Studies on the Rate and Site-Specificity of *P* Element Transposition

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ABSTRACT

A single genetically marked *P* element can be efficiently mobilized to insertionally mutagenize the Drosophila genome. We have investigated how the structure of the starting element and its location along the *X* chromosome influenced the rate and location of mutations recovered. The structure of two *P*[*rosy*$^+$] elements strongly affected mobilization by the autonomous "Jumpstarter-1" element. Their average transposition rates differed more than 12-fold, while their initial chromosomal location had a smaller effect. The lethal and sterile mutations induced by mobilizing a *P*[*rosy*$^+$] element from position 1F were compared with those identified previously using a *P*[*neo*^{R}] element at position 9C. With one possible exception, insertion hotspots for one element were frequently also targets of the other transposon. These experiments suggested that the genomic location of a *P* element does not usually influence its target sites on nonhomologous chromosomes. During the course of these experiments, *Y*-linked insertions expressing rosy$^+$ were recovered, suggesting that marked *P* elements can sometimes insert and function at heterochromatic sites.

P elements are members of a class of transposable elements that transpose by DNA-DNA mediated mechanisms (see review by FINNEGAN 1989). Knowledge of *P* element behavior has led to their use as transformation vehicles and as insertional mutagens (reviewed in ENGELS 1988). The widespread application of *P* elements as genetic tools provides opportunities to analyze fundamental problems relating to *P* element biology that have been difficult to address previously. In this report, we utilized strains generated in two large insertional mutagenesis screens to study two such problems: how the rate and site-specificity of *P* element transposition is controlled.

The movement of individual, naturally occurring *P* elements is difficult to measure. Transposition rates averaged over all the elements within a strain have been estimated by counting the number of new *in situ* hybridization sites that appear in progeny from a dysgenic cross. In several cases about 0.25 new insertion sites per donor *P* element per generation were observed (BINGHAM, KIDWELL and RUBIN 1982; BENZ and ENGELS 1984; EGGLESTON, JOHNSON-SCHLITZ and ENGELS 1988). However, *in situ* hybridization may fail to identify new insertions of very small elements or those landing in regions that are underrepresented in polytene chromosomes. Not all the presumed donor elements may actually be capable of movement. Thus, average transposition rates provide little insight into the rate at which individual elements transpose.

Progress in studying the movement of individual elements has come from schemes in which a genetically marked transposon is mobilized to nonhomologous chromosomes by a single autonomous *P* element (COOLEY, KELLEY and SPRADLING 1988; ROBERTSON *et al.* 1988; reviewed in COOLEY, BERG and SPRADLING 1988). Individual transposons moved at highly variable rates, from 3.1×10^{-4} to 2.1×10^{-1} insertions per element per generation (ROBERTSON *et al.* 1988). Chromosomal position effects associated with the starting location and variations in element size were suggested to cause these differences (reviewed in ENGELS 1988). However, size-independent differences in element structure might also have influenced the results of these experiments; the particular sequences present in a *P* element can exert large effects on its rate of transformation (reviewed by SPRADLING 1986). The extraordinary variation in element mobility would be particularly significant if it resulted from properties intrinsic to particular chromosomal sites rather than as a by-product of constructing artificially large and structurally diverse transposons.

A second area that has been difficult to study is the target specificity of *P* element insertion. Analysis of the mutations recovered during hybrid dysgenesis revealed large gene-specific differences in mutation rates, and suggested that not all genes are targets for *P* element inactivation (SIMMONS and LIM 1980; SIMMONS *et al.* 1984; EEKEN *et al.* 1985; reviewed in KIDWELL 1986, 1987). Nonrandom target selection could be an intrinsic property of the *P* element transposition mechanism. This characteristic is presumably the explanation for the highly nonrandom site pref-

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erences observed on a local level (O'HARE and RUBIN 1983; KELLEY *et al.* 1987; ROIHA, RUBIN and O'HARE 1988). Since a strong consensus nucleotide sequence is not present at sites of insertion, the molecular explanation for local preferences remains obscure.

The initial location of an element probably also influences its choice of target sites. Like the maize transposon Ac (FEDOROFF 1988), *P* elements transpose preferentially into nearby sites located on the same chromosome (RAYMOND and SIMMONS 1981; HAWLEY *et al.* 1988). Whether its starting location affects a *P* element's preferred target sites on heterologous chromosomes has been difficult to determine. Mutational hotspots can be strain-specific during hybrid dysgenesis (ENGELS 1985). However the many preexisting *P* elements in such strains become hotspots for mutations resulting from imprecise excision (ENGELS 1979; VOELKER *et al.* 1984; DANIELS *et al.* 1985; ROIHA, RUBIN and O'HARE 1988), chromosome rearrangement (ENGELS and PRESTON 1981, 1984), secondary insertion (HAWLEY *et al.* 1988; ENGELS 1988) and conversion-like events (GEYER *et al.* 1988). Very few studies corrected for these effects, so the magnitude of true site-dependent insertional preferences remains uncertain.

Mobilizing a single genetically marked *P* element allows the effects of element structure and location on transposition rate and target selection to be directly measured. We therefore examined the transposition frequency of two $P[ry^+]$ elements located at seven different sites on the *X* chromosome. In addition, we compared the lethal and sterile mutations induced by two different elements mobilized from different locations. A preliminary report of some of these experiments was presented previously (COOLEY, BERG and SPRADLING 1988).

MATERIALS AND METHODS

Genetic strains: All cultures were maintained at 25° on standard cornmeal, agar, molasses and yeast medium. The mutations and chromosomes used in this study are described in LINDSLEY and GRELL (1968) and LINDSLEY and ZIMM (1985, 1986, 1987, 1990).

Transposable elements: The $P[ry^+11]$ and $P[ry^+1]$ mutator elements are described in SPRADLING and RUBIN (1983), RUBIN and SPRADLING (1983), and Figure 1. The DNA sequence of the *P* element and *rosy* gene is given in O'HARE and RUBIN (1983), LEE *et al.* (1987), and KELTH *et al.* (1987). Jumpstarter-1 ($P[Js]$) was made by LASKI, RIO and RUBIN (1986) and characterized further by FORREST SPENCER and LYNN COOLEY (COOLEY, KELLY and SPRADLING 1988). The Jumpstarter-1 strain was maintained by outcrossing and tested periodically for the presence of transpositions by Southern blotting (COOLEY, KELLY and SPRADLING 1988).

Measurement of transposition frequencies: $X, P[ry^+]; ry^{42}$ flies containing one of seven different *X*-linked $P[ry^+]$ elements were crossed to a strain bearing the complete *P* element, Jumpstarter-1: $P[Js]ry^{506}/TM3$, *Sb* ry^{RK} *e*^{s} (Figure 2). Single males carrying both mutator and jumpstarter elements (ry^+ Sb^+) were mated in individual vials to ry^{506} virgins. Parents were discarded after seven days. All the daughters from this cross received an *X* chromosome from their fathers and thus were ry^+. Sons received an *X* chromosome from their mothers and therefore were ry^-, unless a transposition event occurred in which $P[ry^+]$ moved to an autosome carried in a *Y*-bearing sperm. Transposition events were scored in two ways. The "transposition frequency" was calculated as the fraction of progeny chromosomes containing a $P[ry^+]$ insertion. In the protocol of Figure 2, transposition events cannot be observed on average in 5 of the 9 major chromosome arms. Therefore, the fraction of ry^+ males was multiplied by 9/4 to correct for the failure to detect such insertions. This correction ignores small differences in target size between the chromosomes, insertions on the *Y* chromosome, and the probable existence of an increased frequency of "local" insertions near the site of the starting element.

