19. J. A. Edighoffer *et al.*, paper presented at the Ninth International Conference on Free-Electron Lasers, Williamsburg, VA, 14 to 18 September 1987.
20. A. Ts. Amatuni *et al.*, "Generation of coherent induced undulator radiation," *Yerevan Physics Institute Report EON-727 (42)-84* (1984).
21. E. D. Shaw *et al.*, *Nucl. Instrum. Methods A* **250**, 44 (1986).
22. A. Renieri *et al.*, paper presented at the Ninth International Conference on Free-Electron Lasers, Williamsburg, VA, 14 to 18 September 1987.
23. M. Billardon, *et al.*, *Nucl. Instrum. Methods A* **250**, 26 (1986).
24. J. Murphy and C. Pelligrini, *J. Opt. Soc. Am.* **1B**, 530 (1984).
25. L. R. Elias, *Phys. Rev. Lett.* **42**, 977 (1979).
26. M. W. Berns *et al.*, *Science* **213**, 505 (1981).
27. A. Wittin *et al.*, *Phys. Rev. A* **34**, 493 (1986).
28. D. H. Sliney, *Clin. Chest Med.* **6**, 2 (1985).
29. J. A. Parrish and T. F. Deutsch, *IEEE J. Quant. Electron.* **QE-20**, 1386 (1984).
30. J. A. Dixon, *Lasers in Surgery*, vol. 21, no. 9 of *Current Problems in Surgery* (Yearbook Medical, Chicago, 1984).
31. K. K. Jain, *IEEE J. Quant. Electron.* **QE-20**, 1401 (1984).
32. D. S. J. Choy, *ibid.*, p. 1420.
33. P. Peng *et al.*, *Appl. Phys. B* **42**, 73 (1987).
34. W. M. Yen *et al.*, *J. Phys. Colloq.* **44**, C1-413 (1982).
35. R. Srinivasan, *Science* **234**, 559 (1986).
36. D. J. Erlich, *Nucl. Instrum. Methods A* **239**, 397 (1985).
37. R. B. Hall, *Laser Focus* **18**, 57 (1982).
38. A. Hartford *et al.*, *J. Appl. Phys.* **51**, 4471 (1980).
39. J. Wolfrum *et al.*, West German Patent 293,853 (1981).
40. *Aviation Week Space Technol.* (18 August 1986), p. 40.
41. *Report to The American Physical Society of the Study Group on Science and Technology of Directed Energy Weapons* (American Physical Society, New York, 1987).
42. G. Edwards *et al.*, paper presented at the Ninth International Conference on Free-Electron Lasers, Williamsburg, VA, 14 to 18 September 1987.
43. C.-M. Tang *et al.*, *Nucl. Instrum. Methods A* **250**, 278 (1986).
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Research Articles

Insertional Mutagenesis of the *Drosophila* Genome with Single P Elements

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A versatile genetic method for identifying and cloning *Drosophila melanogaster* genes affecting any recognizable phenotype is described. Strains are constructed in which the insertion of a single P transposable element has caused a new mutation, greatly simplifying the genetic and molecular analysis of the affected gene. Mutagenesis is initiated by crossing two strains, each of which contains a specially designed P element. One element (jumpstarter), encoding P element transposase, efficiently mobilizes the second nonautonomous transposon (mutator), whose structure facilitates selection and cloning of new insertion mutations. Random mutator transpositions are captured in individual stocks that no longer contain jumpstarter, where they remain stable. This method was used to construct 1300 single P element insertion stocks which were then screened for recessive mutations. A library of single-element insertion strains will allow the structure and function of *Drosophila* genes to be readily correlated, and should have many other applications in *Drosophila* molecular genetics.

IN BACTERIA, GENETICALLY MARKED TRANSPOSABLE ELEMENTS are very useful for genetic manipulation in vivo (*1*). Strains containing single, marked transposons allow highly efficient insertional mutagenesis, genetic mapping, and the construction of specific chromosome rearrangements. In contrast, the potential of transposable elements as genetic tools in higher organisms has been less fully realized. Transposon insertion mutations have occasionally allowed the isolation of specific genes in organisms as diverse as *Drosophila*, maize, nematode, and mouse (*2*). However, the high copy number and low transposition rate of most eukaryotic transposons have limited the general applicability of this approach.

An exception is the family of small nonretroviral *Drosophila* transposons called P elements (*3*). Several properties of P elements make them unusually useful for controlled genetic manipulation. Transposition of both full-length (complete) and internally deleted (defective) P elements is dependent on an element-encoded transposase whose production is limited to germline cells by tissue-specific splicing (*4*). In P strains, which contain 30 to 50 complete and defective P elements, transposition is repressed by a cytoplasmic state known as P cytotype. The molecular basis of cytotype is unknown; repressor molecules may be produced by certain defective elements or from incompletely spliced transposase messenger RNA (*4*). Most laboratory strains lack P elements; the absence of repressor activity in such a strain constitutes the M cytotype. P elements are released from repression in the progeny of crosses between females of the M cytotype and P strain males. Activation of P elements in the germline cells of such flies leads to a syndrome of traits (including new insertion mutations) known as "hybrid dysgenesis" (*5*). P element transposition can therefore be experimentally regulated by controlling either the supply of transposase or the P cytotype.

P elements have been used extensively as insertional mutagens activated by "hybrid-dysgenic" crosses between wild P strains and laboratory strains (*5*). However, the heterogeneous collection of elements present in wild P strains greatly limits the utility of this approach. Newly induced insertion mutations are highly unstable; they are frequently lost unless stabilized in the P cytotype by crossing to a P strain. Stabilized strains retain dozens of P elements,

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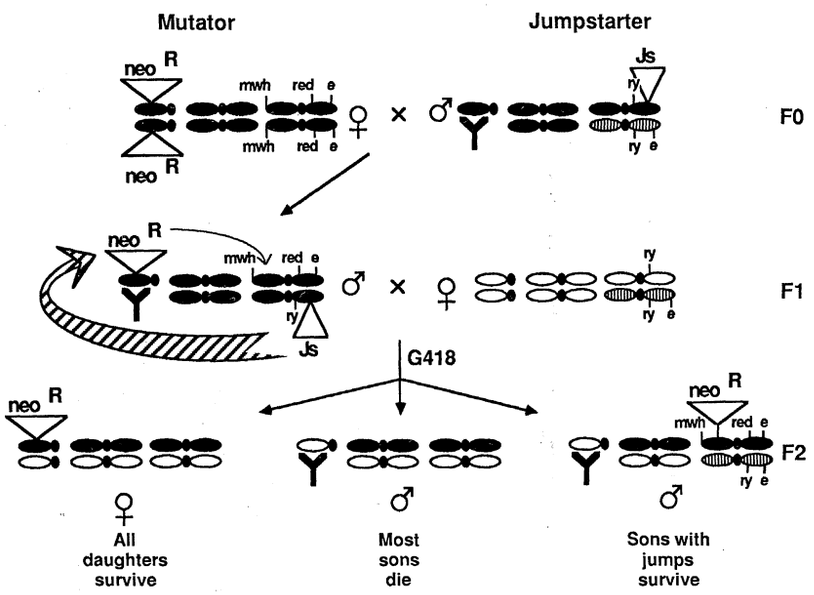

