. The results of all these mapping experiments are summarized in Tables 2 and 3, and plotted in Figure 4.

The new transpositions that were obtained fell into two classes. One group clustered within and around the starting element, creating "double" *P* elements of various structures, including many 5′ head-to-head double *P* elements similar to those previously observed (ROIHA, O'HARE and RUBIN 1988; HAWLEY *et al.* 1988; Eggleston 1990). The second group of insertions was distributed widely throughout much of the euchromatic portion of the minichromosome.

None of the new insertions was located within the large region of centromeric heterochromatin, or in the 40 kb closest to the left telomere. Since the starting element was still present in all the *rosy*$^+$ lines tested, heterochromatic insertions should have been detected by DNA blot hybridization even if the expression of the marker gene within the transposed *P* element was unable to function due to position effects. Since over 1,500 lines were tested, the *Dp8002 PZ* element rarely inserted into these regions.

Comparison of transpositions in the male and female germline: There appeared to be a substantial difference in the number and distribution of local transpositions obtained from male and female germ-

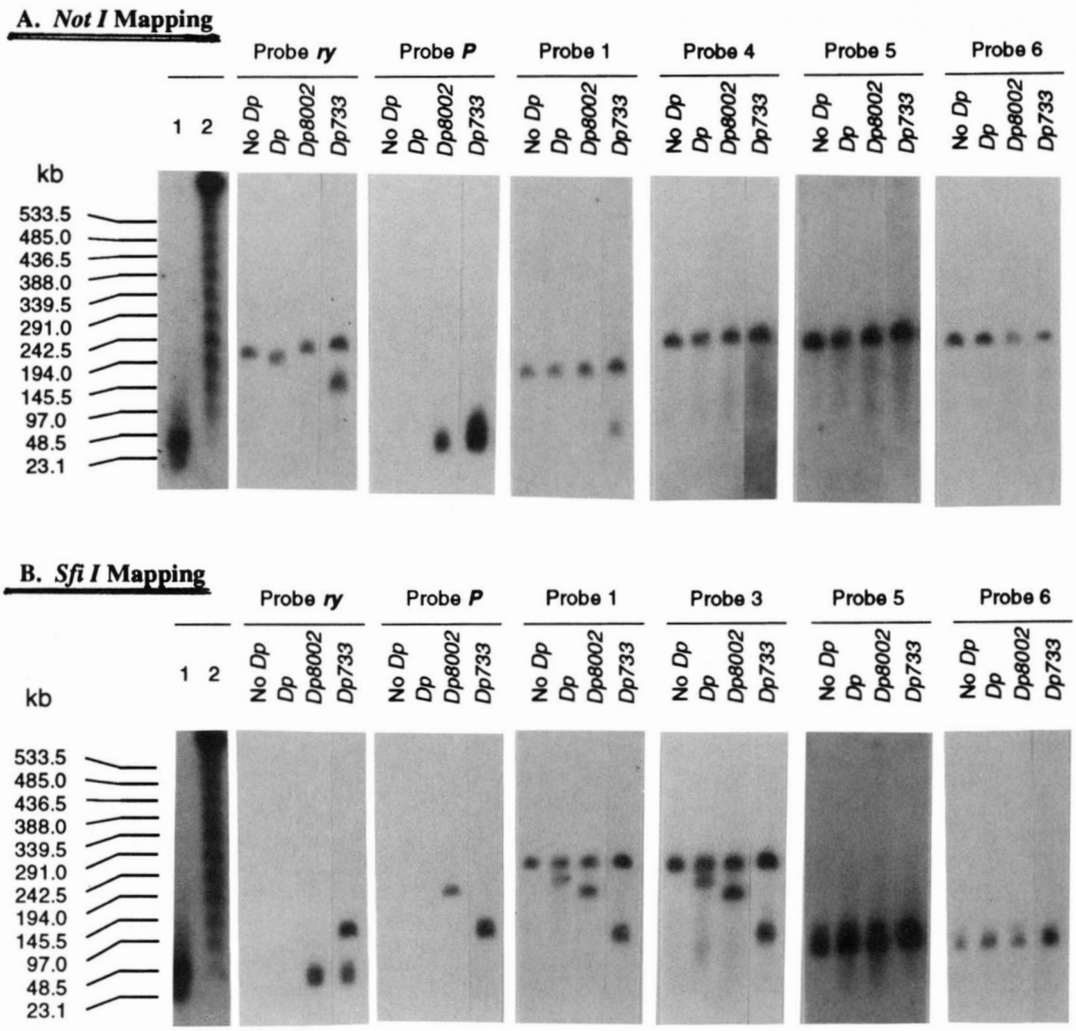

FIGURE 3.—Mapping the local *Dp733* insertion using pulsed-field gel electrophoresis. An example of the physical mapping that was applied to each minichromosome derivative is shown in the case of *Dp733*. High molecular weight genomic DNA was isolated from adult ovaries of flies containing no minichromosome (No *Dp*), containing *Dp1187*, which lacks inserted *P* elements (*Dp*), containing the starting chromosome *Dp8002* (*Dp8002*), or containing the minichromosome *Dp733* (*Dp733*) and analyzed by restriction digestion, pulsed-field gel electrophoresis and DNA blotting as described in the MATERIALS AND METHODS. The probes used are indicated above each blot and are described in detail in Figure 2 and in MATERIALS AND METHODS. Molecular size standards shown in the left panels are oligomerized λ genomic DNA. Details of the analysis are described in the text.