In addition, we defined the "jumping rate" as the percentage of vials containing at least one ry^+ male. By counting only one ry^+ male per vial as a transposition event, we avoided inflated or skewed estimates resulting from premeiotic clusters. In a second set of experiments (crosses "j," Table 1), $X, P[ry^+11]$; *cn*; *mwh* ry^{506} *e* females were mated to *cn*; $P[Js]$ $ry^{506}/TM3$, *Sb* ry^{RK} *e*^{s} males. Single males carrying both mutator and jumpstarter elements (ry^+ cn^- Sb^+) were mated in individual vials to *mwh* ry^{506} *e*/*TM3*, *Sb* ry^{RK} *e*^{s} virgins, and the jumping rate was determined as before.

Mutagenesis: The crosses used to generate and map single $P[ry^+]$ insertion mutations are described in Figure 3. This scheme incorporated three improvements over our previous strategy (COOLEY, KELLEY and SPRADLING 1988). First, transpositions were recovered on chromosomes bearing recessive markers to facilitate recognition of homozygotes. Second, a y^+Y chromosome was incorporated so that sterile *X*/O males could be distinguished from transpositions. Third, stocks containing the marked chromosomes were established from single, isogenized first, second and third chromosomes, to minimize the presence of background mutations. In addition, prior to expanding the mutator and jumpstarter stocks for use in the large scale screen, individual sublines were established from pair matings and characterized in the following ways. Viability and fertility of stocks were examined by counting the number of eggs laid and the subsequent number of adults produced by each of the pair matings. Salivary gland chromosome preparations were examined for the presence of inversions or other gross rearrangements that might affect the future insertion lines. The presence of a Cy-like inversion in some of the lines from the ry^{506} stock (SPRADLING and RUBIN 1982; RUBIN and SPRADLING 1982; BOUROUIS and RICHARDS 1985; COOLEY, KELLEY and SPRADLING 1988) prompted this investigation. Finally, DNA from all stocks used at any point during the screen was examined on a Southern blot to verify that the only *P* elements present were $P[ry^+11]$ and Jumpstarter-1.

The crosses were carried out as follows (see Fig. 3): $X, P[ry^+11](1F)$; *cn*; *mwh* ry^{506} *e* females were mated in bottles to either *cn*; $P[Js]ry^{506}/TM3$, *Sb* ry^{RK} *e*^{s} or X/y^+Y; *cn*; $P[Js]$ $ry^{506}/TM3$, *Sb* ry^{RK} *e*^{s} males. Single males carrying both the mutator and the jumpstarter elements ($ry^+cn^-Sb^+e^+$ males) were selected and mated in vials to three *mwh* ry^{506} *e*/*TM3*, *Sb* ry^{RK} *e*^{s} virgin females. Transposition events were identified as ry^+ sons, and a single e ry^+ male was selected from each vial to establish a stock (e males were chosen to eliminate the $P[Js]$-bearing chromosome from the stock). The chromosomal location of the new insertion was mapped by subsequent crosses. (Note: ry^+ sons could be distinguished from their ry^+ fathers because the fathers were cn e^+, while the sons were cn^+ e.) When a y^+Y chromosome was present

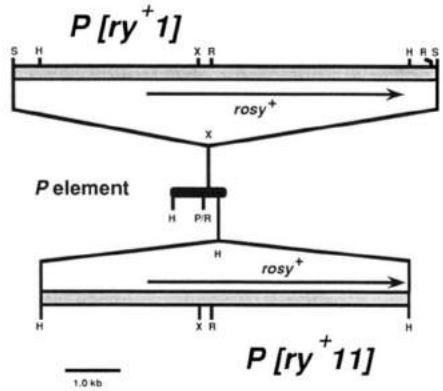

FIGURE 1.—*P*[*ry*$^+$*1*] contains the 8.3-kb *Sal*I fragment from the *rosy* locus (COTÉ *et al.*, 1986) inserted into the *Xho*I site of the *P* element derivative p6.1 (RUBIN and SPRADLING 1982). *P*[*ry*$^+$*11*] contains the 7.2-kb *Hin*dIII fragment of the *rosy* gene inserted into the *Hin*dIII site of the transformation vector pCIW4 (RUBIN and SPRADLING 1983). In both elements, the *rosy* gene is transcribed toward the 3′ *P* element end. Restriction sites: S (*Sal*I), H (*Hin*dIII), X (*Xho*I), R (*Eco*RI), P/R (*Pvu*II in *P*[*ry*$^+$*1*], *Eco*RI in *P*[*ry*$^+$*11*]).

in the male parent, transposition events were distinguished from maternal *XX* ↔ O nondisjunction by scoring the dominant hairy wing effect (GARCIA-BELLIDO 1979; CAMPUZANO *et al.* 1986) in each e ry$^+$ male.

The chromosomal location of each new insertion was determined by a series of additional crosses (Figure 3). Each male containing an independent transposition was crossed to three ry^{506}/*TM3*, *Sb* ry^{RK} e^s virgins. When possible, we chose transpositions that contained the *TM3* balancer chromosome, and were therefore Sb, thus allowing us to score segregation in the progeny of this cross. (Transpositions recovered as *mwh* ry^{506} *e*/*P*[*ry*$^+$*11*], *mwh* ry^{506} *e* were crossed to ry^{506}/*TM3*, *Sb* ry^{RK} e^s virgins. In the next generation, ry$^+$ Sb males and females were collected to establish a third chromosome stock, and, ry$^+$ Sb males were crossed to *Sco*/*CyO*, cn^2; ry^{506} virgins to score segregation. If the cross to *CyO* indicated that the line carried a third chromosome insertion (that is, all the Sb progeny were ry), *mwh* ry^{506} *e* *P*[*ry*$^+$*11*]/*TM3*, *Sb* ry^{RK} e^s virgins and males were collected from the established stock to eliminate the *mwh* ry^{506} *e* chromosome that did not bear the *P*[*ry*$^+$*11*] insertion.)

We analyzed the results of the cross to the ry^{506}/*TM3*, *Sb* ry^{RK} e^s virgins in the following way. If there were no ry$^+$Sb e$^+$ progeny, and, all the ry progeny were also Sb, then the insert was on the third chromosome and we established a balanced stock by crossing ry$^+$Sb e females and males *inter se*. If, however, there were any ry$^+$Sb e$^+$ progeny, then a *P*[*ry*$^+$*11*] insert existed that was not on the third chromosome and it was presumed to be on the second chromosome. In that case, *ry*$^+$; ry^{506}/*TM3*, *Sb* ry^{RK} e^s males were crossed to *Sco*/*CyO*,cn^2; ry^{506} virgins to set up a second chromosome balanced line. (By using ry$^+$Sb e$^+$ males, insertions on both chromosomes *2* and *3* were recovered as second chromosome only lines.) We then crossed ry$^+$ Cy female and male progeny *inter se*.