Fig. 1. Generation of single-insert strains. The mutator strain contains an insert of the *neo*^{R} transposon at position 9C on the X chromosome, unmarked second chromosomes, and third chromosomes bearing *mwh red* and *e* (*42*). The jumpstarter strain contains a single autonomous P element (Js) at position 90A on a *ry*^{506} third chromosome and a *TM3*, *ry*^{RK} *Sb e* balancer chromosome (hatched). F1 sons containing both P elements were recovered from crosses between the two stocks and mated individually to virgin females containing the *TM3*, *ry*^{RK} *Sb e* balancer and *ry*^{506} third chromosomes. This cross was carried out on medium containing G418 at 0.6 mg/ml (Gibco) in culture tubes (13 by 100 mm). Larvae were subjected to heat shock at 37°C for 30 minutes, 3 or 4 days after the cross was initiated to enhance expression of the *hsp70-neo*^{R} gene. At day 17 or 18 the F2 progeny of each cross were scored. All daughters survive G418 selection since they inherit the paternal X chromosome containing the *neo*^{R} insert. In the absence of a transposition event, sons do not survive. Males, one adult per cross, representing presumptive autosomal transpositions were collected and mated to virgin females prior to further analysis. The presence of the recessive markers allowed the selection of males lacking the jumpstarter chromosome. Triangles denote the approximate location of P element insertion on the *Drosophila* chromosomes, which are diagrammed in the following order X, Y; second; third. Hatched chromosomes represent the *TM3*, *ry*^{RK} *Sb e* balancer. The large arrow indicates the dependence of *neo*^{R} transpositions on transposase protein produced by the Js element.

greatly complicating the identification, secondary mutagenesis, and cloning of the particular element responsible for the mutant phenotype. These problems are especially severe when several generations of crosses are required to identify mutants in the desired genes; for example, those in previously uncharacterized autosomal lethal or sterile loci.

We have developed an improved method of insertional mutagenesis which eliminates most of these disadvantages. New mutations are induced by the transposition of a single P element into the genomes of flies lacking any other P transposons. The new P element identifies the location of the induced mutation, allowing rapid mapping, complementation testing, and cloning of the affected gene. Genes responsible for a wide variety of mutant phenotypes were identified among a collection of 1300 insertions induced by our method. These experiments demonstrate that it will be feasible to construct a "library" of single-element insertion strains defining the location and function of a significant fraction of *Drosophila* genes.

Experimental design. Single-element insertional mutagenesis requires a method efficient enough to generate many independent insertion events and which ensures insert stability. P element transformation represents the most obvious approach. In a typical transformation experiment, DNA's encoding a genetically marked defective P element and a complete P element bearing a "wings-clipped" mutation are mixed and microinjected into early *Drosophila* embryos (*6, 7*). Transposase produced by the complete element catalyzes chromosomal integration of the marked, defective element in a small fraction of the embryo's germline cells. The wings-clipped mutation inactivates one terminal repeat of the complete element, preventing it from integrating (*8*). Since microinjected DNA does not persist in subsequent generations, integrated defective elements are stable. New single-insert lines can also be generated by mobilizing (jumping) a transposon already present in the genome. DNA containing the wings-clipped element is microinjected into embryos from a strain containing an integrated defected element, and new insertions are recognized among the progeny by genetic tests (*8*). However, the number of single-insert lines that can be constructed by transformation or by transposon jumping is severely limited because 10 to 100 embryos must be microinjected for each independent insertion event.

If transposon jumping could be initiated by a simple genetic cross, it would eliminate the time-consuming requirement for microinjection. A genomic transposase source would have to mobilize an integrated defective element at high rates, but be located on a single chromosome so that it could be segregated into or out of strains as required. Several previous observations suggested that a single complete P element might fulfill this role. A single integrated complete P element produced sufficient transposase to activate the excision of defective P elements, although at a lower rate than a P strain (*6, 8*). Strains containing integrated complete elements catalyzed the transposition of genetically marked transposons as well (*9, 10*). We therefore sought to determine whether a transposon jumping scheme activated by a single integrated P element could be controlled and still produce transposition rates high enough for large-scale mutagenesis.

Our scheme (Fig. 1) relies on two strains called "mutator" and "jumpstarter" that were constructed by P element–mediated transformation. The jumpstarter strain contains a single complete P element that produces transposase. The mutator strain contains the pUChsneo transposon, a defective P element containing a bacterial gene (*neo*^{R}) conferring resistance to the antibiotic G418 (*11*). To initiate mutagenesis, the mutator strain is crossed to jumpstarter (F0 generation). Male progeny that have inherited both transposons are individually mated to normal females in the presence of G418 (F1 generation). Transposase produced by the jumpstarter element will catalyze, in a small percentage of the germline cells of these males, transposition of the *neo*^{R} mutator to a new site on one of the autosomes. All F2 female progeny are expected to inherit the X-linked *neo*^{R} element from their fathers and thus survive. The F2 males will lack the *neo*^{R} gene and die unless they develop from one of the rare germ cells containing an autosomal transposition of the mutator element. Half the males surviving from each cross will not have inherited the jumpstarter-containing chromosome; in these males the new insertion should be completely stable. Saving only one such son per cross guarantees that each will represent an independent insertion event (*12*).

Origin and maintenance of the jumpstarter strain. Ideally, the P element within the jumpstarter strain would produce high levels of transposase but be incapable of transposition. Such an element would represent a chromosomal analog of the wings-clipped P

element used in transformation experiments (*8*). However, direct introduction of such a crippled element into the genome by P element transformation is impossible. The jumpstarter strain was obtained during an unsuccessful attempt to indirectly construct a strain containing such an immobile complete P element. The element present in jumpstarter arose in vivo from an integrated P[*w,hspP*] transposon (Fig. 2B) containing an inducible source of transposase (*hsp70*-transposase fusion gene) and marked with the *white* gene (*4*). Rarely, imprecise excision events self-catalyzed by this element should delete one of its ends, producing a genomic wings-clipped derivative. A derivative lacking *white* gene function was selected (*13*) and characterized to determine if the loss resulted from a deletion which also removed the 5′ end of the P element. Molecular analysis of the deletion derivative revealed, however, that all *white* and heat shock promoter DNA had been lost, but the 5′ end was retained (*13*). These observations can be reconciled if the derivative that we have used as a jumpstarter element arose by homologous recombination between duplicated 5′ P element sequences, thereby regenerating an essentially normal P element (Fig. 2B).

The element in the jumpstarter strain remained functionally autonomous, since it was capable of transposition (Fig. 3). In two early versions of the stock maintained by inbreeding for several months, new bands resulting from transpositions could be detected in DNA from groups of flies. A subline inbred for approximately 1 year accumulated large numbers of P elements, rendering it useless as a jumpstarter strain. Despite its ability to transpose at these low levels, a strain containing a single copy of the jumpstarter element could be stably maintained with relatively little effort. Simply outcrossing the strain each generation greatly reduced the rate of accumulation of new insertions (Fig. 3). Testing DNA from several outbred sublines every 3 months allowed a single-copy jumpstarter strain to be propagated indefinitely.