lines. Most of the elements that derived from transpositions in males were inserted very close or within the original *P* element (9/13 = 69%). The 7-3 insertion landed about 6 kb to the right of the *Dp8002* element. Three other local insertions (63-1, 90-1, 111-1) were mapped within a 5-kb interval (−246 to −251) known to be a hotspot for *P* element transpositions from other chromosomes (KARPEN and SPRADLING 1992).

In contrast, local transpositions occurred more frequently in the female germline (1.8% *vs.* 0.7%). Furthermore, the transpositions recovered from female germlines were distributed much more widely within the euchromatic regions surrounding the donor element. Only 10 of 21 insertions (48%) where located near or within the starting element, and only two were located in the hotspot region (278 and 617). In practical terms the difference between the male and female derived lines was significant. Insertions lying within or very close to the starting element (or in the hotspot) were less likely to be useful for mutagenesis. Therefore, only 1/13 = 8% of insertions recovered from males were potentially useful, compared to 9/21 = 43% of insertions recovered from females. As described below, rearrangements also occurred more frequently in females.

Terminal and internal deletions: Most of the 20 strains with chromosomal alterations contained terminal deletions (Table 4 and Figure 5). Mapping using pulsed-field gels demonstrated that these minichromosomes had lost nearly the entire region distal to the original insertion at −68. The position of the new telomere varied, however. It ranged from more than 5 kb distal to the 5′ end of the starting element, to within the starting element itself. Complementation tests using mutant alleles and small deletions on the

