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Genetics

Constructing deletions with defined endpoints in *Drosophila*

(deficiency/P element/transposase/ebony gene)

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ABSTRACT **Chromosomes bearing small deletions are valuable tools in *Drosophila* genetics. We have investigated a method for efficiently constructing precise chromosomal deficiencies. Two P transposable elements were positioned within a progenitor strain at the sites of the desired deletion endpoints. Deletions spanning the two transposons were recovered at high frequency when P element transposase was expressed in these flies, but only if the flanking P elements were in a cis rather than a trans configuration. Appropriate progenitor strains can now be constructed to delete virtually any chromosomal region by utilizing an extensive collection of lines containing single P element insertions throughout the *Drosophila* genome.**

Several approaches are available for generating chromosomal "deficiencies." Combinations of Y-autosome translocations with different breakpoints have been used to produce small regions of aneuploidy throughout the genome (1). This approach is useful for the systematic examination of genomic organization. Stable, viable deletions for specific chromosomal regions have been identified among the random rearrangements induced by ionizing radiation. Despite the development of sophisticated genetic procedures to supplement this approach (2), deletions encompassing only about 50% of the genome have been recovered. The utility of these deficiencies has been limited because neither the size nor the endpoints of the deleted region could be precisely specified. We have turned to the use of P transposable elements for the construction of specifically designed deficiencies.

During P/M hybrid dysgenesis, P elements transpose and excise at extraordinary frequencies (3). Recently developed methods harness the chaotic transposon movement characteristic of naturally occurring P strains (4, 5); transposase is produced by a single element that can be introduced and removed by simple genetic crosses, thus activating or stabilizing at will other genetically marked defective transposons. By inducing the transposition of a marked transposon, thousands of stable strains containing single P insertions have been produced that are useful for insertional mutagenesis and enhancer trapping (6, 7). P/M hybrid dysgenesis also results in a high frequency of chromosomal rearrangements whose endpoints correspond to the sites of preexisting P elements (8, 9). We therefore investigated whether the process of P element-induced chromosome rearrangement could also be controlled by using a small number of transposon targets and a single transposase source.

MATERIALS AND METHODS

Genetics. The progenitor stock for the cis strategy was recovered as a meiotic recombinant from females heterozygous for chromosomes carrying each of the single P elements that would define the deletion interval. One chromosome carried an insertion of the pUChsneo (10) P element at 93B and was from line l(3)neo54 (4). The other third chromosome carried a P element marked with the rosy$^+$ gene located at 93F from line $A_{17}O_{15}$-6 (11). The recombinant progenitor stock was wild type for rosy and resistant to the antibiotic G418 (0.6 mg/ml) in the medium. Construction of the trans progenitor strain required recombining the P element encoding transposase, Δ2-3(99B) (5, 12), onto the same chromosome as one of the target elements. The *neo*^{R} target element was chosen since the recombinant could be recognized as rosy$^+$ [from Δ2-3(99B)] and resistant to G418. This stock was maintained on standard fly medium containing G418 to select for the retention of the *neo*^{R} element despite the presence of transposase. The structure and location of both elements were intact when tested on Southern blots from an F_1 progeny chromosome, indicating that the starting chromosome had not mutated prior to the initiation of the experiment.

The mating strategies for generating deletions are diagramed in Fig. 1. The F_1 matings were carried out in bottles containing approximately 30 females and 30 males that were passaged daily for 5 days. The endogenous rosy gene was mutant in all the stocks. The Js-1 Jumpstarter element (4) was present on a chromosome marked with Kinked (*Ki*). The Δ2-3(99B) element (5, 12) was present on a chromosome marked with Stubble (*Sb*).

For genetic notation see ref. 13. The complete genotypes for Fig. 1 were as follows:

Target elements arranged in cis.

F_0: *ry*^{506}, P[*neo*^{R}](93B), P[*ry*$^+$](93F) × *Ki*, *ry*^{506}, P[Js-1]-(90A)/TM3, *Sb*, *ry*^{RK} or *ry*^{506}, *Sb*, P[*ry*$^+$Δ2-3](99B)/TM6

F_1: *ry*^{506}, P[*neo*^{R}](93B), P[*ry*$^+$](93F)/*Ki*, *ry*^{506}, P[Js-1](90A) or *ry*^{506}, *Sb*, P[*ry*$^+$Δ2-3](99B) × *mwh*, *ry*^{506}, *e*

F_2: score *Ki*$^+$ or *Sb*$^+$ progeny for rosy and ebony

Target elements arranged in trans.

