Modified *P* Elements That Mimic the P Cytotype in *Drosophila melanogaster*

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ABSTRACT

Activity of the *P* family of transposable elements in *Drosophila melanogaster* is regulated primarily by a cellular condition known as P cytotype. It has been hypothesized that P cytotype depends on a *P* element-encoded repressor of transposition and excision. We provide evidence in support of this idea by showing that two modified *P* elements, each with lesions affecting the fourth transposase exon, mimic most of the P cytotype effects. These elements were identified by means of two sensitive assays capable of detecting repression by a single *P* element. One assay makes use of cytotype-dependent gene expression of certain *P* element insertion mutations at the *singed* bristle locus. The other measures suppression of transposase activity from the unusually stable genomic *P* element, Δ2-3(99B), that normally produces transposase in both germinal and somatic tissues. The P cytotype-like effects include suppression of sn^w germline hypermutability, sn^w somatic mosaicism, pupal lethality, and gonadal dysgenic sterility. Unlike P cytotype, however, there was no reciprocal cross effect in the inheritance of repression.

HYBRID dysgenesis is the collective term for the syndrome of germline abnormalities associated with mobilization of the *P* family of transposable elements (reviewed by ENGELS 1989). It includes high mutability, chromosome rearrangements, male recombination, and a temperature-sensitive agametic effect called gonadal dysgenic (GD) sterility. Many natural populations of *Drosophila melanogaster* are designated P strains to indicate that they carry many *P* elements scattered throughout their genomes. Hybrid dysgenesis does not occur within P strains, however, due to suppression of *P* element activity by a condition known as the P cytotype. When males from a P strain are crossed to females from a strain lacking *P* elements (M strain), the dysgenic traits appear in the germline of the progeny. These effects are greatly reduced in progeny from the reciprocal cross.

Various studies of the nature and inheritance of P cytotype (ENGELS 1979a, b, 1981; KIDWELL 1985; SVED 1987) indicate that it is determined by a limited form of maternal transmission that is ultimately dependent on the genomic *P* elements. The molecular basis for P cytotype remains unclear. Most models for cytotype (*e.g.*, ENGELS 1981; O'HARE and RUBIN 1983) involve an element-encoded repressor whose production depends in some way on the cytotype of an individual's mother. The primary difficulty in previous studies aimed at determining the precise nature of P cytotype has been the lack of a sufficiently sensitive assay. Scoring for the presence or absence of hybrid dysgenesis in the progeny of M♀ × P♂ crosses has allowed the determinants of cytotype to be mapped to individual P strain chromosomes (ENGELS 1979a) or to regions within chromosomes (KIDWELL 1981), but not to specific sites.

In contrast, the *P* element transposase function has been fairly well characterized. Complete *P* elements are 2907 base pairs (bp) long with 31 bp inverted terminal repeats (O'HARE and RUBIN 1983). Transposase is encoded by four exons, numbered 0–3, which encompass 80% of the complete *P* element length (KARESS and RUBIN 1984). LASKI, RIO and RUBIN (1986) demonstrated that one aspect of *P* element regulation, the restriction of activity to the germline, involves differential splicing of the last two exons. The splice occurs in germ cells leading to production of an 87-kD polypeptide with transposase activity. In somatic cells, however, the splice does not occur, resulting in the production of a truncated polypeptide of about 66 kD. RIO, LASKI and RUBIN (1986) suggested that this 66 kD polypeptide might be the repressor that constitutes P cytotype, a hypothesis that would require its production in the germline, perhaps by alternative processing of the 2–3 splice.

There is indirect evidence that certain internally deleted *P* elements can partially repress *P* element mobility. Strains have been identified that lack complete *P* elements but that appear to have the P cytotype (NITASAKA, MUKAI and YAMAZAKI 1987; HAGIWARA *et al.* 1987). Various internally deleted *P* elements were observed in these strains, including one

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with most of the first three exons intact but a deletion in the fourth exon. Similarly, SIMMONS *et al.* (1987) found partial suppression of *P* element activity in a subline of the Muller-5 Birmingham strain, which also lacks complete *P* elements and transposase activity (BINGHAM, KIDWELL and RUBIN 1982; SIMMONS and BUCHOLZ 1985; ENGELS *et al.* 1987). Another kind of evidence indicating that exon 3 is not necessary for the P cytotype comes from the observation that the P cytotype is fully effective in preventing hybrid dysgenesis in somatic cells where the 2–3 splice is not made (ENGELS *et al.* 1987). This observation was made possible by the use of a modified *P* element known as *P*[*ry*$^+$Δ2-3], which lacks the 2–3 intron, and can therefore produce transposase somatically (LASKI, RIO and RUBIN 1986). The transposase activity of a particularly stable insert of this element, called Δ2-3(99B) (ROBERTSON *et al.* 1988), is effectively suppressed by the P cytotype.

In this report we examine a series of *in vitro*-modified *P* elements for the ability to mimic the P cytotype. We used two new assays for *P* element regulation that are sufficiently sensitive to respond to a single *P* element. The first makes use of a *P* element insertion mutation whose phenotype is conditional upon cytotype. The results of this method agreed with our second assay, which was a more direct test for repression of *P* element activity using the hypermutable *sn*^{w} allele of the *singed* locus. One of the elements identified by these screens was tested further, and found to mimic the effects of the P cytotype in most respects. It failed, however, to show the reciprocal cross effect expected if there were maternal inheritance of repression.

EXPERIMENTAL PROCEDURES

Drosophila stocks: Genetic symbols not described here are in LINDSLEY and GRELL (1968).

π_2—An inbred P strain described previously (ENGELS and PRESTON 1979).

UK4—A wild type M′ strain from Madison, Wisconsin (ENGELS and PRESTON 1980).

ν_6—A wild type Q strain from Madison, Wisconsin (ENGELS and PRESTON 1980).

Sexi and Tübingen—Two wild-type M′ strains taken in 1970 and 1981 from European populations (KIDWELL, FRYDRYK and NOVY 1983; BLACK *et al.* 1987).

M-5-Birmingham—An M′ strain (BINGHAM, KIDWELL and RUBIN 1982).

ry^{506}—An M strain lacking any *P* element sequences. This strain was used by KARESS and RUBIN (1984) and LASKI, RIO and RUBIN (1986) in transformation procedures to place *P*[*ry*$^+$] elements into the genome. We therefore assume that it is essentially isogenic with the resulting *P*[*ry*$^+$] strains except for their single marked *P* elements.