Production of single-insert strains. We used the protocol of Fig. 1 to generate lines containing new autosomal insertions of the neo^R transposon; 17,568 F1 males containing both elements were mated in individual crosses. In 2040 of the crosses, a G418-resistant male survived; 1317 such males were fertile and yielded stocks (*14*). By following the segregation of G418 resistance relative to other genetic markers in a series of subsequent crosses, we mapped 725 of the inserts to the second chromosome and 592 to the third chromosome (*15*). Each strain containing a new neo^R insertion was maintained by means of an appropriate "balancer" chromosome that facilitated recognition of flies homozygous for the insertion. This made it possible to determine whether the insert-bearing chromosome in each strain contained a recessive lethal, sterile, or visible mutation (*15*).

We used complementation tests to further characterize the mutant chromosomes (*16*). These experiments revealed a surprisingly high frequency of "background" mutations in the initial collection of mutant chromosomes. Ten percent of the second chromosome insertion stocks carried an identical lethal mutation associated with a chromosome rearrangement. A similar fraction of the third chromosomes harbored a single, cytologically invisible, lethal mutation. Finally, nearly 20 percent of the third chromosomes contained the same female sterile mutation. In situ hybridization (*17*) demonstrated that these mutations were not associated with P element insertions. The second chromosome lethal mutation was shown to originate from a ry^{506} stock used in the construction of the jumpstarter stock (*18*). Although the mutator stock had been constructed using a single marked third chromosome, we suspect that both background mutations nonetheless arose prior to the initiation of the screen. Careful testing of starting strains should eliminate such background mutations. However, we cannot totally

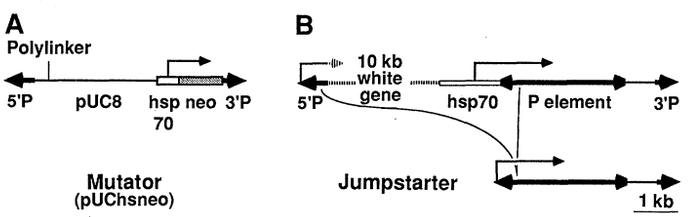

Fig. 2. P element structure. (**A**) The mutator element, pUChsneo (*11*), contains the entire sequence of pUC8 including the amp^R gene and the ColE1 origin of replication, as well as a bacterial neomycin-resistance gene (neo^R) fused to a *Drosophila* heat shock protein promoter (*hsp70*). (**B**) The jumpstarter element was derived from the large transposon shown above, containing DNA that encodes both the *Drosophila white* gene, and a fusion of the *hsp70* promoter to the P element transposase gene (*4*). The *hsp70-P* fusion actually lies in the opposite orientation to the structure originally described (*4, 13*). The jumpstarter element, shown below, is a derivative of this element in which all *white* and *hsp70* DNA is deleted. The thin solid line represents approximately 800 bp of DNA from pBR322 (Bal I to Sal I) that is present in the original $white^+$ transposon. The structure of this region in the jumpstarter element was not verified (*13*). The bracket indicates possible end points of the deletion, which appears to have resulted from a homologous recombination event. Transcription of the transposase gene in the jumpstarter element is assumed to be from the normal P element promoter (indicated by the bent arrow).

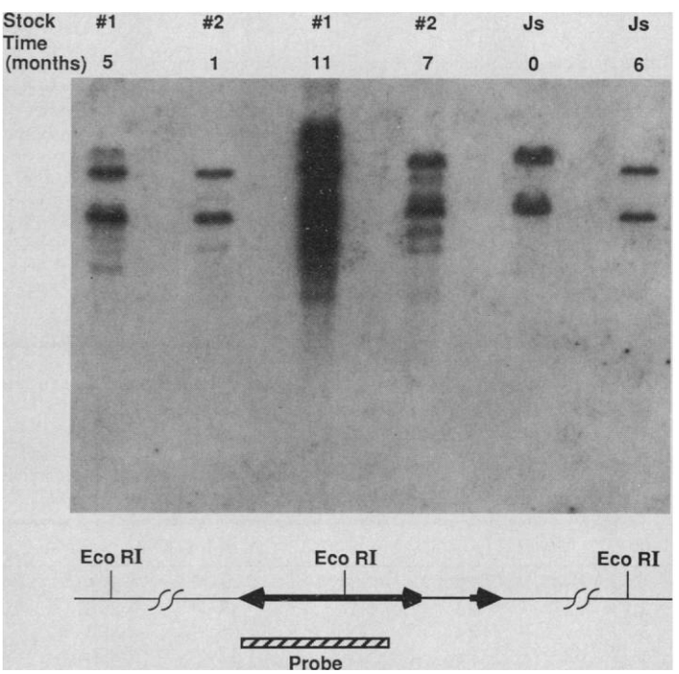

Fig. 3. Stability of the jumpstarter strain. DNA from adult flies of both the progenitor (stock 1 and stock 2) and final (Js) jumpstarter strains was prepared at various times (months) after their establishment from single chromosomes. Stock 1 was started from the original P[*w,hspP*] $white^-$ derivative, while stock 2 is a subline containing a single third chromosome isolated from stock 1. The DNA was digested with Eco RI and analyzed by Southern hybridization. As shown in the diagram, two bands that hybridize to a P element–specific probe are expected from any jumpstarter insertion. Between 1 and 7 months after establishment, several elements, in addition to the original insertion site, accumulated in stock 2. In stock 1, the number of elements increased dramatically between 5 and 11 months. In contrast, the jumpstarter strain, containing only the original insert purified from stock 2, did not accumulate transpositions between 0 and 6 months when maintained by outcrossing. The third chromosome containing the jumpstarter element in this strain is marked with ry^{506} so that it can be recognized after outcrosses to a strain containing *TM3*, ry^{RK} *Sb e*, but lacking P elements. Testing DNA from six to eight jumpstarter sublines every 3 months guaranteed that a single-element line was maintained.

rule out the possibility that P element activity somehow contributed to their appearance.

When strains containing the background mutations were eliminated, the mutation frequencies were similar for both second and third chromosomes (Table 1). About 10 percent of the chromosomes contained lethal mutations and 2 percent produced female sterility. This ratio of lethal to female sterile mutations was similar to that observed in experiments with chemical mutagens (*19*). A small number of strains mutated to male sterility or showed visible abnormalities. One of the visible mutations, *rotated abdomen* ($rt^{neo\ 1}$), reduced fertility and caused abdominal rotation in both adult males and females.

Third chromosome lethal insertions were chosen for detailed characterization. Transposon insertion sites were determined by in situ hybridization to salivary gland chromosomes from each of these strains (Table 2). As expected, nearly all the lethal lines contained a single insertion located apparently at random within either arm of the third chromosome (Fig. 4). Only two lines contained more than one insert. A complementation test was carried out between any lines containing insertions located at approximately the same position on the polytene chromosomes. Three pairs of mutations failed to complement in these tests. If no allelic mutations were missed by relying on insert position, application of the Poisson distribution suggested that a minimum of 582 sites can be mutated to lethality by single-element insertion (*20*).