In 75 lines, the insertion was not balanced by the previous crosses. These lines either contained fourth chromosome insertions, double insertions or remained unstable due to inheritance of *P*[*Js*] through transposition or male recombination. We crossed ry$^+$ Cy males to ry^{506}; +/ci^D virgins, establishing *inter se* crosses to maintain a stock and outcrossing ry$^+$ ci^D males to ry^{506} virgins to verify the previous segregation analysis. Thirty-five of the lines were fourth chromosome insertions, while the remaining 40 contained

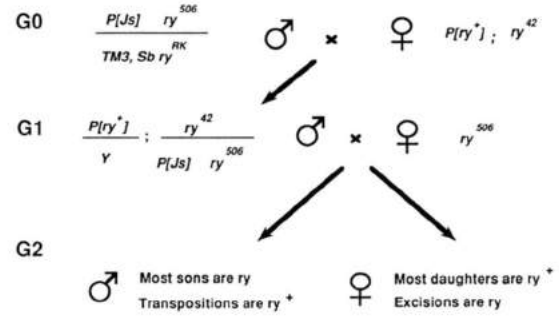

FIGURE 2.—Crossing scheme for identifying *P*[*ry*$^+$] transposition and excision events. See text in MATERIALS AND METHODS and RESULTS.

double, unstable or incorrectly scored insertions.

Mutations not associated with *P*[*ry*$^+$] insertions: Although marked, isogenized chromosomes were utilized in this screen, several anomalous results were obtained. An unusually high fraction of the third chromosome insertions, approximately 1/3, were homozygous lethal. Complementation analysis between nine randomly selected lethal lines revealed that three failed to complement each other. We then crossed virgin females from one of these lines, *bl(3)ry-13*, to all of the remaining third chromosome lethal stocks. A total of 106 lines failed to complement *bl(3)ry-13*. This lethality was assumed to result from a background mutation that occurred in the *P*[*ry*$^+$*11*]; *cn*; *mwh ry e* strain while it was being expanded prior to the initiation of the screen.

Three other unexpected results were observed. (1) Eleven of the 541 second chromosome lines were lethal over the *CyO* balancer. The lethal mutation was readily separable from the *rosy*$^+$ marker in ten such chromosomes. We have observed this kind of lethality in combination with *CyO* in two subsequent mutagenesis screens (L. YUE, C. BERG and A. SPRADLING, unpublished results). Since the presence of a preexisting *Cy* inversion was rigorously excluded, these results suggest that new mutations that are lethal over *CyO* are frequently generated independent of a stable *P*[*ry*$^+$*11*] transposition. (2) Eleven lines carried a mutation at the *sparkling* locus (4, 102D-F) that did not segregate with the *P*[*ry*$^+$*11*] element (four lines carried *P*[*ry*$^+$*11*] on the second chromosome, seven on the third). We never observed any such phenotype in our starting strains or balancer stocks. These lines have not been characterized further. (3) An allele of *transformer-2* was recovered that lacked a *P* element insertion at the *tra-2* locus. We speculate that some mutations result from insertion followed by immediate imprecise excision of the *P* element.

Insertions of an internally deleted element might cause some of the anomalous mutations, if they were recovered in conjunction with a transposed intact *P*[*ry*$^+$*11*] within the same *Y*-bearing sperm. The anomalies cannot result from transposition of an intact Jumpstarter element, since such lines are observed to rapidly display ry$^-$ phenotypes and inconsistent segregation of the *ry*$^+$ marker. Recently, we have identified rosy$^-$ derivatives of a *P*[*ry*$^+$] mutator segregating in some *P*[*ry*$^+$] single insert lines (G. KARPEN, R. GLASER and A. SPRADLING, unpublished results). Thus both a deleted and an intact mutator element can be recovered simultaneously in some males.

Generation of *TM6B*, ry^{CB}: In order to obtain a third chromosome balancer carrying both a dominant larval marker (*Tubby*) and a *rosy* allele, we mutagenized the *TM6B* chromosome, a variant of *TM6* carrying *Hu* and *Tb* (CRAYMER 1984). Canton-S males carrying the *TM6B* chromosome

TABLE 1

Transposition frequencies of mutator elements from various *X* chromosome sites

Site	F_1 crosses[a]	Jumps[b]	Jumping rate[c]	ry^+ males[d]	ry males[e]	Transposition rate[f]	ry females[g]	ry^+ females[h]	Excision rate[i]
P[ry⁺1]									
4D	99	4	4	7	3724	0.42	9	3864	0.23
7D	99	3	3	4	3074	0.29	15	3199	0.47
9AB	103	11	11	21	2567	1.83	14	2716	0.51
12D	107	7	7	10	2817	0.80	11	2950	0.37
P[ry⁺11]									
1F	96	49	51	172	2891	12.63	75	3117	2.35
9E	103	67	36	110	3034	7.87	28	3252	0.85
18A	104	52	50	150	2841	11.28	63	3060	2.02
1F	136[j]	113	83						
9E	211[j]	103	49						
18A	147[j]	85	58						

[a] Number of fertile G_1 crosses as shown in Figure 2.

[b] Jumps are the number of G_1 vials containing at least one ry^+ son. (This value differs slightly from that reported in COOLEY, BERG and SPRADLING (1988) because jumps are defined in that paper as the number of G_1 vials containing at least one ry^+ son that is fertile and does not contain Jumpstarter).

[c] Jumping rate is the percent of jumps/fertile G_1 crosses.

[d] Total number of ry^+ sons in all vials from these crosses; that is, the number of transpositions disregarding premeiotic clusters.

[e] Total number of ry sons; therefore, the number of nontransposition events.

[f] Transposition rate is the percent of the ratio of ry^+ males/ry males × 9/4, since only 4/9 of the chromosomes are recovered. See methods and text.

[g] Total number of ry daughters; that is, the number of excisions disregarding premeiotic clusters.

[h] Total number of ry^+ daughters, therefore, the number of nonexcision events.

[i] Excision rate is the percent of the ratio of ry females/ry^+ females. See methods and text.

[j] Number of fertile G_1 crosses, but with different, marked chromosomes (see Methods).

were irradiated with 4300 rad of γ-rays, then mated to ry^{506} virgins. We obtained 5 new *rosy* alleles on *TM6B* chromosomes among the approximately 27,000 progeny that were screened. Only one of those alleles was viable and fertile and, therefore, retained. That mutation is a 8.23-kb deletion in the 3′ end of the *ry* gene (see COTÉ *et al.* 1986), mapping between the central *Eco*RI site and the 3′ *Hin*dIII site (data not shown).

***In situ* hybridization:** *l(3)ry/TM3, Sb* ry^{RK} e^s males were mated to *TM2, red* ry^{SC} Ubx^{130} e^s/ *TM6B, Hu* ry^{cB} *e Tb ca* females to establish stocks useful in identifying the $P[ry^+]$ chromosomes in larvae. *l(3)ry/TM2, red* or *l(3)ry/TM6, Tb* males were mated to three *mwh red* virgins in yeasted vials. Adults were transferred daily to new vials. Wet yeast was added to the vials three days after egg-laying to ensure production of well-fed larvae. Late third instar larvae carrying the $P[ry^+]$ chromosome were identified as either red^+ (if the starting males carried *TM2*) or Tb^+ (if the starting males carried *TM6*).