Oregon-R—An M strain lacking any *P* element sequences (BINGHAM, KIDWELL and RUBIN 1982).

y sn^w; *bw*; *st*(M)—The *X* chromosome carried a *P* element insertion mutation at the *singed* bristle locus (ENGELS 1979b, 1984; ENGELS and PRESTON 1981). There are two defective *P* elements at *singed* and a third very closely linked (ROIHA, RUBIN and O'HARE 1988), but no others in the genome. The stock behaves as an M strain. The autosomes carry eye color markers.

sn^w; *ry*^{506}—Similar to the above except that the *y* marker has been removed, and the autosomal background is replaced with that of the *ry*^{506} strain described above.

sn^w; *CyO*; *TM6/T(2;3)ap*^{Xa}—Similar to the above except that the autosomes carry dominantly marked balancer chromosomes and a translocation between chromosomes *2* and *3* which is also dominantly marked.

sn^w(P)/*M-5*(P); π_2—The autosomes and most of the *sn*^{w} chromosome, except the area close to the *singed* locus, are derived from the π_2 strain. *M-5*(P) is a dominantly marked *X* chromosomal balancer carrying many *P* elements (ENGELS 1985). This stock has the P cytotype. The *sn*^{w}/*sn*^{w} females appearing in this stock are sterile, as will be discussed below, and the homozygous *M-5*(P) females are removed by periodic selection.

FM7, *y*^{31d} *sn*^{x2} *B*/*Df(1)N19*/*y*$^+$*Y*—An M cytotype stock carrying an *X* chromosome balancer with the female sterile allele, *sn*^{x2}. Its homolog is lethal.

FM7, *y*^{31d} *sn*^{x2} *B*(P)/*Df(1)E128*/*y*$^+$*Y*; π_2—The *FM7*(P) balancer carries many *P* elements (ENGELS 1985). Its homolog is a lethal-bearing P chromosome. All other chromosomes were derived from π_2. This stock has the P cytotype.

ry^{506}*P*[*ry*$^+$ Δ2-3](99B)—The *P* element designated *P*[*ry*$^+$ Δ2-3](99B), hereafter Δ2-3(99B), was found to be essentially immobile, but produces abundant transposase activity (ROBERTSON *et al.* 1988). The genetic background is that of the *ry*^{506} strain.

C(1)DX, *yf*; *ry*^{506} Δ2-3(99B)—A compound-*X* stock created by crossing and backcrossing males from the above strain to females from a compound-*X* (M) strain.

C(1)DX, *yf*; π_2—A P cytotype stock in which all chromosomes except the compound *X* are derived from π_2 (ENGELS 1979b).

***In situ* hybridization to polytene chromosomes:** Biotinylated probes were hybridized to chromosome squashes and labeled with alkaline phosphatase as described (ENGELS *et al.* 1986).

RESULTS

The *singed* locus: Our first assay involves an interaction between P cytotype and certain *P* inserts at the *singed* locus, so this locus will be described briefly. The primary phenotype of *singed* mutants is the "singed" (shortened and gnarled) appearance of the head and thoracic bristles and, less conspicuously, the other cutical hairs. Alleles of *singed* vary from extreme to nearly wild-type bristles. In addition, some *singed* alleles cause female sterility, involving a defect in vitellogenesis in the nurse cells, which are germline tissues (BENDER 1960; PERRIMON and GANS 1983). This female sterility is associated with a distinct phenotype of the eggs, which are smaller than normal, rounded rather than elongated, and vacuolated (BENDER 1960). The filaments (dorsal appendages) on the eggs are shortened and gnarled in a fashion reminiscent of singed bristles. We call this germline phenotype "singed sterility" to distinguish it from GD sterility.

Origin of *singed* alleles: We tested 16 *singed* mu-

TABLE 1

Cytotype-dependence of singed alleles

Allele	Bristles		Eggs	
	M cytotype	P cytotype	M cytotype	P cytotype
sn^{w}	Moderate	Moderate	++++	+
sn^{e}	Extreme	Extreme	++	+
$sn^{(+)}$	Wild type	Wild type	+++++	+++
sn^{7e}	Extreme	Extreme	+	+
sn^{17w}	Moderate	Moderate	+++++	+++++
sn^{34e}	Extreme	Extreme	+++++	+++
sn^{45e}	Extreme	Extreme	+++++	+++++
sn^{46e}	Extreme	Extreme	+++++	++++
sn^{50e}	Extreme	Moderate	+++++	+++++
sn^{52w}	Moderate	Moderate	+++++	+++++
sn^{62e}	Extreme	Extreme	+++++	+++++
sn^{91e}	Extreme	Moderate	+++++	+++++
sn^{93e}	Extreme	Extreme	+++++	+++++
sn^{103e}	Extreme	Moderate	+++++	+++++
sn^{105e}	Extreme	Extreme	+++++	++++
sn^{107e}	Extreme	Extreme	+++++	+

Bristle phenotypes were classified as "extreme" if all macrochaetae were much reduced in length and gnarled. If the bristles were reduced by approximately half and had frequent hooks and bends, the phenotype was classified as "moderate." Eggs were classified into five morphological categories from wild type (+++++) to extreme (+). The most extreme category is characteristic of females homozygous for the null allele, sn^{x2}. Females in categories ++ and + were usually sterile.

tations for cytotype-dependent expression. These were all insertions of defective *P* elements (not producing transposase) in an M genetic background obtained as described elsewhere (ENGELS 1984; ROBERTSON *et al.* 1988). Thus, there is no *P* element insertion or excision activity in these stocks. One of the alleles (sn^{w}) has been described previously for its hypermutability in the presence of transposase (ENGELS 1979b, 1981, 1984). This allele contains two defective *P* elements of lengths 1.0 and 1.2 kb (ROIHA, RUBIN and O'HARE 1988). In the presence of *P* transposase these elements excise, one at a time, to yield either a more extreme allele (sn^{e}) in which only the 1.2-kb insert is present, or a nearly wild-type allele ($sn^{(+)}$) which carries only the 1.0-kb element. The remaining 13 alleles were all obtained by independent events (ROBERTSON *et al.* 1988).