F_0: *Sb*, *ry*^{506}, P[*neo*^{R}](93B), P[*ry*$^+$Δ2-3](99B)/*mwh*, *ry*^{506}, *e* × *ry*^{506}, P[*ry*$^+$](93F)

F_1: *ry*^{506}, P[*ry*$^+$](93F)/*Sb*, *ry*^{506}, P[*neo*^{R}](93B), P[*ry*$^+$Δ2-3]-(99B) × *mwh*, *ry*^{506}, *e*

F_2: score *Sb*$^+$ progeny for rosy and ebony

Progeny mutant for ebony resulting from the cis strategy of matings with the genotype *ry*^{506}, *e*/*mwh*, *ry*^{506}, *e* were mated to *mwh*, *red*. The resulting *mwh*$^+$ progeny were mated to TM2, *Ubx*^{130}, *red*, *ry*^{SC}, *e*^{s}/TM6B, *Tb*, *Hu*, *ry*^{CB}, *e*, *ca* to recover balanced stocks of the new ebony chromosomes for further analysis.

Cytological Analysis of ebony Chromosomes. The balanced ebony stocks were outcrossed to obtain larvae heterozygous for an unrearranged chromosome and an ebony chromosome. Salivary gland polytene chromosomes were prepared from third instar larvae by standard procedures, examined, and photographed.

Molecular Analysis of ebony Deletions. Genomic DNA was isolated using standard procedures from adult flies hetero-

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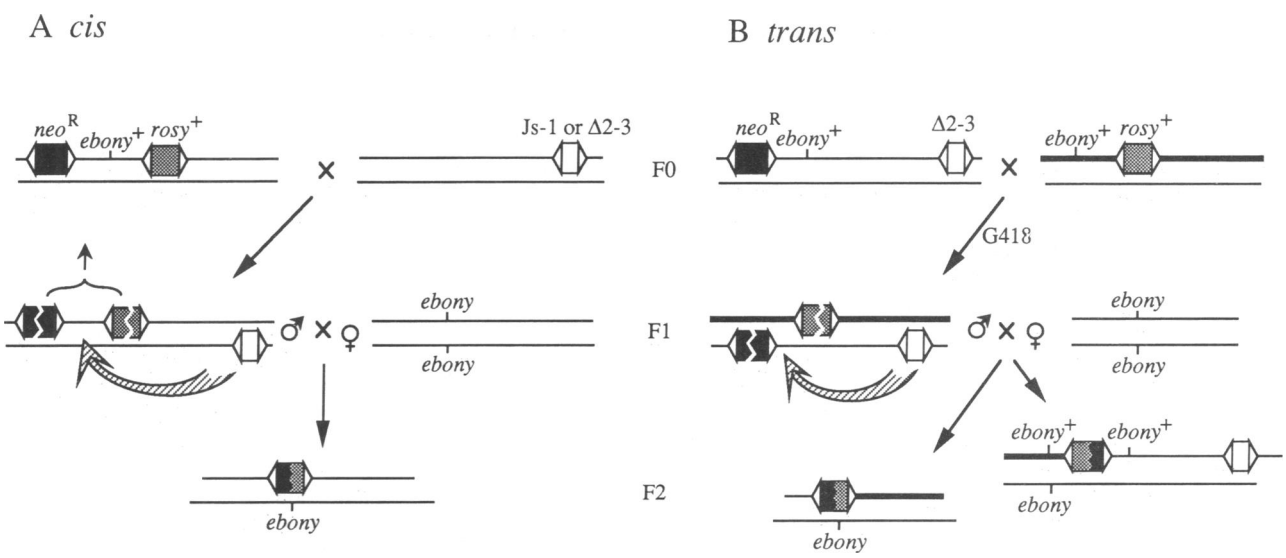