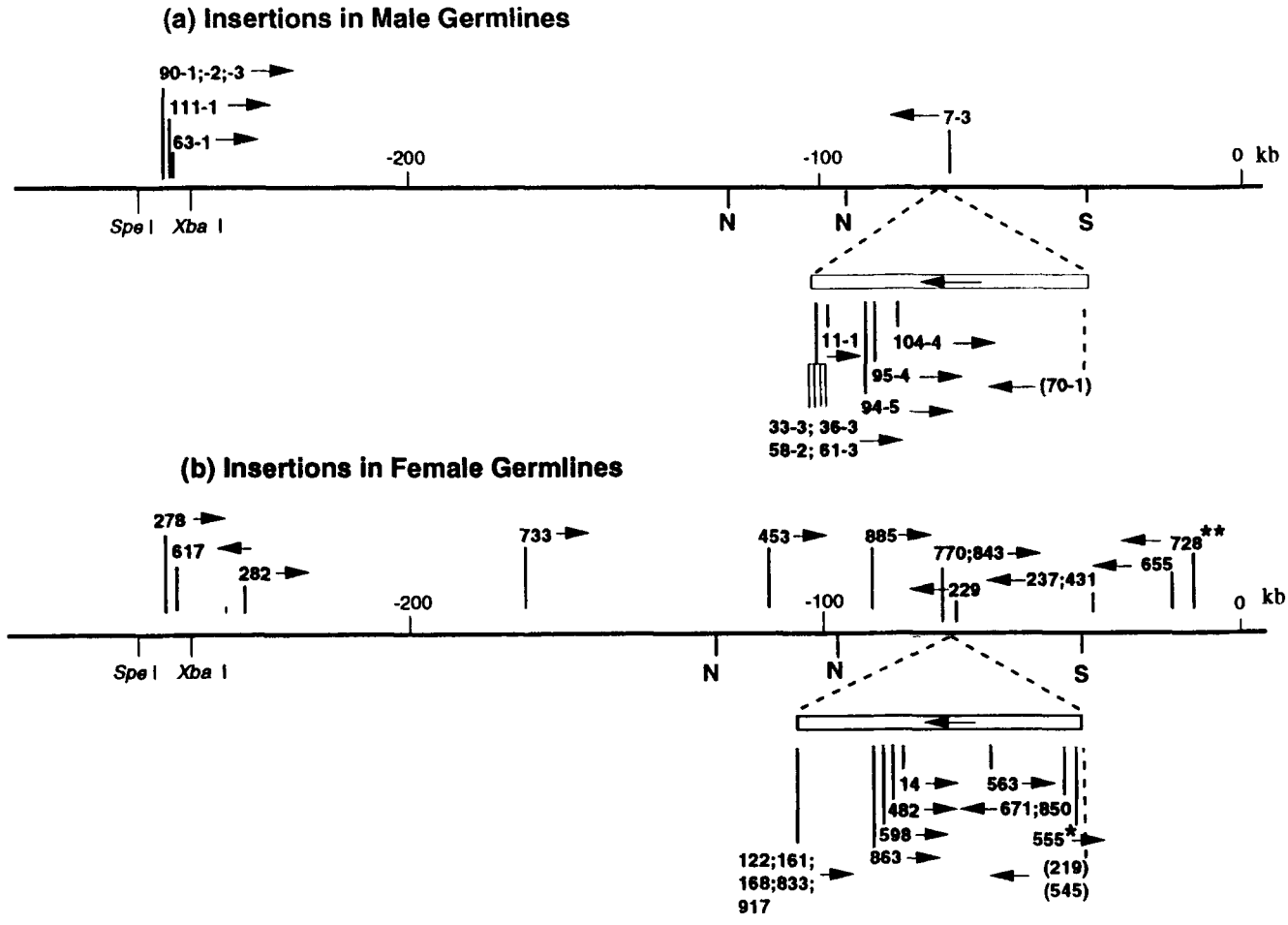

FIGURE 4.—Summary map of transpositions recovered following mobilization of the *PZ* element at coordinate −68 on *Dp8002*. A map representing the distal 290 kb of the minichromosome, including the position of *Not*I (N) and *Sfi*I (S) sites, is shown. The open box indicates the location of the starting *PZ* element at coordinate −68. The location and orientation of the new *PZ* elements mapped in the indicated strains are shown by vertical lines topped with an arrow representing the 5′ end of the *PZ* element. Arrows mark the positions of the *PZ* insertions derived from local transpositions. ***Dp728* also contained a terminal deletion of sequences within the 5′ region and distal to the starting *PZ* element at −68. *This line also contained additional changes (see text). Three derivatives (*Dp70-1*, *Dp219* and *Dp545*) appeared to contain direct tandem repeats of the starting element (at the level of resolution of these experiments). The insertion in these lines is shown at the 3′ region of the starting element. The chromosome region between the *Spe*I and *Xba*I restriction sites (at positions −243 to −253) contains a *P* element insertion hotspot.

tip of the *X* chromosome also revealed that several essential genes located at the minichromosome tip had been deleted on these derivative minichromosomes (Table 5 and Figure 5).

Three minichromosome internal deletions were also isolated from female germlines (Table 4 and Figure 5). These lines were first detected to have changes in the restriction fragments flanking the starting element. Physical mapping studies showed that they had lost large internal genomic DNA segments. *Dp231* and *Dp243* may have derived from a cluster, since they have similar or identical restriction patterns. *Dp322*, *Dp790* and *Dp915* may represent another cluster. As shown in Figure 5, internal deletions occurred on both sides of the starting element and the sizes of the deleted fragments were approximately 40 kb for *Dp322*, *Dp790* and *Dp915*; 40 kb for *Dp537*; and 50 kb for *Dp231* and *Dp243*. Genetic complementation tests showed that all of these internal deletions failed to complement *Df(1)cin-arth*, which deleted four of the essential genes at the tip of the *X* chromosome (Table 5).