Tests for cytotype dependence: Each *singed* allele was tested for cytotype-dependent bristle morphology by crossing mutant males to compound-*X* females from either a P- or M-cytotype stock. We then examined the bristles of the resulting sons, which had the same *X* chromosome as their fathers, but differed in their maternally inherited cytotype. Three of the alleles, sn^{50e}, sn^{91e}, and sn^{103e}, which had extreme singed bristles in the M cytotype, became closer to wild type in the P cytotype (Table 1).

To test for cytotype-dependent egg morphology, we crossed mutant males to P or M cytotype females bearing the *FM7* chromosome, which carries the null allele, sn^{x2}. The eggs of the heterozygous sn^{x2} daughters were examined by squashing 6–10-day-old females between two glass plates and viewing with transmitted light against a dark background. Each female was thus classified into one of five groups, ranging from wild type to extreme singed sterility. As indicated in Table 1, seven of the alleles were cytotype-dependent. The direction of the effect was opposite that of the three bristle cytotype-dependent alleles, with the egg morphology becoming more mutant in the P cytotype.

We interpret cytotype-dependent singed sterility as indicating that a *P* element-encoded product interacts in trans with the *P* inserts in the *singed* locus, thus altering expression of the gene. Several experiments described below are aimed at testing the possibility that this product corresponds to the repressor hypothesized to account for the P cytotype, and at using cytotype-dependence to screen for repressor-making *P* elements. Only the sn^{w} allele was used in these experiments; the other alleles will be described more fully in a subsequent paper.

Tests for singed sterility in other genetic backgrounds: To examine the generality of the correlation between cytotype and sn^{w}-induced sterility, we observed the fertility and egg morphology of sn^{w}/sn^{w} females with autosomes derived from various strains. These females were obtained by crossing each strain of interest to sn^{w}; *CyO*; *TM6/T(2;3)*, ap^{Xa} females, then crossing the ap^{Xa} progeny to each other. Most of the autosomal complement of the ap^{+} daughters from the last cross will be from the strain of interest. Half of these daughters are expected to be sn^{w}/sn^{w} and the rest $+/sn^{w}$. These two genotypes cannot be distinguished by bristle phenotypes because dosage compensation of the sn^{w} allele is not complete. If approximately half of the ap^{+} females were sterile, and the rest proved to be $+/sn^{w}$, as indicated by the presence of both sn^{+} and sn^{w} among their sons, we concluded that the sn^{w}/sn^{w} females were sterile. Egg morphology was examined as described above.

The strains tested included one of the M cytotype (Oregon-R), one of the P cytotype (v_6), and four stocks classified as M′ (*M-5*-Birmingham, UK4, Sexi and Tübingen). The M′ classification indicates that *P* elements are present in the genome, but there is no strong P cytotype. We found that sn^{w}/sn^{w} females were fertile in the Oregon-R background but sterile in the v_6 background, thus confirming our previous conclusions.

The sn^{w}/sn^{w} females were also fertile with the *M-5*-Birmingham autosomes but sterile in the backgrounds of the other M′ strains. In all cases, this sterility was associated with the egg morphology characteristic of singed sterility. According to our interpretation that singed sterility requires *P* element repressor, this re-

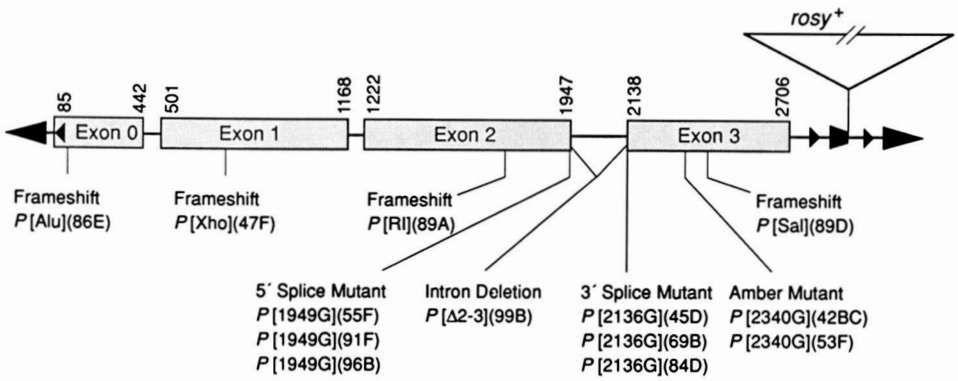

FIGURE 1.—Structure of *Pc*[*ry*] and the *in vitro*-modified elements. Construction of the original autonomous *Pc*[*ry*] element and its modification are described by KARESS and RUBIN (1984) and LASKI, RIO and RUBIN (1986), as is their transformation into the ry^{506} strain. The modified elements are named according to the nature of the modification, and their position of insertion in the genome as determined by in situ hybridization. They correspond to the following strain designations by the above authors: *P*[Alu](86E) = 18; *P*[Xho](47F) = 26; *P*[R1](89A) = 28; *P*[Sal](89D) = 1; *P*[1949G](55F) = 2, (91F) = 6, and (96B) = 14-1; *P*[2136G](45D) = 5, (69B) = 23, and (84D) = 22-1; *P*[2340G](42C) = 2, and (53F) = 6.

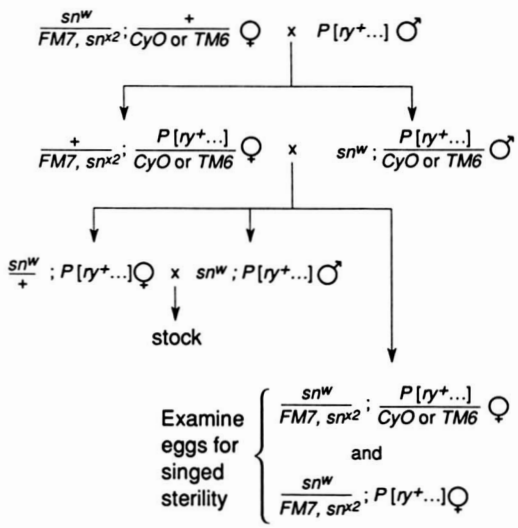

FIGURE 2.—Substitution of the *P*[ry^+ . . .] elements into sn^w/sn^{x2} females. The parental females were obtained by crosses of sn^w; +/*CyO* or *TM6* and *FM7*, $sn^{x2}/Df(1)N19$ (M) stocks.

sult suggests that the *P* elements in at least some M′ strains can produce enough repressor to affect sn^w expression, but not enough to bring about the P cytotype as determined by testcrosses to a P strain and measurement of GD sterility among the offspring. Consistent with this interpretation is the finding that some M′ strains display an intermediate cytotype when tested in this way (KIDWELL 1983, 1985; SIMMONS and BUCKHOLZ 1985; SIMMONS *et al.* 1987).