Chromosomes from the salivary glands of these larvae were prepared and hybridized as described (PARDUE 1986), with the following exceptions. Slides were hybridized with linearized plasmids carrying the $rosy^+$ gene labeled by hexamer-primed reactions (FEINBERG and VOGELSTEIN 1983) using biotin-dUTP from ENZO. Signals were detected using an alkaline phosphatase system (BRL) according to ENGELS *et al.* (1986), except that the final staining period usually required only 10–30 min, rather than 2 hr.

RESULTS

Transposition frequencies of two $P[ry^+]$ elements: To investigate the effects of element location and structure on transposition frequency, we studied two different $P[ry^+]$ elements, $P[ry^+1]$ and $P[ry^+11]$, previously named ry1 and ry11 respectively (SPRADLING and RUBIN 1983). These elements are similar in size (9.3 kb *vs.* 8.2 kb), but differ in the amount of genomic DNA encoding the $rosy^+$ gene and its exact location within a 1.1-kb defective *P* element (Figure 1). Four lines containing single insertions of $P[ry^+1]$ and three lines containing $P[ry^+11]$ each located at diverse positions along the *X* chromosome (SPRADLING and RUBIN 1983) were employed for our studies.

To mobilize these elements we used Jumpstarter-1 (*P[Js]*), a complete *P* element located at cytological position 90A. *P[Js]* is a weak source of transposase as judged by its ability to mobilize the *P* elements present at the sn^w locus (sn^w reverts to sn^+ or sn^e in 6% of progeny) (COOLEY, KELLEY and SPRADLING 1988). Although *P[Js]* is capable of moving itself, it does so only rarely and is easily maintained by outcrossing (see COOLEY, KELLEY and SPRADLING 1988).

Transposition frequencies were measured using the crosses shown in Figure 2 (see also MATERIALS AND METHODS). Flies containing each *X*-linked $P[ry^+]$ element were crossed to the strain bearing the complete *P* element, Jumpstarter-1. Single male G_1 progeny carrying both mutator and jumpstarter elements were then mated in individual vials to ry virgins. Parents were discarded after 7 days. Both transposition and excision frequencies were calculated by scoring the rosy phenotype of the G_2 progeny (Table 1). To eliminate the effects of premeiotic clusters, which generate multiple $rosy^+$ G_2 progeny from a single event, we also calculated a minimum transposition

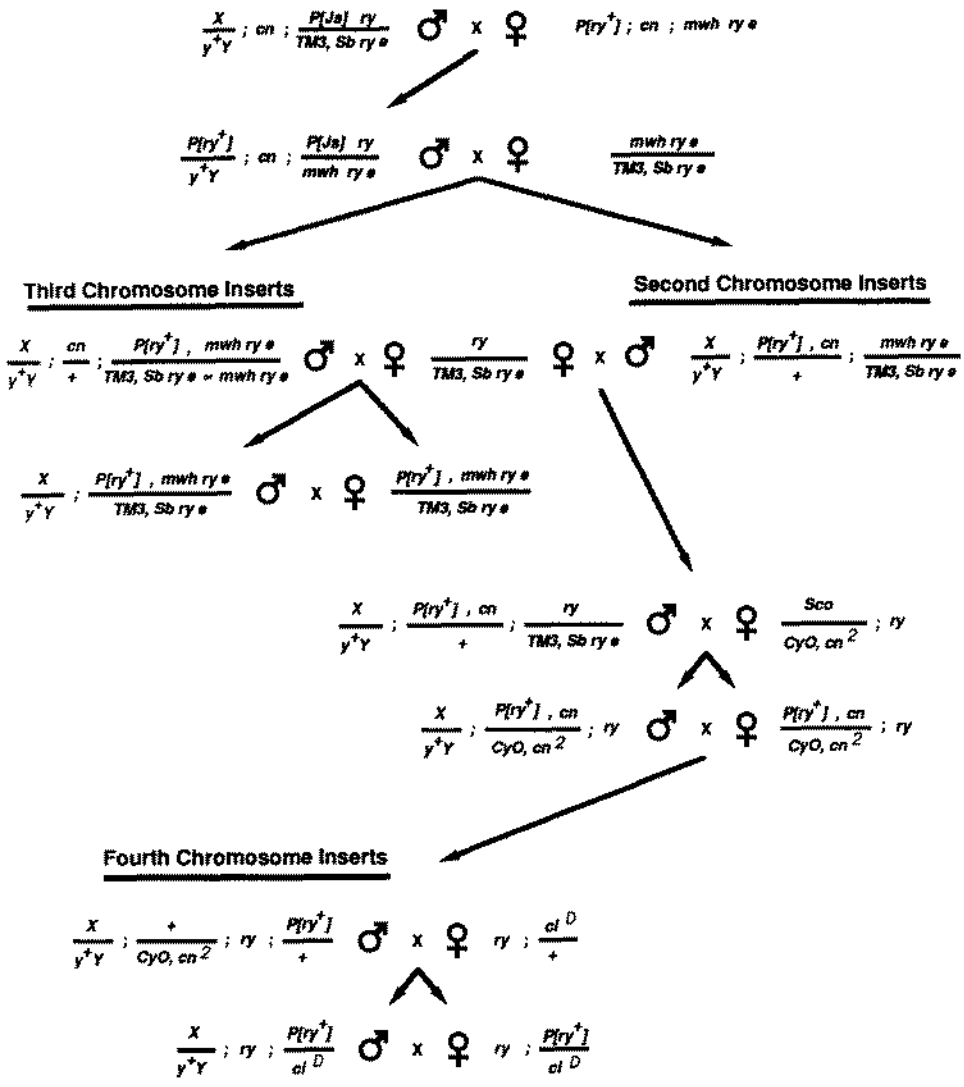

FIGURE 3.—Crosses employed to mutagenize genetically marked autosomes. Stocks were established with single, ry$^+$ sons from individual pair matings. Following segregation analysis, insertion-bearing chromosomes were made homozygous and flies were examined for lethal, sterile, and visible mutant phenotypes. See MATERIALS AND METHODS.

frequency or "jumping frequency" as the fraction of G1 crosses producing at least one transposition. The mobility of the *P*[*ry*$^+$*11*] elements was measured a second time after isogenizing new, marked chromosomes in the mutator and jumpstarter stocks (Table 1, "footnote j").

These experiments revealed several surprising results. The starting location had some influence on the transposition rates, particularly in the case of *P*[*ry*$^+$*1*]. However, there was a highly significant difference (*t*-test; $P < 0.001$) between the transposition rates of the *P*[*ry*$^+$*1*] and *P*[*ry*$^+$*11*] elements regardless of insertion site, an average of 0.83 ± 0.70 *vs.* 10.6 ± 2.45. *P*[*ry*$^+$*1*] insertions also excised at lower frequencies (*t*-test; $P < 0.05$) than *P*[*ry*$^+$*11*] insertions (0.39 ± 0.12 *vs.* 1.74 ± 0.79). Since the two elements are similar in size, some aspect of element structure is likely to influence the frequency of both excision and transposition.