Nonrandom orientation of local insertions: The insertions recovered in both sexes were strikingly nonrandom in orientation relative to the starting *P* element (Figure 4). Nearly all (23/24) of the elements that inserted within or 5′ to the starting element were in opposite (head to head) orientation, while those inserted proximally were usually in tandem orientation (8/9). Even elements located 50–180 kb from the insertion on *Dp8002* usually showed this preference (Figure 4). *Dp671* appeared to be an exception, since it contained an insertion within the starting element (near the 3′ end) in parallel orientation. The hotspot insertion on Dp617 also deviated from the general rule, since it lay in the same orientation as the *Dp8002*

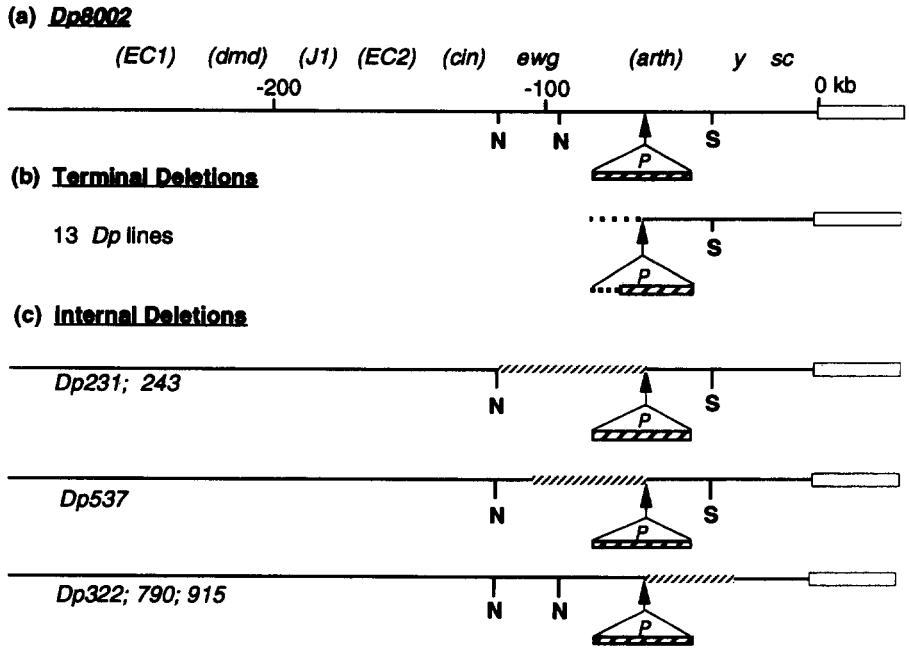

FIGURE 5.—Minichromosome derivatives with terminal or internal deletions recovered following mobilization of the *Dp8002 PZ* element. (a) A map of the distal end of the *Dp8002* minichromosome is shown along with the approximate location of the nine genes (see LINDSLEY and ZIMM 1992). The order of the six loci presented in parentheses is known, but they have not been physically mapped. (b) The structure of thirteen derivatives bearing terminal deletions. Their breakpoints are located within the region shown by a dashed line adjacent to, or within, the starting *PZ* element (see also Table 4). (c) Internal deletions (indicated by hatched lines) in three minichromosome. Abbreviations: *EC1*, *l(1)EC1*; *J1*, *l(1)J1*; *EC2*, *l(1)EC2*; N, *Not*I; S, *Sfi*I.

TABLE 5

Genetic complementation between minichromosome derivatives and *X* chromosomes carrying lethal mutations or deficiencies

	Lethal mutation			Deficiency		
Dp derivative	*l(1)EC1*	*l(1)J1*	*l(1)EC2*	*Df(1)259*	*Df(1)1-96*	*Df(1)cin-arth*
Dp8002	Viable	Viable	Viable	Viable	Viable	Viable
Dp237	ND	ND	ND	ND	ND	Viable
Dp453	ND	ND	ND	ND	ND	Semilethal[a]
Dp655	ND	ND	ND	ND	ND	Viable
Dp733	Viable	Lethal	Viable	Lethal	Lethal	Viable
Dp885	ND	ND	ND	ND	ND	Semilethal[a]
Dp704	Lethal	Lethal	Lethal	Lethal	Lethal	Lethal
Dp911	Lethal	Lethal	Lethal	Lethal	Lethal	Lethal
Dp231	Viable	Viable	Viable	Viable	Lethal	Lethal
Dp537	Viable	Viable	Viable	Viable	Lethal	Lethal
Dp915	Viable	Viable	Viable	Viable	Viable	Viable

[a] Rare male progeny *Df(1)cin-arth/Dp453* or *Df(1)cin-arth/Dp885* were sterile or semisterile; ND = not determined.