In vitro modified *P* elements tested for repressor using singed sterility

Origin of tested elements: The previous experiments indicate the existence of a *P* element repressor product and show that singed sterility can be used to test for its presence. The next step was to identify individual *P* elements that produce this product by a screen of eight in vitro-modified elements. These elements, shown in Figure 1, are derived from *Pc*[*ry*], which has a complete transposase gene plus the wild type *rosy* eye color marker. The frameshift mutant elements were made by KARESS and RUBIN (1984) to demonstrate that each of the four exons is required for transposase production. These are 4 bp insertions created by filling in restriction sites. LASKI, RIO and RUBIN (1986) constructed two elements with synonymous, single base pair substitutions in the conserved dinucleotides of the consensus 5′ donor and 3′ acceptor sites of the third (2–3) intron, to demonstrate that prevention of splicing of this intron abolishes transposase production. They also made an amber mutant in the fourth exon. [In all subsequent discussions, we will refer to these elements by abbreviations such as *P*[Sal](89D), leaving out the ry^+ designation, since it occurs in all elements. The cytological location of the element is indicated in parentheses, as suggested by LINDSLEY and ZIMM (1985).]

Tests for singed sterility: To test these elements for the ability to act as modifiers of the singed sterility phenotype, which we take to indicate repressor, we produced sn^w/sn^{x2} females with one or two copies of the element of interest present on an autosome (Figure 2) and examined their egg morphology. The results (Table 2) showed that two of the elements were associated with singed sterility, thus indicating the production of *P* repressor. One of these elements, *P*[Sal](89D), which carries a frameshift mutation in exon 3, caused nearly complete sterility in sn^w/sn^{x2} females. The other, *P*[1949G](96B), is a mutation in the 5′ splice junction of the 2–3 intron, and had a somewhat weaker singed sterility-inducing effect. We conclude that these two elements are able to mimic the effect of P cytotype on singed sterility of the sn^w allele, implying that they produce a product equivalent in this regard to that which constitutes P cytotype.

TABLE 2

Tests of various *P* elements for singed sterility

Element	$\frac{sn^w}{sn^{z2}}; \frac{P[ry\ldots]}{P[ry\ldots]}$ ++++	+++	++	+	$\frac{sn^w}{sn^{z2}}; \frac{P[ry\ldots]}{\text{Balancer}}$ ++++	+++	++	+
M cytotype control	350	50	26	1				
Frameshift mutants								
P[Alu](86E)	235	62	20	2	140	31	13	0
P[Xho](47F)	472	18	1	0	226	53	2	0
P[R1](89A)	231	141	21	1	211	69	6	0
P[Sal](89D)	0	2	23	106	1	5	71	212
Splice mutants								
P[1949G](55F)	173	4	0	0	331	29	3	0
P[1949G](91F)	131	13	1	0	240	7	0	0
P[1949G](96B)	0	9	39	49	132	80	52	19
P[2136G](45D)	142	19	1	0	250	58	6	0
P[2136G](69B)	113	9	1	0	225	9	1	0
P[2136G](84D)	76	2	0	0	149	11	2	0
Amber mutant								
P[2340G](42BC)	51	17	6	3	72	10	7	1
P[2340G](53F)	87	4	2	0	122	3	0	0
Intron deletion								
Δ2-3(99B)	45	26	8	0	26	21	1	0
"Complete" element								
Pc[*ry* 81.6](multiple sites)	884	189	12	24	264	68	6	0

Egg phenotypes of individual females were scored on a scale from nearly wild type (++++) to extreme singed (+). The categories correspond to the scores in Table 1. Note that genotypes sn^w/sn^w and $+/sn^{z2}$ are essentially wild type and scored as "+++++." The wild type category does not appear in this table since sn^w/sn^{z2} females are slightly affected, and fall into the "++++" category. The *y* sn^w;*bw*;*st*(M) strain was used for the M cytotype control cross.

The other lines, including the two with functional transposase genes, Δ2-3(99B) and *Pc*[*ry* 81.6], produced no detectable singed sterility. However, we cannot conclude that these elements are unable to produce *P* element repressor. One reason is that the marked differences among the three inserts of *P*[1949G] suggest that genomic position may play an important part in determining whether a given element produces *P* repressor. Therefore, it is necessary to test a large number of inserts of a given element before concluding that it is unable to produce repressor. A second reason for not excluding these elements as repressor-makers is that five of the stocks, *P*[Xho](47F), *P*[1949G](55F), *P*[1949G](91F), *P*[1949G](96B) and *P*[2340G](53F), which we assumed to be homogeneous, were later found to be polymorphic for their respective elements. Therefore, we cannot be certain that those elements were actually tested, with the exception of *P*[1949G](96B), which gave a positive result. In addition, the *Pc*[*ry* 81.6] stock was found by in situ hybridization to have multiple and polymorphic *P* elements sites, as was expected since *Pc*[*ry*] is a mobile element and produces transposase. It is likely that at least some of the new *Pc*[*ry*] sites are internally deleted elements. The problems of position effects and polymorphism within stocks are being circumvented in subsequent experiments, and will be reported separately.

Suppression of sn^w hypermutability

A more direct way to test for *P* element repressor is to look for the prevention of *P* element mobility itself. Hypermutability of the sn^w allele is an especially sensitive indicator of *P* element activity. In the presence of *P* element transposase, sn^w mutates to sn^e and $sn^{(+)}$ at high frequencies, but only in the M cytotype (ENGELS 1979a, 1984). As a transposase source, we used the Δ2-3(99B) element described previously (LASKI, RIO and RUBIN 1986; ROBERTSON *et al.* 1988). This element is nearly immobile, but provides transposase both somatically and in the germline.