P[*ry*$^+$*11*] transposed at surprisingly high frequency considering the weak nature of the Jumpstarter-1 element. Nearly 13% of progeny chromosomes contained *P*[*ry*$^+$*11*] transpositions, about half the transposition rate of an average *P* element when mobilized by the 15 complete *P* elements in a typical *P* strain during hybrid dysgenesis. *P*[*ry*$^+$*11*] therefore appears to transpose more readily than many other previously described *P* elements.

Insertional mutagenesis with *P*[*ry*$^+$*11*]: The highly mobile *P*[*ry*$^+$*11*] element was used to generate a new collection of insertional mutations. A protocol similar to previous single *P* element mutagenesis screens was employed (Figure 3) but with several technical improvements (see MATERIALS AND METHODS). From 3267 crosses involving males bearing both *P*[*ry*$^+$*11*] and Jumpstarter-1, we established 1019 lines containing independent transpositions (Table 2). The chromosomal location of each new insertion was mapped genetically using dominantly marked balancer chromosomes (Table 3). An equal number of second and third chromosome insertions is expected, because only half of the insertions on the two second chromosomes are recovered in the absence of Jumpstarter, while all

TABLE 2

Results of $P[ry^+11]$ mutagenesis screen

Mutagenesis step	No.
Total no. of fertile G_1 crosses	3267
G_1 crosses with ry^+ sons (G_2) in which Jumpstarter-1 also segregated away	1201
Dead/sterile G_2 ry^+ sons	182
Established lines	1019

TABLE 3

Segregation analysis of $P[ry^+11]$ insertions

Chromosome	No. of insertions
Y	1
y^+Y	4
2	541
3	398
4	35
Double, unstable or scoring error	40

the insertions on the *mwh* ry^{506} *e* third chromosome segregate from Jumpstarter and are recovered. The observed excess of insertions linked to the second chromosome was in part an artifact of the way the segregation tests were performed. Lines containing insertions on both the second and third chromosomes would have failed to score as third chromosome inserts, and would have been recovered as second chromosome stocks (see MATERIALS AND METHODS). In addition, second chromosome insertions occurring as premeiotic clusters are also recovered more efficiently than third chromosome clusters, since premeiotic transposition increases the chance of obtaining a sperm that does not carry the Jumpstarter chromosome.

Analysis of Y-linked $P[ry^+11]$ insertions: Five $P[ry^+11]$ insertions were linked to the *Y* chromosome (Table 3). Four of these strains carried a y^+Y chromosome, so that insertion might have occurred on the small region of *X* euchromatin present on this chromosome (MULLER 1948). The fifth line (YR) resulted from insertion on a normal *Y* chromosome. To determine whether a *Y*-autosome translocation rather than an insertion was responsible for the observed linkage, ry^+ males from all five *Y* insertion lines were crossed to $ry^{506}/TM3$, *Sb* ry^{RK} e^s virgins and *Sco/CyO*, cn^2; ry^{506} virgins (Figure 3). ry^+ Sb males and ry^+ Cy males were then crossed to ry^{506} virgins to determine if inheritance of ry^+ required only the *Y* chromosome. In four of these five lines, Y1, Y2, Y4, and YR, progeny were recovered in which both $P[ry^+11]$ and *CyO* or $P[ry^+11]$ and *TM3*, *Sb* were present. These results demonstrated that a large *Y*-autosome translocation could not account for the observed *Y* linkage. Line Y3 failed to produce any ry^+ Cy sons indicating that second chromosome material had combined with the *Y* and both chromosomes must be inherited to prevent aneuploidy.

As described above, one line (YR) resulted from insertion on a normal *Y* chromosome. Although insertion of *P* elements into heterochromatin is rare, PRESTON and ENGELS (1989) previously obtained genetic evidence that active *P* elements resided on a *Y* chromosome isolated from a *P* strain by outcrossing. Consistent with the presumed heterochromatic nature of the $P[ry^+11]$ YR insertion, not all progeny of single YR males crossed to ry females expressed the rosy eye color phenotype (2–80% of male progeny). All sons, however, transmitted the gene. In contrast, YR males derived from an attached-*X* stock were >95% $rosy^+$. This behavior may reflect very low levels of rosy expression in YR males due to a heterochromatic position effect on the gene. Eye color variegation is not expected since rosy is not cell autonomous in the eye (see RUSHLOW and CHOVNICK 1984; RUSHLOW, BENDER and CHOVNICK 1984).

Comparing targets of $P[ry^+11]$ and $P[neo^R]$ insertion: Complementation analysis was used to compare in detail the insertion sites derived in these experiments from the $P[ry^+11]$ element at locus 1F with the insertion sites derived from a $P[neo^R]$ element at locus 9C (COOLEY, KELLEY and SPRADLING 1988). Only lines in which a mutant phenotype was associated with transposon insertion could be studied. Lethal and male sterile mutations on the third chromosome were used in these comparisons. To reduce the number of required crosses, we first localized the insertion sites of the $P[ry^+11]$ elements by *in situ* hybridization. All third chromosome lethal and sterile lines were crossed with those insertions located within the same lettered cytogenetic unit (or one unit on either side) and the trans heterozygotes were scored for complementation. The results of these studies are compiled in Table 4 and are plotted in Figure 4. Following localization by *in situ* hybridization, several insertions were recognized as alleles of known mutations following appropriate complementation crosses. The known mutations mutated by $P[neo^R]$ and $P[ry^+11]$ are summarized in Table 5.

The complementation tests revealed that most genes were hit only once. Since a large number of genes are targets for *P* element insertion, this result is not surprising. However, those cases where genes were mutated more than once in the two screens were informative. One previously undescribed gene mutated to male sterility in five independent lines, three with $P[neo^R]$ and two with $P[ry^+11]$. Another probable hotspot was the cell cycle gene *string*, which was mutated twice by both elements. *String* mutations have been reported in other single *P* element mutagenesis

TABLE 4

Third chromosome sterile and lethal lines

Site	Mutant	Site	Mutant
A. Third chromosome *P[ry⁺11]* lethals			
61A	*ry 122*	84A5,7	*ry 16*
61D	*ry 111*	84D/E	*ry 60*
65A	*ry 50*	84F	*ry 78*
66D10	*ry 9, ry 61, ry 149: hairy*	84F	*ry 87*
		89A	*ry 15*
68B/C	*ry 116*	90C	*ry 151*
68D	*ry 98*	90E	*ry 114*
68D/E	*ry 40, neo 17: cyclin A*	91C	*ry 133*
		92F	*ry 141*
70C	*ry 99*	93B1,2	*ry 136*
70F and 75D	*ry 125*	93B	*ry 84*
73A	*ry 89*	93E3,4	*ry 6*
74C	*ry 59*	93F5,6	*ry 93*
75C3,4	*ry 100: all discs small*	94C/D	*ry 107*
		94E	*ry 146*
76D	*ry 119*	94F	*ry 69*
78C/D	*ry 3*	96B	*ry 73*
79DE	*ry 102*	97F	*ry 160*
79E3,4	*ry 127*	99A	*ry 80, ry 129,* neo 61, *neo 62: string*
79F	*ry 85*		
82B/C, 82C1	*ry 103, ry 147*		
82E	*ry 74*	99F	*ry 46, neo 63*
83C1,2	*ry 82*	100A	*ry 105*
B. Third chromosome *P[ry⁺11]* and *P[neo^R]* male steriles			
61D	*neo 1*	88B	*neo 6*
62B	*neo 4*	88D	*neo2, 3, 6* and *ry1, 3*
67DE	*ry 5*		
83D	*neo 5*	96B	*ry 2*
85A4,5	*neo 30*	96D	*ry 4*

experiments as well (EDGAR and O'FARRELL 1989; BIER *et al.* 1989). Two genes were hit once by both elements (*cyclin A*, 68D/E; *l(3)*99F). Four genes were hit more than once by a single element (*hairy*, 66D10; *l(3)ry* 82C1; *l(3)neo* 94D/E, and *l(3)neo* 82E). As discussed below, these results indicated that the targets sites of the two elements overlapped.