P element. In three other derivatives, *Dp70-1*, *Dp219* and *Dp545*, tandem duplications with the starting *PZ* element were present, so it was not possible to determine by mapping if the incoming element had inserted at the 3′ end (as we suspect) or at the 5′ end of the starting element. The transpositions also showed a preference for transposition toward the 5′ end of the starting element (16/23, counting double *P* elements), as observed previously (TOWER *et al.* 1993).

Variegation associated with alterations in *Dp1187*: Both the starting minichromosome and the derivatives generated in these experiments contained a functional *yellow*$^+$ gene. The expression of *yellow*$^+$ from the minichromosome is subject to position-effect variegation, because of its proximity to the heterochromatic sequences lying across the *sc*^{8} breakpoint (KARPEN and SPRADLING 1990). Flies bearing the starting chromosome, *Dp8002*, contain a small fraction of bristles (1–5%) that are phenotypically yellow. Surprisingly, some of the derivative chromosomes produced in these experiments showed greatly increased yellow variegation.

The most dramatic increases in variegation were associated with terminal deletions. Previously, increased yellow variegation was noticed in the case of line *8-61*, which simultaneously underwent a trans-

position near −120 and a deletion of the distal chromosome (TOWER *et al.* 1993). It was difficult to rule out that some other alteration in the *Dp8-61* might have been responsible, although no other changes were seen in its restriction map. Increased variegation was associated in the experiments reported here with all 13 terminal deletions that were independently recovered. Seventy to eighty percent of the bristles on these flies were yellow, and cuticular pigmentation was similarly affected. All these derivatives removed the chromosome region distal to the starting insertion; however, the exact point of deletion varied over more than a 5-kb region, with only a minor effect on extent of variegation. More detailed observations revealed that even internal deletions removing 40 or 50 kb of sequences 5′ to −70 caused increased variegation, although the effect was less than a terminal deletion. Most of the simple insertions showed no changes in variegation that were detectable above the starting level.

When the minichromosomes were transferred into males lacking a *Y* chromosome, a situation known to enhance position-effect variegation, yellow variegation increased dramatically. Flies bearing terminally deleted chromosomes retained only a few small spots of black pigmentation on their abdomens, and rare black bristles in *X*/O males (<1%). In the enhanced background, the internal deletions also showed increased levels of variegation compared to the parent minichromosome.

Complementation analysis of local insertions: Some of the derivative minichromosomes were subjected to complementation analysis with chromosomes deficient for portions of the 1A-1B region, and with alleles of certain lethal complementation groups. (Strains defining all the complementation groups were not available or not tested.) The results of these experiments are presented in Table 5. These experiments showed that at least three of the chromosomes bearing local transposition events were associated with new recessive phenotypes. *Dp733*, containing a new insertion at −170, failed to complement a *l(1)J1* allele and all deficiencies lacking this locus. Thus, an essential portion of the *l(1)J1* locus is likely to be located near the site of the insertion. Two other insertion-bearing chromosomes, *Dp453* (−110) and *Dp885* (−78) were semilethal in combination with *Df(1)cinarth*, which is deleted for the corresponding region of the normal *X*. Rare surviving males were sterile or semisterile. These insertions are likely to disrupt the *erect wing* (*ewg*) complementation group (FLEMING, DESIMONE and WHITE 1989), however a test of allelism was not done. The strong variegation associated with the terminal deletion on the *Dp728* chromosome rendered it difficult to analyze the effects of its new insertion at −10. Variegation for some of the lethal genes was also evident from the complementation analysis of chromosomes bearing internal deletions. For example, *Dp231* (deficient between −120 and −70), failed to fully complement genes such as *l(1)J1*, that lie distal to −120 on the minichromosome (data not shown). This was interpreted to result from variegation for some of the lethal genes on this chromosome.