Table 1. Recessive phenotypes in lines homozygous for neo^R insertion.

	Second chromosome	Third chromosome
Lethal	74 (10%)	65 (11%)
Semilethal	31 (4%)	31 (5%)
Female sterile*	12 (2%)	15 (2.5%)
Male sterile*	5 (0.7%)	9 (1.5%)
Visible	3 (0.4%)	2 (0.3%)
Viable, other†	600 (82%)	470 (79%)
Total	725	592

*The sterile categories include a few leaky lines with fertility ≤10 percent of wild type. †This category includes lines with background sterile or lethal mutations (see text).

Table 2. Cytological localization of neo^R inserts in third chromosome lethal stocks. For explanation of *Drosophila* cytogenetic nomenclature, see (*42*).

Line	Site	Line	Site	Line	Site
1	61C	22	73C	44	89A
2	61D	23	73F	45	89B
3	61D	24	74F/75A	46	89B
4	62A	25	75B	47	89C
5	62A	26	75C	48	90E
6	62A	27	76A	49	91A/B
7	62B	28	77B	50	91D
8	62F	29	78C	51	92A
9	63C	30	79D	52	92B
10	63C	31, 32*	82E	53	92B + 87A
11	65A	33	83C	54	93B
12	66A	34	84D	55	93D
13	66D	35	84DE	56, 57*	94D/E
14	66D/E	36	85C	58	96B + 90CD
15	67A	37	85D	59	97C/D
16	67B	38	86F	60	97E/F
17	68D/E	39	87D/E	61, 62*	99A
18	68F	40	87E	63	99F
19	70A	41	88C	64	ND†
20	73B	42	88D	65	NA†
21	73B	43	88E		

*Pairs indicate independent insertion lines that are allelic. †ND: Not determined.

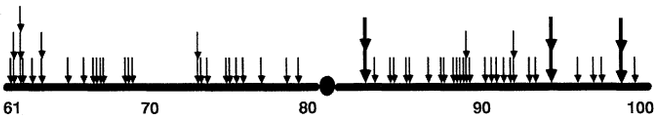

Fig. 4. The chromosomal location of P element insertions from stocks with homozygous lethal mutations on the third chromosome. The insertion sites (Table 3) were determined by in situ hybridization to salivary gland chromosomes (*17*). Cytogenetic nomenclature is described in (*42*). Double and triple arrows indicate cytologically indistinguishable insertion sites. The three large double arrows indicate independent insertions that are allelic.

Transposon insertion is responsible for most of the mutations. Although it is well established that P element insertion can cause mutations, the proportion of mutations arising in dysgenic crosses that are caused by element insertion remains less certain. In addition to promoting P element insertion, transposase induces chromosome rearrangement (*21*) and has been proposed to stimulate the movement of other families of transposable elements (*22*). Other transposons present in the strains used might become activated independently of P element interactions (*23*). For our method to be useful in identifying genes, most of the mutations recovered must be caused by insertion of the single P element present on the mutant chromosome.

We used two tests to investigate whether the new insertions caused the lethal mutations. Mutant chromosomes whose insertion maps within the boundaries of a chromosomal deficiency should fail to complement that deficiency if the insertion is responsible for the mutation. Eleven of the third chromosome lethal mutations contained insertions lying close to or within the reported boundaries of deficiencies that were available to us. Crosses showed that in eight of these (73 percent), the mutation was exposed by the deletion (Table 3). In the other three cases the mutator element was close to, but could not be unambiguously localized within the deletion. Thus 70 percent to 100 percent of these mutants mapped near the site of transposon insertion.

To directly correlate insertions with mutant phenotypes, we carried out reversion tests (Fig. 5). Chromosomes bearing lethal mutations were reexposed to transposase by crossing to the jump-starter strain. Reversion of the lethal phenotype was detected by the appearance in a subsequent generation of viable adults homozygous for markers on the mutant third chromosome. Of ten third chromosome insertion stocks tested from the lethal class, transposase-dependent reversions were recovered in five (Table 4). The reversion frequencies were similar to those reported for other P element mutations (5×10^{-2} to 10^{-3}) (*5*). Phenotypic reversion correlated with the loss of detectable transposon sequences in genomic DNA in the three cases analyzed (Fig. 6). Thus the neo^R insertion must have been responsible for the mutation in these strains. Because P element excision rates vary widely depending on the site of insertion, some strains may have failed to revert because the excision rate from their insertion sites was too low to be detected. Damage incurred during insertion might also have prevented precise excision. However, we cannot eliminate the possibility that a small fraction of the mutations may not have been caused by the P element insertion.

Additional study of several mutant strains reinforced the evidence from mapping and reversion experiments that most of the mutations were caused by insertion of the neo^R transposon. Several laboratories have begun to study the phenotypes exhibited by the neo^R lines in order to identify genes disrupting fertility, neural development, or other processes. So far, seven strains containing mutations allelic to previously identified genes have been recognized on the basis of distinctive mutant phenotypes. In six of these cases, the insertion was found to reside at the previously established cytogenetic locus

(*24*). Two of the new sterile mutations also mapped to the site of the *neo*^{R} insertion. Complementation tests among the third chromosome male sterile mutations revealed that three strains contained allelic mutations. The insertions in these three lines were located at cytologically indistinguishable sites in region 88D. Taken together, these findings demonstrate that single-element mutagenesis has a very high success rate in identifying genes causing a wide variety of mutant phenotypes.

Is single-element insertional mutagenesis applicable to most *Drosophila* genes? Specific genes mutate at very different rates under conditions of hybrid dysgenesis (*5*). Somewhat more than 50 percent of *Drosophila* genes are thought to be readily mutable by P element insertion (*5*). Our results suggest that a similar proportion of genes is likely to be identified by the single-element insertion method. The third chromosome was estimated to contain at least 582 essential loci readily mutable by single P element insertion (*20*), compared to an estimated 1650 lethal genes mutable in an extensive study with the chemical mutagen ethylmethanesulfonate (EMS) (*25*).

Cloning the *rotated abdomen* gene. To test the ease with which chromosomal DNA flanking *neo*^{R} insertions could be isolated, we selected the *rt*^{neo1} line. The insertion in this strain mapped at 68C, very near the position of a locus with an identical phenotype called *rotated abdomen* (*26*) (Fig. 7). A complementation test confirmed that the *neo*^{R} insertion mutant was an *rt* allele. DNA prepared from adults containing this insertion was digested to completion with an endonuclease that cleaves once in the polylinker within the *neo*^{R} element (Fig. 7D), ligated, and used to transform *Escherichia coli* cells to ampicillin resistance. The recovered DNA flanking the insertion site was used to probe a cosmid library of adult DNA; overlapping cosmids were isolated which together represent about 50 kb of continuous genomic DNA (Fig. 7E). The cosmids hybridized only to polytene chromosome band 68C, confirming that the correct DNA had been isolated (*27*). Thus, as expected, DNA surrounding the insertion sites in our strains can be readily cloned.