DISCUSSION

Transposition frequencies: Measurement of transposition rates of seven specific *P* insertions revealed that chromosome position does influence mobility, at least in the case of the *P[ry⁺1]* elements. This element transposed six times more frequently when located at 7D than at 9AB sites. This difference could not be ascribed to premeitotic clusters because the jumping rates also differed significantly (χ^2; $P < 0.05$). In contrast, only weak position effects were observed among the three *P[ry⁺11]* insertions. The insertion at

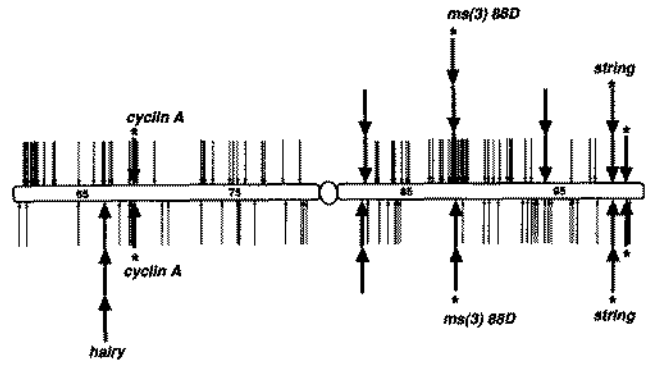

FIGURE 4.—Comparison of the insertion sites of third chromosome lethals and male steriles generated by insertion of *P[neo^R]* (above the line) and *P[ry⁺11]* (below the line). Thin arrows represent single hits, thick arrows represent multiple hits. Starred arrows represent loci targeted by both elements. Insertion sites for the lethal *P[neo^R]* alleles were obtained from Table 2 in COOLEY, KELLEY and SPRADLING (1988). Insertion sites for all the male steriles and for the lethal *P[ry⁺11]* alleles are listed in Table 4, this paper.

9E was mobilized slightly less frequently than the same element located at 1F or 18A in two separate experiments. The excision rate at the 9E site was also lower, however, these differences were not statistically significant.

Differences in the structure of the *P[ry⁺1]* and the *P[ry⁺11]* elements had a large effect on transposition frequency. Averaged over the different starting sites, *P[ry⁺11]* elements transposed greater than 12-fold more frequently than *P[ry⁺1]* elements. Differences in element structure rather than position effects probably also account for most previously reported differences in *P* element transposition rates (ROBERTSON *et al.* 1988). Although the diverse elements tested transposed in 0.03–20% of progeny, a nearly 10^3-fold difference, no single element varied more than 9-fold at different chromosomal sites.

The greater efficiency of the *P[ry⁺11]* element may result from the location in the parent *P* element where the *rosy* gene is inserted. Position 882, the insertion site in *P[ry⁺11]*, lies just 12 nucleotides 5′ to the site of the internal deletion in the defective *P* element. In the *P[ry⁺1]* elements, however, *rosy* is inserted at position 733, 149 bp farther 5′. Although the region between these two insertion sites is not essential for transposition (RUBIN and SPRADLING 1983; MULLINS, RIO and RUBIN 1989), maintenance of the first 882 bp might facilitate higher levels of transposition.

Target site specificity: There are several ways in which the initial site or structure of a transposon might affect its target sites. The distance between the initial site and individual targets is likely to vary within germ cell nuclei where transpositions occur. If transpositions usually take place within a small radius of the starting site, then nonrandom, three-dimensional organization of chromosomes in these cells would generate site-specific differences in observed target sites.

TABLE 5

Genes independently mutated by elements originating from different sites

Gene[a]	Function	Ref[b]	Independently mutated?
all discs small[c]	Disc development	1, [c]	Yes
bag of marbles[d]	Female fertility	2	No
big brain	Neurogenesis	2	?
chickadee[e]	Female fertility	2, 3, 4	Yes
cyclin A[f]	Cell cycle	1, 2, 5	Yes
dorsal	d/v polarity	3, 4	Yes
fasciclin III[g]	Nervous system	4, 6	Yes
flipper	Growth	1, 4	Yes
hairy	Segmentation	1, 7	Yes
kelch[e]	Female fertility	2	No
ms(3)88D[h]	Male fertility	1, 2	Yes
rotated abdomen	Morphogenesis	2, 4	Yes
staufen	a/p polarity	1	No
string	Cell cycle	1, 2, 5	Yes
vasa	Germ line	3, 4, 5	Yes

[a] All genes are described in LINDSLEY and ZIMM (1986, 1990) unless otherwise noted.

[b] References: (1) this paper; (2) COOLEY, KELLEY and SPRADLING (1988); (3) L. YUE, C. BERG and A. SPRADLING (unpublished); (4) A. C. SPRADLING, unpublished; (5) BIER *et al.* (1989); (6) BELLEN *et al.* (1989); (7) INGHAM *et al.* (1985).

[c] A third chromosome disc mutation recovered in the screen described in DEAROLF, HERSPERGER and SHEARN (1988).

[d] MCKEARIN and SPRADLING (1990).

[e] Second chromosome female sterile mutations recovered in the screen described in SCHÜPBACH and WIESCHAUS (1989).

[f] LEHNER and O'FARRELL (1989).

[g] Patel, Snow and GOODMAN (1987).

[h] This paper, Table 4B.

Alternatively, the local DNA sequence near the starting site or of the element itself might influence target selection. For example, following cleavage at a potential target site, a sequence-dependent interaction with the initial site might be required to bring the target close enough for transposition to occur.

The amount of overlap between the target sites of the $P[ry^+11]$ (1F) and $P[neo^R]$ (9C) insertions can be estimated using the Poisson distribution. A minimum of 582 third chromosome target sites for lethal mutation by the $P[neo^R]$ (9C) element must exist, based on three coincident mutations among 63 lines tested (COOLEY, KELLEY and SPRADLING 1988). If the $P[ry^+11]$ (1F) insertions are selected from the same pool of target sites, then about 2 internal coincidences would be expected among the 45 lethal $P[ry^+11]$ insertions. Three additional lethal insertions would be expected to correspond to sites mutated in the $P[neo^R]$ collection. If the elements select different genes as targets, then a lower level of overlap should exist between genes mutated by $P[ry^+11]$ and $P[neo^R]$.