DISCUSSION

Local transposition occurs frequently following mobilization of the *P* element on *Dp8002*: The fraction of progeny chromosomes that contained new *P* element insertions on the minichromosome (0.7 or 1.8%) was much higher than expected if local transposition was not preferred. Comparing these figures to the insertion rate onto the minichromosome from a distant site [0.015%, see KARPEN and SPRADLING (1992) and TOWER *et al.* (1993)], yielded local enhancements of 0.7%/0.015% = 47-fold or 1.8%/0.015% = 120-fold in the male a female germlines, respectively. Only 3 or 4% of progeny contained transpositions to other chromosomes that comprise >99% of the genome. Even if insertions into or very close to the starting *P* element are subtracted, the frequency of minichromosome insertions in males and females (0.21 and 0.96%) and the corresponding enhancements (14- and 64-fold) are still substantial. Therefore, the greatly elevated frequency of local insertions seen in a companion study (TOWER *et al.* 1993) appears to be a general property of *P* elements mobilized from diverse genomic locations.

Local transpositions from the starting site at −68 were associated with a strong orientation preference relative to the starting element. This preference did not simply represent an inherent property of these DNA sequences. Insertions onto *Dp1187* that were derived from a *P* element on the *X* chromosome (KARPEN and SPRADLING 1992) had an approximately equal chance of inserting into the hotspot in either orientation (20/36 = 56% were oriented as preferred in these experiments). There was no inherent tendency for *P* elements to insert with their 5′ ends facing the −79 region, since 4/4 elements that inserted between −70 and the hotspot in these experiments had the opposite orientation to the one observed here (KARPEN and SPRADLING 1992).

Transposons mobilized from the euchromatic starting site in *Dp8002* therefore displayed a similar directional and orientational preference to those seen following mobilization of *PZ* elements in the hotspot and at *cactus* (TOWER *et al.* 1993). The tendency of insertions within or 5′ to the starting element to reside in opposite orientation was particularly strong; 39 of 40 insertions in the three sites were consistent with the internal and 5′ rule. Evidence that insertions on the

3′ side of the starting element are frequently oriented in tandem was first obtained in these experiments; 13 of 16 insertions at all three sites followed this 3′ rule. The 5′ directional preference was less pronounced; 27 jumps to the 5′ were recorded compared to only 13 to the 3′ side.

The increased transposition rate and orientation preference suggested that *P* elements can transpose by two pathways, one leading to preferential reinsertion near the initial site, and one in which sites are selected from among the genome at large. The local pathway accounted for 20–40% of all transpositions in our experiments; the larger fraction were recovered in females and accounted for the higher rate in this sex (Table 1).

The frequent occurrence of local transpositions may help explain a variety of previous observations of *P* element movement. It strongly supports the contention that a *P* element inserted at the *singed* locus frequently gave rise to head to head derivatives by short range transposition (HAWLEY *et al.* 1988). Local transpositions in the vicinity of resident *P* elements probably contribute heavily to the strain specificity of insertional mutagenesis reported in hybrid dysgenesis experiments (see ENGELS 1989).

Difference of *P* transpositions between male and female germlines: A greater number of independent local insertions outside the starting element were recovered among the progeny of females compared to males (11/1, 150 *vs.* 4/1, 900, $P < 0.05$). These rates might be due to some intrinsic difference between the transposition mechanism or its efficiency in male and female germline cells. Female germline cells produce many fewer mutations per gamete following an equal dosage of radiation, presumably because they repair DNA damage more efficiently than male cells (see ASHBURNER 1990). Repair enzymes whose levels differed between males and females might affect the efficiency of local transposition. Alternatively, target sites might differ between the sexes. "Enhancer-trap" *P* element insertions recovered from males or females were preferentially expressed in the germline tissue of the sex from which they were derived (BOWNES 1990). The preference for local insertion in our experiments might indicate that more regions of the minichromosome have a conformation permissive for transposition during oogenesis than during male germ line development.

Mechanism of local transposition: *P* elements are thought to transpose by a conservative mechanism (ENGELS 1989; ENGELS *et al.* 1990). Increases in the total number of elements can take place following transposition because the double-strand break resulting from element excision is efficiently repaired by gene conversion (ENGELS *et al.* 1990; GLOOR *et al.* 1991), and because transposition may preferentially occur from a replicated site to an unreplicated site during the S phase.

All the chromosomes containing new local transpositions that were analyzed retained a copy of the original element. This was also the most common situation observed previously (TOWER *et al.* 1993); however, several local insertions were accompanied by excision of the starting element, and this situation could be selected for by "reversion jumping." Because wild-type sequences corresponding to the −68 region are present only on the normal *X* chromosome, which is not known to pair with the minichromosome, the gap produced by excision may almost always invade the sister strand, leading to regeneration of the starting element. The excised *P* element might subsequently reinsert onto either the sister or the repaired original strand to give rise to the double *P* elements.