Genomic density of vital loci. Previous attempts to determine the fraction of P element insertions that cause recessive lethality yielded divergent results. In a relatively small number of transformant lines that had not been tested for the presence of background mutations, rates of recessive lethality between 15 and 33 percent were associated with the insertions (*28*). In contrast, Simmons *et al.* estimated that only 1 percent of X chromosome P element insertions induced lethal mutations (*29*). Death of germline cells in males carrying new X-linked lethal insertions may explain the low percentage obtained by these authors (*30*). Our results allow the fraction of lethal insertions to be directly determined in the absence of significant numbers of background mutations or germ cell death. Approximately 10 percent of the P element insertions that we analyzed on both the second and third chromosomes caused lethal mutations. Recently a similar number (11 percent) of about 1000 lines containing single insertions of a mutator element marked with the *rosy* gene (*31*), and of more than 200 lines containing *rosy* insertions generated by germline transformation (*32*), were shown to be associated with recessive lethality.

Table 3. Deficiency mapping of third chromosome lethal mutations.

Line	*neo*^{R} insertion site	Df(3) tested*	Lethality uncovered?
14	66D/E	h-i22:66D10–11;66F1–2	No
17	68D/E	vin5:68A2–3;69A1–2 vin7:68C8–11;69B4–5	Yes
18	68F	vin5:68A2–3;69A1–2 vin7:68C8–11;69B4–5	Yes
25	75B	W^{R10}:75B1–2;75C2–4 W^{R4}:75B9–10;75C2–4†	Yes
28	77B	in61j:76F;77D	Yes
29	78C	Asc:78D1–2;79A4–C1	No
35	84DE	p712:84D4–6;85B6 + T(2;3)25D;85B	No
39	87D/E	kar-lG27:87B5;87D6	Yes
41	88C	red3l:87F12–14;88C1–3	Yes
52	92B	Dl5:91F5–13;92E1–11	Yes
55	93D	e-GC3:93C;94A	Yes

*The deficiencies (Df) are described in (*44*). †See (*45*).

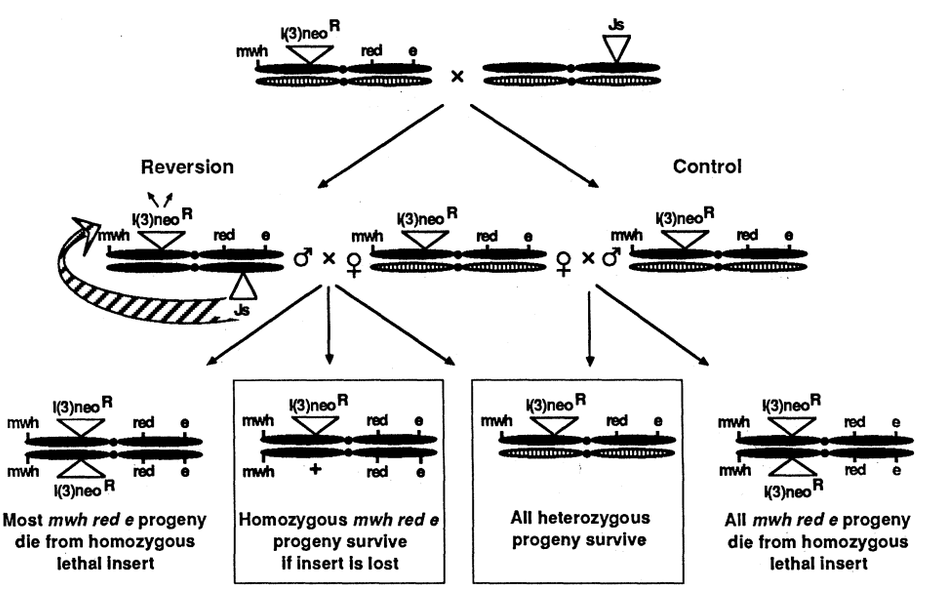

Fig. 5. Jumpstarter-dependent reversion of lethal mutations. The crosses used to generate reversions of third chromosome lethal mutations are diagrammed (only the third chromosome is shown). Balanced *neo*^{R} insertion stocks [*l(3)neo*^{R}] marked with *mwh red e* were crossed to the jumpstarter strain. For the "reversion" cross, males containing both jumpstarter and the *neo*^{R} insertion were chosen. Precise excision of the *neo*^{R} element, catalyzed by jumpstarter-dependent transposase production in the germline of these flies, would be expected to revert a lethal insertion mutation. For the "control" cross, male siblings heterozygous for the insertion chromosome and the *TM3* balancer were chosen. Since no transposase is present in the germline of these flies, the insert should remain stable. Both classes of males were backcrossed to female virgins of the original lethal strain. Revertants were recognized by the presence of progeny homozygous for the genetic markers. Our observation that a low frequency of male recombination is induced in males containing jumpstarter (*43*) required that revertants be distinguished from recombinants in these experiments. Since the insert-containing chromosomes are marked with *mwh red e*, we studied insertions lying between two or these genes. Only if surviving homozygotes retained both markers were they considered to be revertants. The frequency of male recombination was low enough to rule out the possibility that the revertants were double recombinants. Hatched symbols indicate the *TM3* balancer chromosome. Triangles indicate P elements. Classes of progeny that survive from the last cross are boxed. The original site of transposon insertion on the revertant chromosome is indicated by a plus sign (+).

The frequency of lethal insertions in different organisms should reflect differences in the number and structure of vital loci. In yeast 12 percent of 216 random insertions were haploid inviable, while an additional 14 percent were associated with reduced growth rate (*33*). Mutagenic disruptions of the mouse genome occur after the microinjection of cloned DNA into embryos or by retroviral insertion. A recent compilation of these experiments revealed that 2 of 48 retroviral (4 percent) and 9 of 110 cloned DNA insertions (8 percent) were associated with mutations (*34*). Some of the latter lesions may not be caused by the inserted DNA, since chromosome rearrangements frequently accompany the integration of exogenous DNA in mice (*35*). Yeast, *Drosophila*, and mice therefore appear to differ only two- to threefold in the proportion of insertions that are lethal, much less than the hundredfold differences in genome size existing between these three organisms. Unfortunately, the significance of these similar frequencies of lethal mutation is clouded by the strong site specificity of P element insertion (*36*), and the possible insertional preferences of exogenous DNA or retroviruses within the mouse genome.

Table 4. Progeny from reversion tests.

Line	Control		Reversion	
	mwh red e/TM3	*mwh red e*	*mwh red e/TM3*	*mwh red e*
3	1024	0	899	0
14	1112	0	1170	0
15	1601	0	1092	0
18	426	0	208	1*
20	1317	0	785	0
37	1239	0	1183	4
43	428	0	153	1*
49	1318	0	1015	0
52	1834	0	2456	25*
54	976	0	921	1

*These revertants were analyzed by Southern hybridization (see Fig. 6).