About the predicted degree of overlap between the two distributions was observed. Two genes were mutated twice by $P[ry^+11]$ and one gene three times (Table 4A). Three genes were mutated by both $P[ry^+11]$ and $P[neo^R]$ elements. However the existence of insertional hotspots complicated the comparison. The *string* locus was mutated twice in both samples, indicating that it represents a mutational hotspot. Targets mutable to male sterility also overlapped, and corresponded to hotspots. One locus in region 88D mutated in 3 of 7 $P[neo^R]$ insertions. Complementation analysis revealed that this same gene was disrupted in 2 of 5 $P[ry^+11]$ lines. Additional evidence of overlap among hotspots exists for the *cyclin A* as well as *string* loci. Two *string* alleles and two *cyclin A* alleles were recovered among mutations induced following transposition of another *X*-linked but different mutator *P* element (BIER *et al.* 1989). The existence of hotspots means that the total number of third chromosome loci mutable to lethality is actually much greater than the 582 derived from the Poisson distribution. Therefore we could conclude that the $P[neo^R]$ and $P[ry^+11]$ elements target an overlapping set of sites for insertion; however, whether only insertional hotspots overlap or if all sites are identical cannot be determined from our data. Evidence that many genes can be mutated from different starting sites is summarized in Table 5.

One locus in our experiments appeared to be a preferred target of the $P[ry^+11]$ element. The *hairy* gene was mutated by three $P[ry^+11]$ (1F) insertions but was not mutated by $P[neo^R]$ (9C). It is unlikely that mutation to lethality at *hairy* requires a sequence present only within the $P[ry^+11]$ element, since *P* elements lacking any heterologous sequences can cause lethal *hairy* mutations (INGHAM *et al.* 1985). Our experiments do not completely exclude the probability on statistical grounds that the *hairy* gene is equally mutable with both the $P[ry^+11]$ (1F) and $P[neo^R]$ (9C) transposons. Given a hotspot that receives three mutations, the chance that all three will fall within the $P[ry^+11]$ set would be $(1/2)^3 = 0.125$ if the screens had been of equal size, or, $(45/(45 + 63))^3 = 0.072$, when the actual number of lethals in each group is considered. Nonetheless, these observations argue that starting location or structure may influence target site selection in certain cases.

Much higher mutation rates have been observed for several genes in single element screens than during hybrid dysgenesis (KIDWELL 1987). For example, two alleles of *big brain* were recovered among 725 second chromosomes examined in the $P[neo^R]$ screen compared to 0/6000 in the dysgenic screen carried out by YEDVOBNIK *et al.* (1985, 1988). Likewise, three of 398 $P[ry^+11]$ insertions produced *hairy* mutations, compared to only seven of 20,000 chromosomes mutagenized during hybrid dysgenesis (INGHAM *et al.*, 1985). Similarly, mutations at *staufen* and *all discs small* were obtained at higher frequency by mobilizing a specific element compared to hybrid dysgenesis. Target site preferences resulting from differences in the structure

and starting sites of the mutagenic elements might explain some of these observations. Greater mutability could be due to the larger size of the specific elements: insertion of a 15-kb element carrying *lac-Z*, plasmid sequences and a ry^+ marker might have a more deleterious effect on a gene than inserting a 1 kb *P* element deletion-derivative. Rate discrepancies could be more apparent than real, however, since the number of elements actually mobilized during hybrid dysgenesis has rarely been monitored. Clearly more studies comparing the target sites of specific elements will be necessary to resolve these issues.

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LITERATURE CITED

BELLEN, H., C. O'KANE, C. WILSON, U. GROSSNIKLAUS, R. PEARSON and W. GEHRING, 1989 *P* element-mediated enhancer detection: a versatile method to study development in *Drosophila*. Genes Dev. **3**: 1288–1300.

BENZ, W. K., and W. R. ENGELS, 1984 Evidence for replicative transposition of *P* elements in *Drosophila melanogaster*. Genetics **107**: s10.

BIER, E., H. VAESSIN, S. SHEPHERD, K. LEE, K. MCCALL, S. BARBEL, L. ACKERMAN, R. CARRETTO, T. UEMURA, E. GRELL, L. Y. JAN and Y. N. JAN, 1989 Searching for pattern and mutation in the *Drosophila* genome with a *P-lacZ* vector. Genes Dev. **3**: 1273–1287.

BINGHAM, P. M., M.G. KIDWELL and G. M. RUBIN, 1982 The molecular basis of *P-M* hybrid dysgenesis: the role of the *P* element, a *P* strain-specific transposon family. Cell **29**: 995–1004.

BOUROUIS, M., and G. RICHARDS, 1985 Remote regulatory sequences of the *Drosophila* glue gene *sgs3* as revealed by *P* element transformation. Cell **40**: 349–357.

CAMPUZANO, S., L. BALCELLS, R. VILLARES, L. CARRAMOLINO, L. GARCIA-ALONSO and J. MODOLELL, 1986 Excess function *Hairy-wing* mutations caused by *gypsy* and *copia* insertions within structural genes of the *achaete-scute* locus of *Drosophila*. Cell **44**: 303–312.

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.

COTÉ, B., W. BENDER, D. CURTIS and A. CHOVNICK, 1986 Molecular mapping of the *rosy* locus in *Drosophila melanogaster*. Genetics **112**: 769–783.

CRAYMER, L., 1984 Report on new mutants. Drosophila Inform. Serv. **60**: 234–236.

DANIELS, S., M. MCCARRON, C. LOVE, S. CLARK and A. CHOVNICK, 1985 Dysgenesis induced instability of *rosy* locus transformation in *Drosophila melanogaster*: analysis of excision events and the selective recovery of control element deletions. Genetics **109**: 95–117.

DEAROLF, C., E. HERSPERGER and A. SHEARN, 1988 Developmental consequences of awd^{b1}, a cell autonomous lethal mutation of *Drosophila* induced by hybrid dysgenesis. Dev. Biol. **129**: 159–168.

EDGAR, B., and P. O'FARRELL, 1989 Genetic control of cell division patterns in the *Drosophila* embryo. Cell **57**: 177–187.

EEKEN, J., F. SOBELS, V. HYLAND and A. SCHALET, 1985 Distribution of MR-induced sex-linked recessive lethal mutations in *Drosophila melanogaster*. Mutat. Res. **150**: 261–275.

EGGLESTON, W., D. JOHNSON-SCHLITZ and W. ENGELS, 1988 *P-M* hybrid dysgenesis does not mobilize other transposable element families in *Drosophila melanogaster*. Nature **331**: 368–370.

ENGELS, W. R., 1979 Extrachromosomal control of mutability in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **76**: 4011–4015.

ENGELS, W. R., 1985 Guidelines for *P* element transposon tagging. Drosophila Inform. Serv. **61**: 1–7.

ENGELS, W. R., 1988 *P* elements in *Drosophila*, pp. 437–484 in *Mobile DNA*, edited by D. BERG and M. HOWE. ASM Publications, Washington, D.C.

ENGELS, W. R., and C. R. PRESTON, 1981 Identifying *P* factors in *Drosophila* by means of chromosome breakage hotspots. Cell **26**: 421–428.

ENGELS, W. R., and C. R. PRESTON, 1984 Formation of chromosome rearrangements by P factors in Drosophila. Genetics **107**: 657–678.

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

FEDOROFF, N., 1988 Maize transposable elements, pp. 375–411 in *Mobile DNA*, edited by D. BERG and M. HOWE. ASM Publications, Washington, D.C.