One possibility for the high frequency of local integration and its orientational preference would invoke proteins associated with the excised transposon. When *P* elements contain DNA sequences derived from the *engrailed* locus, their insertion site-specificity is altered (KASSIS *et al.* 1992), including a greatly increased frequency of insertions near the *engrailed* locus. Proteins on the excised *P* element may cause it to become associated with a chromosome region containing proteins or DNA sequences to which they can bind. Proteins bound to *P* element termini might frequently tether the excised element to the unexcised copy on the sister strand in a fixed orientation, leading eventually to a local insertion event. Alternatively, the two sister *P* elements may not dissociate readily from a complex following DNA replication, so that when one is excised as the first step in transposition, opportunities for insertion into nearby DNA sequences are increased. An excised copy that escapes binding to its sister would be free to move to other regions of the nucleus and to integrate at distant sites.

Internal and terminal deletions: Deletions extending into flanking chromosomal DNA from one end of a starting *P* element that is exposed to transposase regularly arise in a small fraction (~0.1%) of progeny (TSUBOTA and SCHEDL 1986; SALZ, CLINE and SCHEDL 1987). A similar frequency of such events was also seen in our experiments (excluding terminal deletions, which would not have been recovered in previous studies). The ability of closely positioned *P* elements to precisely delete the intervening chromosome segment when activated by transposase (COOLEY, THOMPSON and SPRADLING 1990) suggests a two-step model for the origin of these deletions. Rarely, in developing germline cells, local transposition may be followed by a precise deletion prior to the completion of gametogenesis. Consistent with this model, more internal deletions were recovered in female germlines where the frequency of local transposition was also higher.

Terminal deletions were recovered frequently in these experiments (13/1, 150 = 1.1%), although less frequently than when the starting *P* element was closer to the end of the minichromosome [3% (TOWER *et al.* 1993)]. Two models were considered to explain how terminal deletions were generated. In maize, closely linked *Ac* elements have been shown to cause chromosome breakage (DOONER and BALACHEW 1991). In our experiments, chromosomes containing two linked *P* elements susceptible to terminal deletion may have been frequently generated by local transposition during germ cell development. This model predicts that sequences derived from the transposed copy would sometimes still be present at the termini of the deleted chromosomes. Alternatively, terminal deletions might result from failure to repair the gaps created by element excision. If only one of the two ends produced by excision of the starting element successfully invaded the sister chromatid, a terminally deleted chromosome would be produced. This model is attractive because the different distances the strand was repaired against its sister prior to chromosome separation could explain the variation in the position of the deletion endpoint relative to the starting element. No *P* element sequences (that might have originated from a second transposon) should be found distal to the starting element according to this model.

Comparison to local *Ac* transposition: Preferential transposition near the starting element was originally discovered through studies of *Ac-Ds* elements in *Zea mays*. (VAN SCHAIK and BRINK 1959; GREENBLATT and BRINK 1962; GREENBLATT 1984; DOONER and BELACHEW 1989). The local *P* transpositions shared many but not all features characteristic of local *Ac* jumps. Reinsertion of *Ac* elements frequently occur close to the starting element (DOWE, ROMAN and KLEIN 1990; WEIL *et al.* 1992; ATHMA, GROTEWOLD and PETERSON 1992). However, insertions into the starting element have rarely been recovered in maize (DÖRING, TILLMANN and STARLINGER 1984; OSBORNE *et al.* 1991), possibly because only events causing phenotypic changes have been studied. Local *Ac* transpositions can extend over several map units. However, the small size of the minichromosome may not have allowed the full extent of local *P* transposition to be tested. Local internal deletions can be generated following mobilization of transposable elements in maize (DOONER *et al.* 1991). Unlike *P* elements, *Ac* transposition was frequently accompanied by loss of the starting element. This appears to represent a true difference between the two systems. The gap resulting from excision of the starting element may be repaired less frequently in the case of nonlocal transpositions in maize compared to *P* elements (BARAN *et al.* 1992). In addition, constraints on the orientation of local *Ac* insertions were not observed (ATHMA, GROTEWOLD and PETERSON 1992), except possibly for insertions very close to the starting element (MORENO *et al.* 1992).