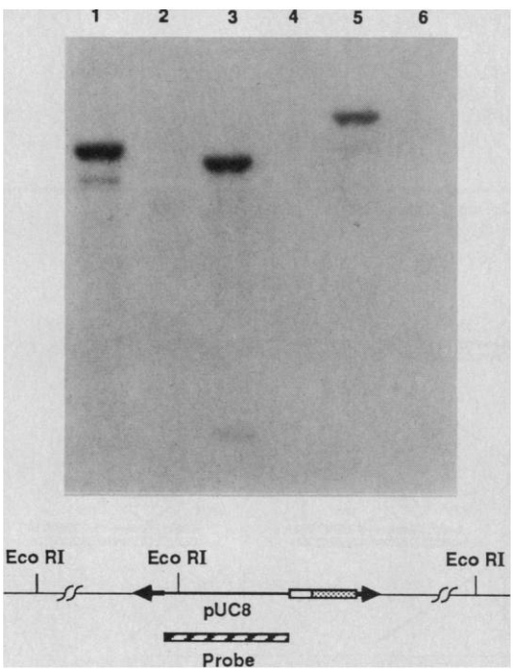

Fig. 6. P element sequences in DNA from control and revertant strains. Revertants recovered (Fig. 5) were crossed to the *TM3* balancer, and four stocks were started from individual progeny containing one of the two *mwh red e* chromosomes present in the revertant individual. Of these sublines 50 percent are expected to contain the revertant chromosome; these sublines produced viable *mwh red e* individuals. DNA from adult flies carrying the original lethal neo^R inserts and from the sublines containing the revertant chromosomes was analyzed for the presence of the neo^R transposon by Southern hybridization. Revertants isolated from three different single-insert stocks are shown. (Lanes 1 and 2) DNA from line 43 and revertant. (Lanes 3 and 4) DNA from line 18 and revertant. (Lanes 5 and 6) DNA from line 52 and revertant. The pUC8 probe is expected to label two Eco RI fragments from each neo^R insertion as shown at the bottom.

Refinements in the mutagenesis method. Several improvements can make it possible to more efficiently generate stable, single-insert lines. In our screen only 8 percent of the F1 males containing both elements yielded useful insertions within their progeny (Fig. 1, F1 cross). However, substituting a more readily mobilized mutator element is likely to raise this frequency much closer to the maximum of 100 percent, where each F1 cross produces one new line. Specific P elements vary widely in their ability to undergo P element transformation depending on both the size and structure of the element (*7*). These variables probably also affect the jumping frequency of integrated mutator elements. Recently, an average jumping frequency of 40 percent was obtained in a large-scale screening with a mutator element marked with the *rosy* gene that had been chosen for efficient mobilization (*31*). Both a higher transposition frequency per germline cell as well as improved fecundity of stocks grown in the absence of G418 contributed to the increase. A strain containing two or three copies of this mutator element on the X chromosome should therefore generate a jumping frequency close to 100 percent when activated by our jumpstarter strain.

An alternative way to increase the mutator element jumping frequency would be to use a different jumpstarter element that produced higher levels of transposase. Our jumpstarter strain generated average to below average levels of transposase compared to other lines containing single autonomous P elements (*37*). Transposons producing much higher transposase levels have been constructed (*10, 38*). However, control experiments will be required to determine whether the use of high level jumpstarter strains in single-element mutagenesis screens induces a larger proportion of mutations that are independent of the mutator insertion. Jumpstarter elements producing high transposase levels might be used to generate single-insert lines containing inefficient mutator elements that cannot be mobilized at maximal rates with the existing jumpstarter.

The fact that our jumpstarter element underwent a low level of self-catalyzed transposition caused few problems. We estimated the fraction of potentially unstable lines containing both the mutator and jumpstarter elements generated by rare jumpstarter transpositions during the screen as follows. Six of 175 lines mapped by in situ hybridization contained two insertions. G418 selection ensured that at least one insertion in each line contained the neo^R gene. However, in two of four double-insert lines that we tested, the second element was found to be jumpstarter which led to instability of the inserts. Therefore, a negligible percentage of the lines produced in our screening, approximately 3 out of 175 or 1.7 percent, will be unstable because of jumpstarter insertions. Likewise, the effort required to maintain the jumpstarter strain in single copy was minimal. On the basis of this experience we would expect other single complete P elements producing average levels of transposase to function equally well as jumpstarter elements.

Eventually it should be possible to construct a wings-clipped jumpstarter element lacking the minor problems associated with studying transposition-competent jumpstarter elements. Robertson *et al.* (*10*) have described a strain containing an integrated modified P element (*4*) that produces transposase but appears to be stable. However, even a transposition-defective jumpstarter element would probably retain the potential to destroy its own capacity for transposase production by self-catalyzed deletion. Placing transpo-

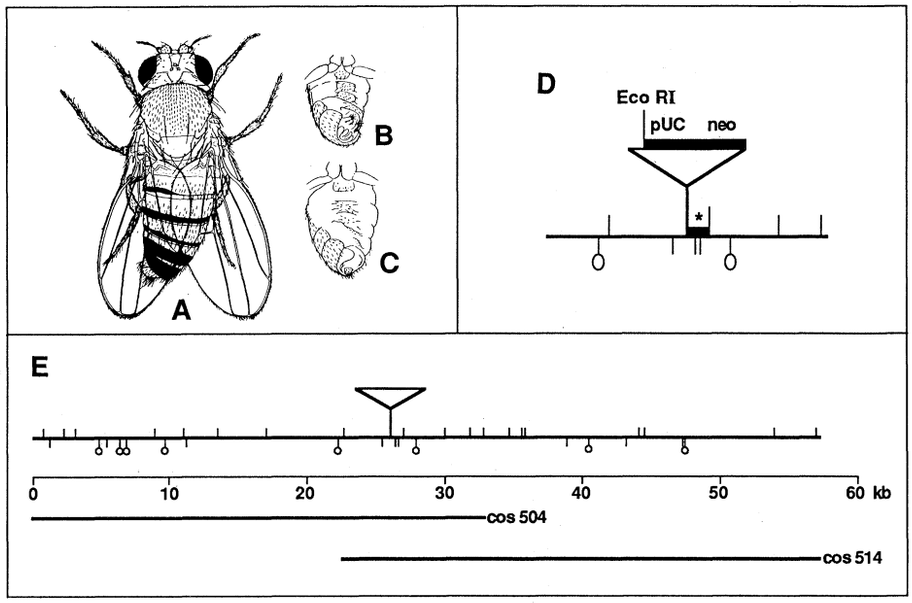

Fig. 7. Cloning *rotated abdomen* by plasmid rescue. The *rt* phenotype is shown. (**A**) Dorsal view of a female. (**B**) Ventral view of a male. (**C**) Ventral view of a female. (**D**) DNA was extracted from rt^{neo1} flies and digested to completion with Eco RI. The genomic DNA was then ligated under dilute conditions to circularize the restriction fragment containing pUC8 and flanking genomic DNA (indicated by a heavy line). Clones were recovered by transforming JA300 cells to ampicillin resistance. Unique genomic DNA flanking the insertion site was isolated (*), and used as a probe to recover cosmids from a library of *Drosophila* Canton S genomic DNA inserted in the cosP*neo* vector (*11*). (**E**) The upper line shows the restriction map of the region defined by two of these cosmids (504 and 514). A scale in kilobase pairs (kb) is shown. Vertical lines above indicate Eco RI sites, vertical lines below indicate Sal I sites and vertical lines with circles below indicate Bam HI sites [A, B, and C are from (*26*)].

sase transcription under experimental control by fusing its structural gene to a regulated promoter might minimize the likelihood of self-destruction. Alternatively, transposase production might be regulated by incorporating a defective transposon (or transposons) capable of inducing the P cytotype (producing repressor) within the jumpstarter strain. Until reliable control methods are developed, jumpstarter strains containing either transposition competent or wings-clipped elements can be regularly tested for transposase activity (*39*).