FEINBERG, A. P., and B. VOGELSTEIN, 1983 A technique for radiolabeling DNA restriction endonuclease fragments to high specificity. Anal. Biochem. **132**: 6–13.

FINNEGAN, D. J., 1989 Eukaryotic transposable elements and genome evolution. Trends Genet. **5**: 103–107.

GARCIA-BELLIDO, A., 1979 Genetic analysis of the *achaete-scute* system of *Drosophila melanogaster*. Genetics **91**: 491–520.

GEYER, P., K. RICHARDSON, V. CORCES and M. GREEN, 1988 Genetic instability in *Drosophila melanogaster*: *P* element mutagenesis by gene conversion. Proc. Natl. Acad. Sci. USA **85**: 6455–6459.

HAWLEY, R. S., R. STEUBER, C. MARCUS, R. SOHN, D. BARONAS, M. CAMERON, A. ZITRON and J. 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.

INGHAM, P.W., S. M. PINCHIN, K. R. HOWARD and D. ISH-HOROWICZ, 1985 Genetic analysis of the *hairy* locus in *Drosophila melanogaster*. Genetics **111**: 463–486.

KEITH, T., M. RILEY, M. KREITMAN, R. C. LEWONTIN, D. CURTIS and G. CHAMBERS, 1987 Sequence of the structural gene for xanthine dehydrogenase (*rosy* locus) in *Drosophila melanogaster*. Genetics **116**: 67–73.

KELLEY, M. R., S. KIDD, R. L. BERG and M. W. YOUNG, 1987 Restriction of *P* element insertions at the *Notch* locus of *Drosophila melanogaster*. Mol. Cell. Biol. **7**: 1545–1548.

KIDWELL, M. G., 1986 *P-M* Mutagenesis, pp. 59–81 in *Drosophila: A Practical Approach*, edited by D. B. ROBERTS. IRL Press, Oxford.

KIDWELL, M. G., 1987 A survery of success rates using *P* element mutagenesis in *Drosophila melanogaster*. Drosophila Inform. Serv. **66**: 81–86.

LASKI, F. A., D. C. RIO and G. M. RUBIN, 1986 Tissue specificity of *Drosophila P* element transposition is regulated at the level of mRNA splicing. Cell **44**: 7–19.

LEE, C., D. CURTIS, M. MCCARRON, C. LOVE, M. GRAY, W. BENDER and A. CHOVNICK, 1987 Mutations affecting expression of the *rosy* locus in *Drosophila melanogaster*. Genetics **116**: 55–66.

LEHNER, C., and P. O'FARRELL, 1989 Expression and function of *Drosophila* cyclin A during embryonic cell cycle progression.

Cell **56**: 957–968.

LINDSLEY, D. L., and E. H. GRELL, 1968 *Genetic Variations of Drosophila melanogaster*. Carnegie Inst. Wash. Publ. 627.

LINDSLEY, D. L., and G. ZIMM, 1985 The genome of *Drosophila melanogaster*, Part 1: Genes A-K. Drosophila Inform. Serv. **62**.

LINDSLEY, D. L., and G. ZIMM, 1986 The genome of *Drosophila melanogaster*, Part 2: Lethals, maps. Drosophila Inform. Serv. **64**.

LINDSLEY, D. L., and G. ZIMM, 1987 The genome of *Drosophila melanogaster*, Part 3: Rearrangements. Drosophila Inform. Serv. **65**.

LINDSLEY, D. L., and G. ZIMM, 1990 The genome of *Drosophila melanogaster*, Part 4: Genes L-Z. Drosophila Inform. Serv. **68**.

MCKEARIN, D., and A. C. SPRADLING, 1990 Bag of marbles: a gene required for the initiation of both male and female gametogenesis in Drosophila. Genes Dev. **4**: 2242–51.

MULLER, H. 1948 Report on new mutants. Drosophila Inform. Serv. **22**: 73–74.

MULLINS, M., D. RIO and G. M. RUBIN, 1989 Cis-acting sequence requirements for *P* element transposition. Genes Dev. **3**: 729–738.

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

PARDUE, M. L., 1986 In situ hybridization to DNA of chromosomes and nuclei, pp. 111–137 in *Drosophila: A Practical Approach*, edited by D. B. ROBERTS. IRL Press, Oxford.

PATEL, N., P. SNOW and C. GOODMAN, 1987 Characterization and cloning of *fasciclin III*: a glycoprotein expressed on a subset of neurons and axon pathways in *Drosophila*. Cell **48**: 975–988.

PRESTON, C., and W. R. ENGELS, 1989 Spread of *P* transposable elements in inbred lines of *Drosophila melanogaster*. Prog. Nucleic Acid Res. Mol. Biol. **36**: 71–85.

RAYMOND, J., and M. SIMMONS, 1981 An increase in the X-linked lethal mutation rate associated with an unstable locus in *Drosophila melanogaster*. Genetics **98**: 291–302.

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 melanogaster*. Genetics **118**: 461–470.

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.

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

RUBIN, G. M., and A. C. SPRADLING, 1983 Vectors of *P* element mediated gene transfer in *Drosophila*. Nucleic Acids Res. **11**: 6341–6351

RUSHLOW, C., W. BENDER and A. CHOVNICK, 1984 Studies on the mechanism of heterochromatic position effect at the *rosy* locus of *Drosophila melanogaster*. Genetics **108**: 603–615.

RUSHLOW, C., and A. CHOVNICK, 1984 Heterochromatic position effect at the *rosy* locus of *Drosophila melanogaster*: cytological, genetic and biochemical characterization. Genetics **108**: 589–602.

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

SIMMONS, M., and J. LIM, 1980 Site specificity of mutations arising in dysgenic hybrids of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **77**: 6042–6046.

SIMMONS, M. J., J. D. RAYMOND, N. JOHNSON and T. FAHEY, 1984 A comparison of mutation rates for specific loci and chromosome regions in dysgenic hybrid males of *Drosophila melanogaster*. Genetics **106**: 85–94.

SPRADLING, A. C. 1986 *P* element-mediated transformation, pp. 175–197 in *Drosophila: A Practical Approach*, edited by D. B. ROBERTS. IRL Press, Oxford.

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

SPRADLING, A. C., and G. M. RUBIN, 1983 The effect of chromosomal position on the expression of the *Drosophila xanthine dehydrogenase* gene. Cell **34**: 47–57.

VOELKER, R., A. GREENLEAF, H. GYURKOVICS, G. WISELY, S. HUANG and L. SEARLES, 1984 Frequent imprecise excision among reversions of a *P* element-caused lethal mutation in *Drosophila*. Genetics **107**: 279–294.

YEDVOBNICK, B., M. A. T. MUSKAVITCH, K. A. WHARTON, M. E. HALPERN, E. PAUL, B. G. GRIMWADE and S. ARTAVANIS-TSATKONAS, 1985 Molecular genetics of *Drosophila* neurogenesis. Cold Spring Harbor Symp. Quant. Biol. **50**: 841–854.

YEDVOBNICK, B., D. SMOLLER, P. YOUNG and D. MILLS, 1988 Molecular analysis of the neurogenic locus *mastermind* of *Drosophila melanogaster*. Genetics **118**: 483–497.

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