Several models have been proposed to explain the enhanced recovery of transposons on the starting chromosome in maize (BRINK and WILLIAMS 1973; GREENBLATT 1984; DOONER and BELACHEW 1989). Most postulate relationships between the replication of the donor and target sites, and the incoming element. At present, the mechanistic bases for the observed differences in the behavior of local *Ac* and *P* transpositions remain unclear.

Local *P* transpositions into heterochromatin: *P* elements have only rarely been shown to insert into heterochromatic chromosome regions (see ENGELS 1989). A few exceptions are known, including insertions in the heterochromatin of chromosome *4* (SPRADLING and RUBIN 1983), the *Y* chromosome (BERG and SPRADLING 1991; KARPEN and SPRADLING 1992; P. ZHANG and A. C. SPRADLING, unpublished data), and a subtelomeric region (KARPEN and SPRADLING 1992). The reason insertions did not occur more widely within heterochromatic regions has been difficult to determine. The highly repetitive sequences characteristic of these regions may preclude *P* element insertion due to the absence of certain sequence requirements for target site cleavage. Alternatively, *P* elements may transpose into heterochromatin at diverse positions, but marker genes associated with these elements become unable to function due to position effects, so that such insertion cannot be detected genetically.

Our experiments should have allowed these possibilities to be distinguished. Any heterochromatic transposition that retained the starting *P* element would have been revealed by DNA blotting in our experiments regardless of the ability of the *PZ* element inserted into the heterochromatin to express *rosy*$^+$. None were recovered, despite the fact that centric heterochromatin was located only 70 kb from the starting element. Several factors might have rendered heterochromatic insertions difficult to detect even by Southern analysis. If no *Eco*RI or *Xba*I sites were present near the insert, it might not have been recognized. Recently, we proposed that the reductions in heterochromatin content observed in many polytene cells result from the elimination of heterochromatic sequences during the development of many somatic cells (KARPEN and SPRADLING 1990). Insertions into sequences that underwent elimination in tissues used for Southern analysis would also have been missed. However, the telomeric and centromeric region of the minichromosome are known to be present in the expected amounts (relative to imaginal disc DNA) in the adult DNA used for the initial screening (data not shown). Consequently, it seems unlikely that many

germline insertions escaped detection due to their elimination in somatic cells.

There remain several possible reasons why *P* elements might avoid insertions into most heterochromatic regions. Active genes are thought to be absent from many heterochromatic regions, and they may be required to make a region accessible to insertion. If replication of the donor and target DNA has to be synchronized, then the replication of heterochromatin late in S phase might prevent it from acquiring transpositions from euchromatic sites. Finally, the repetitive DNAs present in heterochromatic regions may mostly lack appropriate target sequences for insertion.

Use of local transposition in single element insertional mutagenesis: Local transposition was previously proposed as a means to increase the chance of tagging desired genes in maize (DOONER and BELACHEW 1989), tomato (OSBORNE *et al.* 1991), and in tobacco (DOONER *et al.* 1991). Our data demonstrate that particularly in the female germline, transposition can be significantly enhanced into a broad region surrounding the starting element. The rate of insertion was increased more than 100-fold, and several complementation groups located near the site of the insertion on *Dp8002* were mutated, despite the very small sample size used. Even if an insertion that inactivates the gene of interest is not obtained, the insertions and internal deletions produced by local transposition are likely to assist mapping its position relative to other genes in the region. Chromosomes containing two nearby *P* elements produced by local transpositions could be used to generate a series of deletions extending in both directions from the starting element (COOLEY, THOMPSON and SPRADLING 1990).

In practical terms, it should be possible to design an effective local mutagenesis strategy for virtually any region of the Drosophila genome. Many stocks bearing single insertions at known chromosome locations are already available (COOLEY, KELLEY and SPRADLING 1988; BIER *et al.* 1989; BELLEN *et al.* 1989; BERG and SPRADLING 1991; KARPEN and SPRADLING 1992). Consequently, starting elements located within 200 kb of most targets should frequently be available. Several methods could be used to detect chromosomes bearing local insertions. These include an induced phenotype, an altered dosage of a marker gene located on the starting element, or an altered phenotype associated with the starting element (TOWER *et al.* 1993). Local transposition could also be combined with rapid molecular methods for identifying transpositions within the region of interest (HAMILTON *et al.* 1991). Local transposition screens are likely, therefore, to enhance the general utility of single *P* element insertional mutagenesis.

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