FIG. 1. Strategies for deleting DNA between P elements located in cis (on the same homolog) (*A*) or in trans (*B*). (*A*) A stock containing in cis both a pUChsneo (10) element (*neo*^{R}) at locus 93B and a rosy$^+$ P element at 93F was crossed to a transposase-producing [Js-1 or Δ2-3(99B)] stock (F_0 generation). The F_1 males were mated to rosy, ebony virgin females. F_2 progeny were scored for rosy (probable excisions of only the right element) and for ebony (putative deletions spanning from the right to the left elements). (*B*) A stock was constructed just prior to its use that contains a recombinant chromosome harboring both the *neo*^{R}(93B) element and Δ2-3(99B). The *neo*^{R}(93B), Δ2-3(99B) stock was crossed to the stock containing the rosy$^+$(93F) element (F_0) (in the presence of G418) and F_1 males were mated to rosy, ebony virgin females. Breaks in flanking elements, followed by reciprocal religation of the chromosome fragments, would give rise to both deletions including ebony and duplications of the wild-type ebony gene (F_2). Only the deletions would be detected in this experiment. Horizontal lines represent chromosomes. Hexagons represent P elements. Broken hexagons indicate transposase-induced breaks. The large, hatched arrows indicate transposase.

zygous for each of 10 independent deletions for ebony. DNA was digested with either *Pst* I or *Eco*RI, covalently linked to nylon filters (GeneScreen), and hybridized sequentially with probes homologous to DNA contained within either P element. The restriction fragments listed by number in Fig. 3 were detected with the following probes: fragments 1 and 2 with a 550-base-pair *Xma* III/*Nae* I fragment from the *neo* gene in pUChsneo (10); fragments 3, 4, and 7 with pUC8; fragment 5 with pπ25.1 (14); fragments 6 and 8 with p302.77 (15); fragments 9 and 10 with *pry*$^+$ (16).

RESULTS AND DISCUSSION

The first step in generating a defined rearrangement was to construct "deletion progenitor" strains containing a defective P element at each desired endpoint. A region on the third chromosome that included the ebony gene (specifying body color) was selected for these tests. Two previously con-

Table 1. Recovery of ebony progeny from deletion progenitor strains with elements arranged in cis or in trans

	Cis				Trans	
	Js-1*		Δ2-3(99B)†		Δ2-3(99B)†	
Jumpstarter	No.	%	No.	%	No.	%
F_2 scored	4358		3884		4071‡	
rosy	181	4	3084	79	2706	66
ebony, rosy	1	0.02	10	0.3	0	
ebony, rosy$^+$	2	0.05	35	0.9	0	

The frequencies of rosy and ebony, rosy, and ebony, rosy$^+$ are reported without correcting for premeiotic clusters. A minimum value for the total frequency of ebony in the cis experiment with Δ2-3(99B) when premeiotic clusters are eliminated was 0.5%. See Fig. 1 for explanation of the experiments.

*See ref. 4.

†See refs. 5 and 12.

‡This number represents only the *ry*^{506}, P[*ry*$^+$](93F) chromosomes scored (see Fig. 1), which is about half the total number of chromosomes scored (since the trans event involves both homologs). It is reported as such for comparison to the cis experiment.

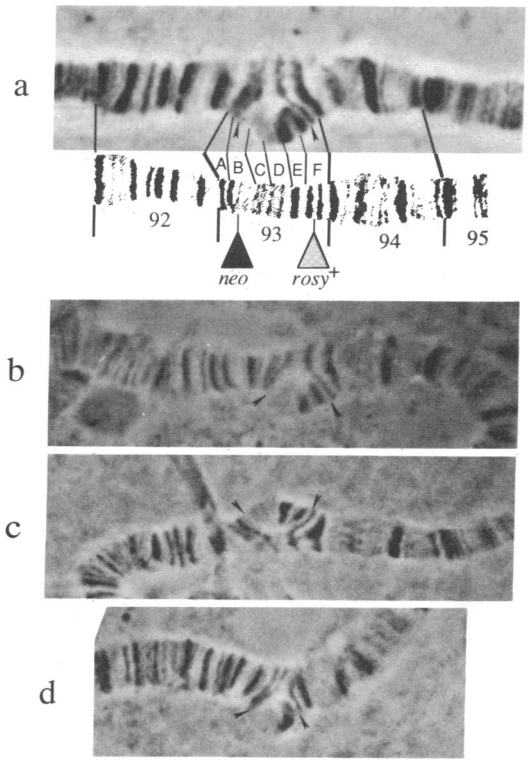

FIG. 2. Polytene chromosome cytology in region 92-95 from four ebony chromosomes independently derived from the cis experiment. In each of the four cases shown (*a–d*), the normal homolog bulges from the interval of the third chromosome where pairing is disrupted by the loss of the deleted interval. The endpoints of the deleted intervals correspond exactly to the original sites of P element insertion (indicated by small arrowheads). For comparison, a map (17) of this portion of the chromosome is shown under *a* and the sites of the two flanking P elements are indicated by triangles. The ebony locus is at position 93D. (*a*) Deletion 1. (*b*) Deletion C. (*c*) Deletion K. (*d*) Deletion I (see Fig. 3).