Repression of somatic mosaicism: To test for repressor in somatic cells, we crossed females from the stocks homozygous for each *P*[*ry*. . .] element (see Figure 2) to Δ2-3(99B) males, yielding sons of the genotype

$$\frac{sn^w}{Y}; \frac{P[ry\ldots]}{+}; \frac{+}{\Delta 2\text{-}3(99B)} \text{ or } \frac{sn^w}{Y}; \frac{P[ry\ldots]}{\Delta 2\text{-}3(99B)}$$

depending on whether the *P*[*ry* . . .] element was on chromosome *2* or *3*. Each sn^w male was classified as a mosaic if any of the 44 major macrochaetae was clearly different from sn^w. The presence of any nonmosaics was taken as an indication of repressor production in somatic cells. The results (Figure 3) show that high frequencies of non-mosaics were produced only in the presence of elements *P*[Sal](89D) and *P*[1949G](96B).

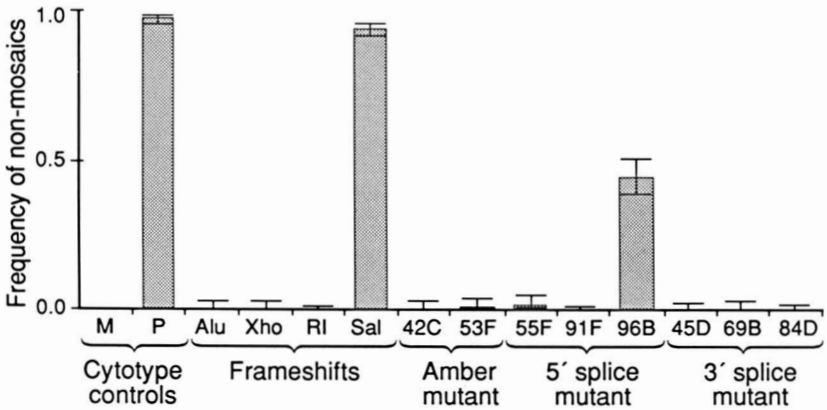

FIGURE 3.—Tests of suppression of somatic mosaicism. Males carrying the sn^w allele and one copy of Δ2-3(99B) plus one copy of the element to be tested were scored for somatic mosaicism at the *singed* locus. In the M and P cytotype controls, *y* sn^w; *bw*; *st* (M) and $sn^w(P)/M\text{-}5$(P); π_2 females were used instead of one of the $P[ry^+ \ldots]$ lines. Sample sizes averaged 196 flies (92–440) for the modified elements. The controls included 1886 M cytotype flies and 1008 P flies. The 95% confidence intervals were computed from the binomial distribution.

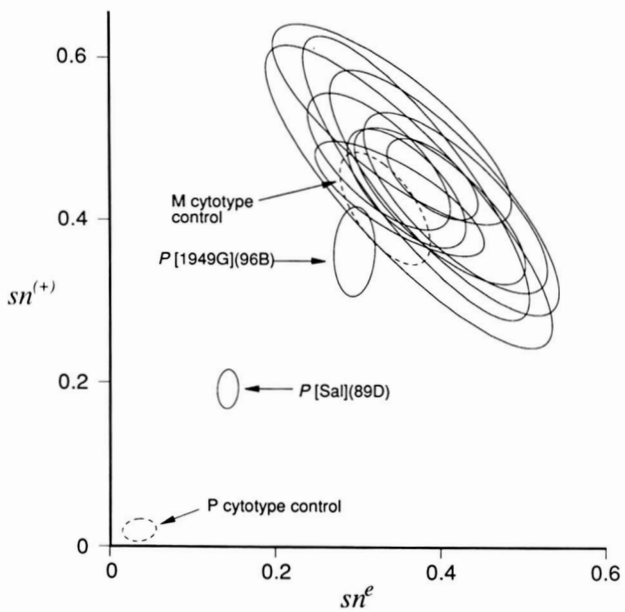

FIGURE 4.—Tests of suppression of germline mutability. The progeny of sn^w males used in Figure 3 were scored for sn^e and $sn^{(+)}$ phenotypes. For each tested element, 20 males were crossed individually to *C(1)DX, y f*; (P) females, and an average of 1431 sons were scored. The use of P cytotype females in this cross prevented *singed* mosaicism in the progeny, thus facilitating the identification of *singed* alleles. Each pair of mutation rates is represented by a confidence region based on approximate bivariate normality of the rate estimates. The size of the confidence region was arbitrarily chosen to be 63%. Variances were computed by a method that is unbiased by the existence of premeiotic events (ENGELS 1979c), and the covariance was obtained by an analogous procedure, also unbiased for clustering (W. ENGELS, unpublished results). The negative correlation in many of the tests arises from trinomial sampling within each set of progeny. The elements and control strains tested are the same as in Figure 3, but many of them are unlabeled here since they are statistically indistinguishable from each other and from the M cytotype control.

These are the same elements indicated by our singed sterility assay above to be repressor makers. Moreover, the *P*[Sal](89D) element appears to produce considerably more repressor function than *P*[1949G](96B), which is also in agreement with the previous finding.

We then measured germline mutability in the same set of males by crossing them to *C(1)DX,y f*; π_2 females and scoring the *singed* phenotypes of the sons. The resulting mutation rates of $sn^w \rightarrow sn^e$ and $sn^w \rightarrow sn^{(+)}$ are shown in Figure 4. Again, we see evidence of suppression by the element *P*[Sal](89D), which resulted in the lowest mutation rate to both sn^e and $sn^{(+)}$ of any element. The second lowest in both rates was that of *P*[1949G](96B), indicating somewhat weaker repressor function. The difference between *P*[1949G](96B) and the other elements was relatively slight, but statistically significant by the rank sum test ($P = 0.00003$). Our previous conclusion that the elements *P*[Sal](89D) and *P*[1949G](96B) suppress sn^w hypermutability was thus extended to the germline.

Characteristics of repression by *P*[Sal](89D)

The *P*[Sal]*89D) element represses pupal lethality: The remaining sections of this report deal with the properties of the *P*[Sal](89D) element and the extent to which it mimics the P cytotype. We begin by testing its ability to prevent a lethal interaction that occurs when *P* element transposase is combined with the many nonautonomous *P* elements that are found in the M′ strain known as *M-5*-Birmingham (ENGELS *et al.* 1987). This lethality occurs at the pupal stage, and is thought to result from chromosome breakage events during imaginal disc development in larvae. It is highly temperature sensitive, being more severe at elevated temperatures.