Applications in *Drosophila* molecular genetics. The idea of using strains containing single, transposase-producing P elements to activate the movement of marked defective elements is extremely versatile. The scheme shown in Fig. 1 can be modified to allow mutagenesis of any chromosome. To mutate genes on the X chromosome, for example, a mutator element located on an autosome would be used. A jumpstarter element integrated on chromosome two would allow more efficient mutagenesis of the third chromosome than in the scheme of Fig. 1.

Transposon mobilization can also facilitate the analysis of gene function by germline transformation. Once one insertion of any construct is obtained, the additional independent insertions necessary for evaluating position effects can be generated without further microinjection (*9, 10*). Since large constructs and transposons containing certain genomic sequences transform inefficiently, the savings in time can be very substantial (*7*).

Secondary mutations generated at the site of transposon insertion are frequently useful in analyzing gene function (*40*). Single-insert lines generated with a mutator element containing a marker gene affecting a visible phenotype such as eye color are particularly useful for secondary mutagenesis. Unlike the neo^R strains, partial or imprecise excisions of a visibly marked element can be recovered regardless of whether they revert the mutant phenotype associated with the insertion.

Single-insert strains are particularly advantageous for identifying and cloning previously undescribed genes affecting a phenotype of interest. In contrast, transposon tagging by means of P-M hybrid dysgenesis may sometimes be more efficient for cloning previously identified loci. However, a modification of the procedure described here may be useful for cloning single, genetically defined genes. By increasing the number of defective elements in the mutator strain, it should be possible to obtain an average number of insertions per target chromosome similar to that in crosses between P and M strains. For example, Robertson *et al.* (*10*) have used a single chromosome containing 17 unmarked defective elements as a mutator strain. Since the jumpstarter element can still be segregated away from the new insertions, this approach should efficiently generate stable multiple insert strains.

An insertion library of single-insert strains. Single-insert lines are likely to retain significant long-term value as a genetic resource for analyzing the *Drosophila* genome. Lines containing single-transposon insertions generated by transformation have begun to be catalogued (*41*). However, our method should greatly accelerate the construction of a "library" of stable single-insert strains that together define a significant fraction of the genes in the *Drosophila* genome. The main library would contain lines showing recessive or dominant phenotypes caused by insertions in separate genes. Complementation tests would only be necessary between strains whose insertions mapped by in situ hybridization to the same chromosome region. Retaining only one insertion per gene would lessen the burden of stock maintenance, and minimize the number of strains that would have to be studied in order to identify genes affecting a phenotype of interest. Since many potentially interesting phenotypes cannot be readily detected, additional single-insert lines without any known phenotype would also be retained and grouped according to insert location. In this portion of the library, many lines with inserts in the same gene would accumulate until their allelism was revealed by suitable molecular or genetic tests. Genes producing subtle, nonlethal changes might be identified and cloned more easily with these strains than by alternative methods.

Strains bearing single insertions at known locations would be useful for genetic purposes other than insertional mutagenesis. Each insertion represents a point of correlation between genetic and physical maps. The genetic marker within each insertion can be used in recombination experiments to localize point mutations. Each insert represents a cytogenetically defined starting point for chromosome walks. X-ray treatment of a single-insert strain encoding an easily scored phenotype could be used to generate deletions that include the insertion. Highly specific chromosome rearrangements might be produced with single-insert strains. In the presence of transposase, specific rearrangements occur at high frequency with breakpoints at the site of preexisting P elements (*21*). Two (or more) defective elements could be introduced at known positions by recombination between appropriate single-insert strains from the

library. Following a cross to jumpstarter, rearrangements, including deletions with breakpoints at these two sites, would be expected to occur with high frequency. Since such rearrangements are frequently imprecise (*21*), they might be recognized among progeny that had lost the activity of the element's marker gene. Thus libraries of single P element insertion strains are likely to facilitate in many ways the molecular genetic analysis of the *Drosophila* genome.