structed strains containing single P element insertions that flanked the ebony region were used. An element inserted at cytogenetic position 93B and marked with a neomycin resistance gene, obtained from strain l(3)neo54 (4), defined the left endpoint. The desired right endpoint, at 93F, was defined by an insertion marked with the rosy$^+$ gene (specifying eye color) in strain $A_{17}O_{15}$-6 (11). Crossing l(3)neo54 and $A_{17}O_{15}$-6 yielded a strain containing both transposons in a trans configuration. A deletion progenitor strain with both transposons in cis (on the same chromosome) was generated in a subsequent generation by meiotic recombination and recognized by selection for resistance to the antibiotic G418 and rosy$^+$ eye color.

Deletions spanning the 93B to 93F region were expected following introduction into each of the two progenitor strains of a transposase-producing P element. The induction of chromosomal breaks at the site of both the *neo*^{R} and rosy$^+$ P elements, followed by ligation of the chromosome fragments, would give rise to the desired deletion (Fig. 1). Putative deletions would be detected by scoring the progeny for new ebony mutants. Both a strong transposase source, Δ2-3(99B) (5, 12), and a weak source, Jumpstarter-1 (4), were tested with the cis progenitor strain. However because the elements producing transposase were located on the same chromosome as the target elements, combining them with the trans progenitor strain was more complex. The *neo*^{R}(93B) left boundary element was therefore recombined onto the Δ2-3(99B) chromosome prior to initiating the experiment.

The results of introducing a transposase-producing element into both the cis and trans progenitor strains, and scoring progeny flies for ebony and rosy, are summarized in Table 1. The number of rosy flies resulting from excision of the right boundary element served as a measure of transposase activity; when Δ2-3(99B) was used, nearly 80% of the progeny lacked a functional rosy gene. Flies carrying a total of 48 new ebony mutations were recovered from the cis deletion progenitor strain (Table 1). The frequency of ebony flies was at least 10-fold higher using Δ2-3(99B) rather than Jumpstarter-1 (0.07% compared with 1.2%), presumably due to the higher level of transposase produced by the former element. Both ebony, rosy and ebony, rosy$^+$ flies were recovered, indicating that the rosy gene in the right element was inactivated in some but not all of the chromosomes. Among 4071 chromosomes scored, no ebony flies were recovered from the trans experiment. Thus the frequency of putative deletions that can be recovered between these particular P elements is much lower when they reside in trans rather than in cis.

To determine if chromosome deletions were responsible for the new ebony mutations, stocks were established from a number of ebony flies and 12 were analyzed in detail. Cytological examination was carried out on salivary gland chromosomes from flies heterozygous for each of the ebony chromosomes and an unrearranged chromosome. Two of the 12 ebony chromosomes analyzed had complex rearrangements, possibly involving the endogenous rosy locus, and were not examined further (data not shown). The remaining 10 ebony chromosomes had a deficiency with endpoints exactly corresponding to the insertion sites of the flanking elements at 93B and 93F (see Fig. 2). This confirms that the majority of the ebony flies recovered in the cis experiment contained the predicted deletion.