Table 3 shows the results of several crosses involving the *X* chromosome of *M-5*-Birmingham and the Δ2-3(99B) element as a source of somatic transposase. The *Stubble* (*Sb*) mutation was used as a dominant marker to follow *P*[Sal](89D). There were twelve classes of offspring that carried both the *M-5*-Birmingham *X* chromosome and the somatic transposase source, and that were reared at a sufficiently high temperature that pupal lethality would be expected. These classes are indicated in the table by boldface numbers. Six of them carried the *P*[Sal](89D) element, and lived. The other six lacked it, and almost all of them died. Therefore, the *P*[Sal](89D) element provides an essentially complete rescue from lethality. The only class in which this rescue is significantly less

TABLE 3

Rescue from pupal lethality by *P*[Sal](89D)

Female parent[a]	Male parent[a]	Temp.	No. of Progeny			
			Sb♀	*Sb*♂	*Sb*⁺♀	*Sb*⁺♂
Δ2-3	*Birm1*; $\frac{Sb\ Sal}{+}$	19°	145	152	127	165
		25°	**222**[b]	507	**4**	286
		28°	**415**	285	**0**	281
Δ2-3	*Birm1*; $\frac{Sal}{Sb}$	25°	**2**	1363	**1165**	1223
		28°	**0**	1067	**885**	982
$\hat{X}\hat{X}$; Δ2-3	*Birm1*; $\frac{Sal}{Sb}$	25°	796	**0**	736	**511**
		28°	691	**0**	653	**50**
ry^{506}	*Birm1*; $\frac{Sal}{Sb}$	25°	400	450	296	314
		28°	338	356	192	247
$\hat{X}\hat{X}$; ry^{506}	*Birm1*; $\frac{Sal}{Sb}$	25°	404	330	381	320
		28°	436	437	441	311

[a] Abbreviations: *Birm1* = the *X* chromosome from *M-5* Birmingham. This chromosome is derived from the *M-5* balancer marked with *white*[a] and *Bar*, and contains approximately 20 defective *P* elements. Sal = *P*[Sal](89D). $\hat{X}\hat{X}$ = *C(1)DX, yf/Y*. Δ2-3 = Δ2-3(99B).

[b] Boldface indicates progeny classes where pupal lethality might occur.

than complete is that of males reared at 28° which are hemizygous for the *M-5*-Birmingham *X* chromosome. Pupal lethality is known to be most severe in males of this kind (ENGELS *et al.* 1987). We conclude that *P*[Sal](89D) acts as a highly effective repressor of the pupal lethal effects of somatic hybrid dysgenesis.

The *P*[Sal](89D) element represses GD sterility: One of the most common effects of hybrid dysgenesis is GD ("gonadal dysgenic") sterility in which the germline of dysgenic males or females fails to develop (ENGELS and PRESTON 1979; SCHAEFER, KIDWELL and FAUSTO-STERLING 1979; NIKI and CHIGUSA 1986). It was first described in the progeny of crosses between M and P strains, but it also occurs from the interaction of Δ2-3(99B) and the *M-5*-Birmingham genome. Like pupal lethality, this effect is thought to be the result of cell lethality except that it is limited to the germline (ENGELS *et al.* 1987). To test whether *P*[Sal](89D) can prevent this GD sterility, we dissected approximately 50 females (or as many as were available) from each phenotypic class of the crosses in the top three rows of Table 3, and scored them for the presence of oocytes in each ovary. Only 4 out of 304 ovaries from females carrying the *P*[Sal](89D) element lacked oocytes, and were thus considered dysgenic, whereas over half (65/106) of the ovaries from the classes lacking *P*[Sal](89D) were dysgenic. Moreover, the ovaries from the classes lacking *P*[Sal](89D) were necessarily drawn almost entirely from the 19° group where the effects of dysgenesis are expected to be much less severe. These data show that *P*[Sal](89D) is a powerful repressor of GD sterility.

TABLE 4

Lack of reciprocal cross effects of *P*[Sal](89D)

Female parent	Male parent	sn^{+} & $sn^{(+)}$	Total	Rate (% ± SE)
$\hat{X}\hat{X}$; Δ2-3(99B)	sn^{w}; ry^{506}	282	462	61.0 ± 4.5
sn^{w}; ry^{506}	Δ2-3(99B)	253	419	60.4 ± 8.7
sn^{w}/+; *P*[Sal](89D)	Δ2-3(99B)	422	1577	26.8 ± 2.1
$\hat{X}\hat{X}$; Δ2-3(99B)	sn^{w}; *P*[Sal](89D)	254	1688	15.1 ± 1.0

Sons bearing the sn^{w} allele were collected from each cross, mated to compound-*X* females of the P cytotype, and the *singed* phenotype of the male progeny were scored. The first two rows (controls) came from ten matings each, and the last two rows came from 20 matings each. Standard errors were computed by a method that is not biased by premeiotic (clustered) events (ENGELS 1979c).

The repressor function maps to *P*[Sal](89D): We used the pupal lethal interaction discussed above to show that the repressor function on the *P*[Sal](89D) chromosome maps at or very near the *P*[Sal](89D) element itself. We performed the cross

$$\frac{Birm1}{w\ m\ f};\ \frac{Sb\ Sal}{+}\ ♀ \times w;\ Δ2\text{-}3♂$$

and scored the sons for the *Stubble* phenotype. [See the legend of Table 3 for abbreviations and LINDSLEY and GRELL (1968) for other genetic symbols.] Note that *Stubble* lies at cytological position 89B which is quite close to *P*[Sal](89D), thus providing a convenient marker.

Among the surviving *Birm1* sons, 595 were *Sb* and only 15 were *Sb*⁺, whereas those with the *w m f* chromosome were about equally divided (451 and 494). Given the interchromosomal effect due to crossover suppression on the *X* chromosome (LUCCHESI and SUZUKI 1968), this result indicates that the repressor lies about 1 map unit from *Stubble*, placing it at or very near the *P*[Sal](89D) element.