REFERENCES AND NOTES

1. N. Kleckner, J. Roth, D. Botstein, *J. Mol. Biol.* **116**, 125 (1977).
2. P. M. Bingham, R. Levis, G. M. Rubin, *Cell* **25**, 693 (1981); N. V. Fedoroff, D. B. Furtek, O. E. Nelson, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 3825 (1984); I. Greenwald, *Cell* **43**, 583 (1985); E. M. Rinchik, L. B. Russell, N. G. Copland, N. A. Jenkins, *Genetics* **112**, 321 (1986).
3. G. M. Rubin, in *Mobile Genetic Elements*, J. A. Shapiro, Ed. (Academic Press, Orlando, FL, 1983), p. 329; W. R. Engels (*30*).
4. F. A. Laski, D. C. Rio, G. M. Rubin, *Cell* **44**, 7 (1986); D. C. Rio, F. A. Laski, G. M. Rubin, *ibid.*, p. 21.
5. M. Kidwell, in Drosophila: *A Practical Approach*, D. B. Roberts, Ed. (IRL Press, Oxford, 1986), p. 59.
6. A. C. Spradling and G. M. Rubin, *Science* **218**, 341 (1982); G. M. Rubin and A. C. Spradling, *ibid.*, p. 348.
7. A. C. Spradling, in Drosophila: *A Practical Approach.*, D. B. Roberts, Ed. (IRL Press, Oxford, 1986), p. 175.
8. R. E. Karess and G. M. Rubin, *Cell* **38**, 135 (1984).
9. S. M. Mount, M. M. Green, G. M. Rubin, *Genetics* **118**, 221 (1988); A. C. Spradling, unpublished data.
10. H. Robertson *et al.*, *Genetics* **118**, 459 (1988).
11. H. Steller and V. Pirrotta, *EMBO J.* **4**, 167 (1985).
12. P element transpositions frequently occur premeiotically, resulting in clusters of progeny that have undergone identical events (*3*).
13. F. Spencer, unpublished observ .ions.
14. About one-third (723) of the males were sterile or died before they could produce stocks. Some of these may have been X/O products of maternal nondisjunction that inherited the paternal neo^R X chromosome. Since an average of 60 progeny were obtained per cross, the frequency of sterile males [$700/(17{,}000 \times 60) = 6 \times 10^{-4}$], is similar to the expected frequency of nondisjunction [C. Bridges, *Genetics* **1**, 1 (1916)]. The use of a genetically marked Y chromosome allows X/O males to be avoided (*31*). Age, background genotype, and exposure to G418 probably contributed to the sterility of some males.
15. *Ebony* male survivors with the third chromosome genotype *mwh red e/TM3, e* (Fig. 1) were mated to virgin females with *st/TM3, e* third chromosomes in the presence of G418. If only *ebony* (*mwh red e/TM3, e*) progeny survive selection, the neo^R insertion must have resided on the third chromosome. The presence of $ebony^+$ (*st/TM3, e*) progeny indicated a second chromosome insertion of neo^R. This class also contained a small fraction of fourth chromosome insertions as well as a few lines containing both second and third chromosome insertions. The genetic markers on the chromosomes of the balanced strains allowed homozygous flies to be recognized (if present) and the phenotype of recessive mutations to be determined. For a description of F2 genetic screens, see D. B. Roberts [in Drosophila: *A Practical Approach*, D. B. Roberts, Ed. (IRL Press, Oxford, 1986), p. 1]. All the genetic symbols are described in (*42*).
16. A small subset of the mutant lines in each class was crossed in all pairwise combinations, and failure to complement for lethality or sterility was determined. For example, 14 third chromosome lethal lines were crossed and one large complementation group of six members were discovered. A single strain from the large group was then crossed to all the lethal lines, revealing a total of 70 chromosomes containing this mutation. We assume that this complementation group was present on a fraction of the input chromosomes because of its high frequency. In a subsequent screen with different stocks, no mutations in this complementation group were obtained (*31*).
17. In situ hybridization was carried out as described [M. L. Pardue, in Drosophila: *A Practical Approach*, D. B. Roberts, Ed. (IRL Press, Oxford, 1986), p. 111], except that signals were detected with biotinylated probes and an alkaline phosphatase detection system (BRL) as described by W. R. Engels, C. R. Preston, P. Thompson, and W. B. Eggleston [*Focus* **8**, 6 (1986)].
18. The presence of this rearrangement in *ry* strains has been noted previously [(*6*); M. Bourouis and G. Richards, *Cell* **40**, 349 (1985)].
19. M. Gans, C. Audit, M. Masson, *Genetics* **81**, 683 (1975); D. Mohler, *ibid.* **85**, 259 (1978); K. Komitopoulou, M. Gans, L. H. Margaritis, F. C. Kafatos, M. Masson, *ibid.* **105**, 897 (1983).
20. For a rough estimate, we calculated the total number of loci sampled (N) based on the recovery of 56 loci with single alleles [$N(1)$] and 3 loci with two alleles [$N(2)$]. Since $N(0) = N(1)^2/2N(2) = (56)^2/6 = 523$, then $N = 523 + 56 + 3 = 582$. Since the mutation rate of individual genes with P elements varies widely (*5*), this represented a minimum estimate.
21. W. R. Engels and C. R. Preston, *Cell* **26**, 421 (1981); *Genetics* **107**, 657 (1984); H. Roiha, G. M. Rubin, K. O'Hare, *ibid.*, in press.
22. Evidence that hybrid dysgenesis may stimulate the transposition of transposable elements other than the P element has been presented [G. M. Rubin, M. G. Kidwell, P. M. Bingham, *Cell* **29**, 987 (1981); T. I. Gerasimova, Y. V. Ilyin, L. J. Mizrokhi, L. V. Semjonova, G. P. Georgiev, *Mol. Gen. Genet.* **193**, 488 (1984)]. However, attempts to directly demonstrate the mobilization of specific transposons by P element transposase have been negative [R. C. Woodruff, J. L. Blount, J. N. Thompson, Jr., *Science* **237**, 1206 (1987); W. Eggleston, D. Johnson-Schlitz, W. Engels, *Nature (London)* **331**, 368 (1988)].
23. R. K. Blackman, R. Grimaila, M. M. D. Koehler, W. M. Gelbart, *Cell* **49**, 497 (1987); G. Yannopoulos, N. Stamatis, M. Monastirioti, P. Hatzopoulos, C. Louis, *ibid.*, p. 487.
24. The five mutations were *rotated abdomen* (Fig. 7), *vin* [M. E. Akam, D. B. Roberts, G. P. Richards, M. Ashburner, *Cell* **13**, 215 (1978)], allelism demonstrated by Y. N. Jan (personal communication), *big brain* (two alleles), allelism demonstrated by Y. N. Jan (personal communication), and *kelch* and *chickadee*, loci previously identified and mapped by T. Schüpbach (personal communication).
25. G. Jurgens, E. Wieschaus, C. Nusslein-Volhard, H. Kluding, *Wilhelm Roux Arch. Entwicklungsmech. Org.* **193**, 283 (1984).
26. T. H. Morgan and C. B. Bridges, *Carnegie Inst. Washington Publ.* **327**, 190 (1923).
27. L. Cooley, R. Kelley, A. Spradling, unpublished data.
28. A. C. Spradling and G. M. Rubin, *Cell* **34**, 47 (1983); C. C. Laurie-Ahlberg and L. F. Stam, *Genetics* **115**, 129 (1987); see also citations in (*33, 34*).
29. M. J. Simmons *et al.*, *Genetics* **111**, 869 (1985).
30. W. R. Engels, in *Mobile DNA*, D. Berg and M. Howe, Eds. (ASM Publications, Washington, DC, in press).
31. C. Berg, D. McKearin, A. Spradling, unpublished data.
32. D. T. Thompson and A. Spradling, unpublished data.
33. M. G. Goebl and T. D. Petes, *Cell* **46**, 983 (1986).
34. T. Gridley, P. Soriano, R. Jaenisch, *Trends Genet.* **3**, 162 (1987).
35. L. Covarrubias, Y. Nishida, M. Terao, P. D'Eustachio, B. Mintz, *Mol. Cell. Biol.* **7**, 2243 (1987).
36. M. R. Kelley, S. Kidd, R. L. Berg, M. W. Young, *ibid.*, p. 1545; H. Roiha, G. M. Rubin, K. O'Hare, *Genetics*, in press.
37. In crosses to the sn^w strain (*39*), our jumpstarter strain produced mutations in about 6 percent of the progeny. In contrast, the frequency of sn^w destabilization in several lines transformed with a complete P element varied between 2 and 39 percent (*4, 6*).
38. H. Steller and V. Pirrotta, *Mol. Cell Biol.* **6**, 1640 (1986).
39. W. R. Engels, *Science* **226**, 1194 (1984).
40. L. L. Searles, A. L. Greenleaf, W. E. Kemp, R. A. Voelker, *Mol. Cell Biol.* **6**, 3312 (1986); S. Tsubota and P. Schedl, *Genetics* **114**, 165 (1986); H. Salz, T. H. Cline, P. Schedl, *ibid.* **117**, 221 (1987).
41. J. Merriam, S. Smalley, A. Merriam, B. Dawson, *Drosophila Inf. Service* **63**, 173 (1986).
42. D. Lindsley and E. H. Grell, "Genetic variations in *Drosophila melanogaster*," *Carnegie Inst. Washington Publ.* 627 (1968).
43. C. Berg and A. Spradling, unpublished observations.
44. D. Lindsley and G. Zimm, *Drosophila Inf. Serv.* **65** (1987).
45. W. A. Segraves, thesis, Stanford University (1988).

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