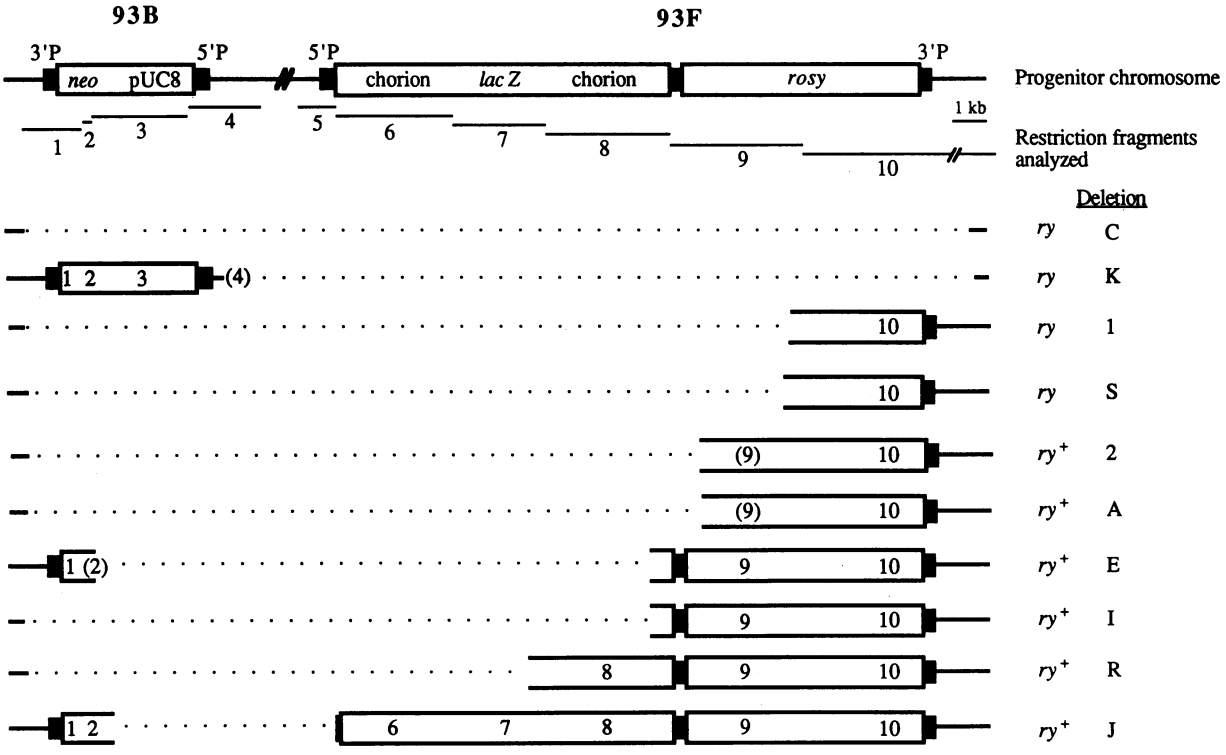

FIG. 3. Deletion endpoints in 10 ebony chromosomes analyzed by Southern blotting to genomic DNA. The two flanking P elements used to generate ebony deletions are diagramed at the top. Thin lines represent chromosomal DNA and black rectangles represent P element DNA. The relative orientation of the two elements was assigned on the assumption that the deletions were polar; sequences proximal to the deletion should be absent whenever more distal sequences were deleted. The restriction fragments that were analyzed are indicated below the diagram (fragments 1–4 were the result of digestion with *Pst* I and fragments 5–10 were from *Eco*RI digestion). The approximate endpoints of 10 deletions are indicated by truncated diagrams of the original elements (the deleted DNA is indicated by a dotted line). The endpoints were assumed to be within the first restriction fragment not detected in Southern hybridizations. The presence of a restriction fragment in Southern blots is indicated by the appropriate number within the truncated diagram. Restriction fragment numbers that appear in parentheses indicate cases where hybridization was detected to restriction fragments of altered size. Deletions 1 and 2 were made using Js-1 and the lettered deletions were made using Δ2-3(99B) (see Fig. 1). ebony chromosomes marked *ry* no longer carry a functional rosy gene, whereas in those marked *ry*$^+$, the rosy gene is intact.

Molecular analysis of the deletion endpoints by Southern blotting suggested that the deletion endpoints rarely coincided precisely with the P element termini. As in the case of previously studied P element-catalyzed rearrangements, the breaks were usually imprecise (9, 18–21), occurring within or near the flanking P elements. Genomic DNA isolated from adults carrying each of 10 independent ebony deletions was analyzed using probes homologous to DNA contained in the two flanking P element constructs (Fig. 3). The relative orientation of the two elements was assigned on the assumption that sequences would be deleted in a polar fashion. Regions proximal to the deletion should be absent whenever more distal sequences had also been removed. All results obtained were consistent with this expectation. Breakpoint locations were assumed to be within the first absent restriction fragment. For example, the endpoints at 93F for deletions 1 and S were probably within restriction fragment 9. Comparison of deletions 1 and S with 2 and A suggests that the endpoints of deletions 2 and A were closer to the left end of fragment 9 since deletions 2 and A retain rosy$^+$ activity. Also, in deletions 2 and A, a new band was detected instead of fragment 9, suggesting that a significant amount of DNA from the rosy gene remained. In several cases, no homology to one or both flanking P element(s) remained in the deletion (93B element of deletions 1, 2, A, C, I, R, and S and 93F element of C and K).