Lack of reciprocal cross effect on the inheritance of *P*[Sal](89D) repressor: One of the characteristics of the P cytotype is its partial maternal inheritance which results in the well known reciprocal cross effect in hybrid dysgenesis. We used sn^{w} hypermutability in the germline to test for a reciprocal cross difference in repression brought about by *P*[Sal](89D). The first two crosses in Table 4 are controls indicating that Δ2-3(99B) causes about 60% mutability of sn^{w} in both reciprocal crosses. The *P*[Sal](89D) element is present in the other two crosses, being matroclinous in the third and patroclinous in the fourth cross. It is clear that sn^{w} hypermutability is suppressed by at least half in the presence of *P*[Sal](89D), as was seen previously in Figure 4. However, suppression was not stronger when *P*[Sal](89D) was derived from the mother. If anything, the reverse was true. We conclude that the effect of *P*[Sal](89D) differs from the P cytotype in its lack of any detectable reciprocal cross effect.

TABLE 5

Lack of suppression of GD sterility by *P*[Sal](89D) in crosses to a P strain

Female parent	Daughters: 1 or more eggs	Daughters: No eggs	% GD sterile
sn^w/M-5; π_2 (P)	165	10	5.7
sn^w/+; *P*[Sal](89D)	15	216	93.5
sn^w; ry^{506} (M)	1	178	99.4

For each test, ten females were mated to π_2 males and the progeny raised at 28°. After aging on yeasted food for four days, female progeny were squashed between glass plates. The absence of eggs was taken to indicate GD sterility. The sn^w allele is present in all crosses but does not play a role in this experiment.

The *P*[Sal](89D) element does not suppress hybrid dysgenesis from the π_2 strain: Several experiments described above show that *P*[Sal](89D) is an effective suppressor of *P* element activity when a single element, Δ2-3(99B), is used as the transposase source. The experiment in Table 5 shows that *P*[Sal](89D) is much less effective in suppressing hybrid dysgenesis, as measured by GD sterility, when transposase comes from the many complete *P* elements present in the P strain, π_2. This result and the lack of maternal inheritance indicate that the suppression properties of *P*[Sal](89D) differ in at least some respects from those of the P cytotype.

DISCUSSION

Kinds of regulation in the P-M system of hybrid dysgenesis: Part of the original definition of hybrid dysgenesis was that it does not occur in nonhybrids and it occurs predominantly in one of the two reciprocal crosses (KIDWELL, KIDWELL and SVED 1977). Subsequent work showed that a cellular condition called the P cytotype is responsible for both of these properties (ENGELS 1979a). A hypothetical gene product encoded by at least some *P* elements was proposed as the basis for the P cytotype (ENGELS 1979b). According to this model the reciprocal cross effect is analogous to zygotic induction in bacteriophage λ. That is, individuals in a P strain and hybrids from a nondysgenic cross (P♀ × M♂) have transposase-making elements but also maternally derived repressor. Hybrids from the reciprocal cross lack the repressor, and are therefore dysgenic. The present results confirm at least one tenet of this model—that some *P* elements are capable of producing a repressor of *P* element activity.

There is recent evidence that the P cytotype is not the only mechanism that can regulate *P* element mobility. Other mechanisms are indicated by the relative lack of *P* element mobility within many natural M′ strains even though the genome harbors one or more complete *P* elements (KIDWELL 1985; SIMMONS and BUCHOLZ 1985; BLACK *et al.* 1987; SIMMONS *et al.* 1987; JACKSON, BLACK and DOVER 1988). In most cases this regulation differs from the P cytotype by its lack of maternal transmission and by its unimodal distribution (reviewed in ENGELS 1989). Titration of transposase by nonautonomous *P* elements (SIMMONS and BUCHHOLZ 1985) has been suggested to explain part of this regulation. Another suggestion (BLACK *et al.* 1987; JACKSON, BLACK and DOVER 1988) is that certain small defective *P* elements called "*KP* elements," which are found in some of the M′ strains in question, might be involved. Further work will be needed to resolve what may be a complex set of regulatory mechanisms.

Cytotype-dependent gene expression: We have described a series of *singed* mutations, all of which are due to *P* element insertions, whose phenotypes are dependent upon cytotype. Some of them are cytotype-dependent for bristle morphology and others are cytotype-dependent for female fertility. The mechanism of this dependence is not known, but for at least one of the latter cases, J. PATTERSON and K. O'HARE (personal communication) have shown that a female-specific *singed* transcript is missing in the P cytotype but is present in the M cytotype.

Several other cases of cytotype-dependent mutations have been found. The $vg^{21\text{-}3}$ mutation (WILLIAMS, PAPPU and BELL 1988) has an extreme *vestigial* wing phenotype in M genomic backgrounds but is nearly wild type in P backgrounds. Recent work has shown that the *P*[Sal](89D) element is sufficient to restore the wing phenotype to essentially wild type (W. BENZ and W. ENGELS, unpublished results). In addition, D. COEN and D. ANXOLABÉHÈRE (personal communication) have studied a *P* transposon carrying a modified *white* gene whose expression is closer to the null phenotype in P backgrounds. This element is also affected by *P*[Sal](89D) in the same way. The evidence that cytotype itself is responsible for the differential expression of all of these cases remains circumstantial, based primarily upon a correlation with cytotype as measured by *P* element regulation. In the case of the singed sterility alleles and the $P[w^+]$ element, the mutant phenotype is enhanced by the P cytotype, and in all other cases it is suppressed. MCCLINTOCK also found both suppression and enhancement of expression in the *Spm* transposon system in maize (reviewed by FEDOROFF 1989). This analogy extends to the finding of differential transcription of the affected gene (MASSON *et al.* 1987).

Is suppression by the *P*[Sal](89D) and *P*[1949G]-(96B) elements the same as that of the P cytotype? Although the effects of these elements seem to mimic the P cytotype in many ways, the analogy is not perfect. First, the reciprocal cross difference, which defines cytotype, has not been observed for *P*[Sal]-(89D). Second, the *P*[Sal](89D) element has only been

found to suppress *P* element activity when the transposase source is Δ2-3(99B), and not when it is an entire P strain genome. One interpretation is that the repressor encoded by *P*[Sal](89D) and other such elements is different from that of the P cytotype.