The ability to construct "designer deletions" now offers an alternative method for producing chromosomal deficiencies. In many cases, the ability to predict the location of the deficiency endpoints would be a significant advantage: complementation groups could be precisely mapped, haploinsufficient loci could be avoided. Successful application of this strategy will depend on several factors. First, extensive collections of single element insertion lines must be available to provide a diverse choice of endpoints. Since many groups are currently generating such lines and storing them at the Howard Hughes P Element Stock Center (Bloomington, IN) this is unlikely to be a significant limitation. Second, it remains to be tested if the relative orientation of the P elements affects the efficiency of deletion formation. Presently there is no evidence to suggest that orientation affects P element-catalyzed rearrangements. Third, since the progenitor strain with elements in trans did not yield deletions, it remains uncertain if interchromosomal events can be obtained at usable frequencies with this strategy. Fortunately, insertions bearing different markers are available to produce cis progenitor strains. Finally, not all intervals will contain an easily scored marker that can be used to recognize deletions. One option is to detect deletions directly by polytene chromosome analysis, particularly if deletions form more frequently in the case of closely spaced boundary elements. Surprisingly, selecting progeny chromosomes based on changes in markers within the flanking elements may not be useful in recognizing deleted chromosomes. Our results suggest that if this approach is to succeed, the markers must lie in the deletion-proximal end of the elements. A deletion-distal marker such as rosy$^+$ in the 93F transposon did not provide useful information. Nearly 80% of the progeny in the cis experiment were rosy, and a majority of the deletions recovered were rosy$^+$. Despite these limitations, however, it is clear that P elements can now provide another useful tool to the *Drosophila* geneticist.

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1. Lindsley, D. L., Sandler, L., Baker, B. S., Carpenter, A. T. C., Denell, R. E., Hall, J. C., Jacobs, P. A., Miklos, G. L. G., Davis, B. K., Gethman, R. C., Hardy, R. W., Hessler, A., Miller, S. M., Nozawa, H., Parry, D. M. & Gould-Somero, M. (1972) *Genetics* **71,** 157–184.
2. Gubb, D., McGill, S. & Ashburner, M. (1988) *Genetics* **119,** 377–390.
3. Engels, W. R. (1989) in *Mobile DNA*, eds. Berg, D. E. & Howe, M. M. (Am. Soc. Microbiol., Washington), pp. 437–484.
4. Cooley, L., Kelley, R. & Spradling, A. (1988) *Science* **239,** 1121–1128.
5. Robertson, H. M., Preston, C. R., Phillis, R. W., Johnson-Schlitz, D., Benz, W. K. & Engels, W. R. (1988) *Genetics* **118,** 461–470.
6. Cooley, L., Berg, C. & Spradling, A. (1988) *Trends Genet.* **4,** 254–258.
7. O'Kane, C. J. & Gehring, W. J. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 9123–9127.
8. Engels, W. R. & Preston, C. R. (1981) *Cell* **26,** 421–428.
9. Engels, W. R. & Preston, C. R. (1984) *Genetics* **107,** 657–678.
10. Steller, H. & Pirrotta, V. (1985) *EMBO J.* **4,** 167–171.
11. Orr-Weaver, T. L. & Spradling, A. (1986) *Mol. Cell. Biol.* **6,** 4624–4633.
12. Laski, F. A., Rio, D. C. & Rubin, G. M. (1986) *Cell* **44,** 7–19.
13. Lindsley, D. & Grell, E. (1968) *Genetic Variations of Drosophila melanogaster* (Carnegie Institution of Washington, Washington), Publ. No. 627.
14. O'Hare, K. & Rubin, G. (1983) *Cell* **34,** 25–35.
15. Spradling, A. C. (1981) *Cell* **27,** 193–201.
16. Spradling, A. C. & Rubin, G. M. (1983) *Cell* **34,** 47–57.
17. Lefevre, G., Jr. (1975) in *The Genetics and Biology of Drosophila*, eds. Ashburner, M. & Novitski, E. (Academic, London), Vol. 1A, pp. 1–25.
18. Daniels, S. B., McCarron, M., Love, C., Clark, S. H. & Chovnick, A. (1985) *Genetics* **109,** 95–117.
19. Tsubota, S. & Schedl, P. (1986) *Genetics* **114,** 165–182.
20. Salz, H. K., Cline, T. W. & Schedl, P. (1987) *Genetics* **117,** 221–231.
21. Roiha, H., Rubin, G. M. & O'Hare, K. (1988) *Genetics* **119,** 75–83.