Another possibility, however, is that the product of *P*[Sal](89D) is qualitatively identical to the P cytotype repressor, but differs in amount and distribution. O'KANE and GEHRING (1987) have shown that genomic position can affect tissue specificity and developmental timing of expression of a β-galactosidase gene driven from the *P* element's promoter. Thus, the *P*[Sal](89D) element, by virtue of its position, might fail to produce sufficient repressor in the oocytes to yield an observable maternal effect. In a true P strain there are probably several repressor-making *P* elements, and the likelihood of at least one of them being expressed appropriately for a maternal effect is correspondingly greater. Similarly, the inability of *P*[Sal](89D) to suppress GD sterility in the presence of the π_2 genome might be explained by the larger number of both transposase- and repressor-making elements in the π_2 genome. The addition of one more repressor-maker in a genotype with many such elements might make relatively little difference. By isolating individual repressor-making *P* elements from the π_2 genome, and further analyzing the nature and distribution of the *P*[Sal](89D) repressor, we hope to determine whether the *P*[Sal](89D) repressor corresponds to the P cytotype.

The nature of the *P*[Sal](89D) repressor: Our results identify a transacting product of the *P*[Sal](89D) and *P*[1949G](96B) elements which alters expression of cytotype-dependent alleles and can repress *P* element mobility. The exact nature of this product and its relationship to the *P* element transposase gene remain unclear. Both elements carry modifications preventing the expression of the fourth exon of the transposase gene, but the first three exons are intact. These findings are consistent with the possibility (RIO, LASKI and RUBIN 1986) that the 66-kD protein, produced when the third and fourth transposase exons are not spliced, can function as a repressor. Further analysis of the *P*[Sal](89D) and *P*[1949G](96B) products along with additional tests of other in vitro-modified elements will be needed to determine what this repressor is and how it operates.

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LITERATURE CITED

BENDER, H. A., 1960 Studies on the expression of various *singed* alleles in *Drosophila melanogaster*. Genetics **45:** 867–882.

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.

BLACK, D. M., M. S. JACKSON, M. G. KIDWELL and G. A. DOVER, 1987 KP elements repress P-induced hybrid dysgenesis in *D. melanogaster*. EMBO J. **6:** 4125–4135.

ENGELS, W. R., 1979a Hybrid dysgenesis in *Drosophila melanogaster*: rules of inheritance of female sterility. Genet. Res. **33:** 219–236.

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

ENGELS, W. R., 1979c The estimation of mutation rates when premeiotic events are involved. Environ. Mutagen. **1:** 37–43.

ENGELS, W. R., 1981 Germline hypermutability in Drosophila and its relation to hybrid dysgenesis and cytotype. Genetics **98:** 565–587.

ENGELS, W. R., 1984 A trans-acting product needed for *P* factor transposition in Drosophila. Science **226:** 1194–1196.

ENGELS, W. R., 1985 A set of P-cytotype balancer chromosomes. Drosophila Inform. Serv. **61:** 70–71.

ENGELS, W. R., 1989 *P* elements in Drosophila, pp. 437–484 in *Mobile DNA*, edited by D. E. BERG and M. M. HOWE. American Society of Microbiology, Washington, D.C.

ENGELS, W. R., and C. R. PRESTON, 1979 Hybrid dysgenesis in *Drosophila melanogaster*: the biology of male and female sterility. Genetics **92:** 161–175.

ENGELS, W. R., and C. R. PRESTON, 1980 Components of hybrid dysgenesis in a wild population of *Drosophila melanogaster*. Genetics **95:** 111–128.

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

ENGELS, W. R., W. K. BENZ, C. R. PRESTON, P. L. GRAHAM, R. W. PHILLIS and H. M. ROBERTSON, 1987 Somatic effects of *P* element activity in *Drosophila melanogaster*: Pupal lethality. Genetics **117:** 745–757.

FEDOROFF, N., 1989 Maize transposable elements, pp. 375–411 in *Mobile DNA*, edited by D. E. BERG and M. M. HOWE. American Society for Microbiology, Washington, D.C.

HAGIWARA, N., E. NAKAMURA, E. T. MATSUURA and S. I. CHIGUSA, 1987 Hybrid dysgenesis in natural populations of *Drosophila melanogaster* in Japan. II. Strains which cannot induce P-M dysgenesis may completely suppress functional *P* element activity. Genet. Res. **50:** 105–111.

JACKSON, M. D., D. M. BLACK and G. A. DOVER, 1988 Amplification of *KP* elements associated with the repression of hybrid dysgenesis in *Drosophila melanogaster*. Genetics **120:** 1003–1013.

KARESS, R. E., and G. M. RUBIN, 1984 Analysis of transposable element functions in Drosophila. Cell **38:** 135–146.

KIDWELL, M. G., 1981 Hybrid dysgenesis in *Drosophila melanogaster*: the genetics of cytotype determination in a neutral strain. Genetics **98:** 275–290.

KIDWELL, M. G., 1983 Evolution of hybrid dysgenesis determinants in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **80:** 1655–1659.

KIDWELL, M. G., 1985 Hybrid dysgenesis in *Drosophila melano-*

gaster: nature and inheritance of *P* element regulation. Genetics **111:** 337–350.

KIDWELL, M. G., T. FRYDRYK and J. B. NOVY, 1983 The hybrid dysgenesis potential of *Drosophila melanogaster* strains of diverse temporal and geographical natural origins. Drosophila Inform. Serv. **59:** 63–69.

KIDWELL, M. G., J. F. KIDWELL and J. A. SVED, 1977 Hybrid dysgenesis in *Drosophila melanogaster*: a syndrome of aberrant traits including mutation, sterility, and male recombination. Genetics **86:** 813–833.

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.

LINDSLEY, D. L., and E. H. GRELL, 1968 *Genetic Variations of Drosophila melanogaster*. Carnegie Inst. Wash. Publ. 6.

LINDSLEY, D. L., and G. ZIMM, 1985 The genome of *Drosophila melanogaster*, Part 1: genes A–K. Drosophila Inform. Serv. **62:** 1–227.

LUCCHESI, J. C., and D. T. SUZUKI, 1968 The interchromosomal control of recombination. Annu. Rev. Genet. **2:** 53–86.

MASSON, P., R. SUROSKY, J. A. KINGSBURY and N. V. FEDOROFF, 1987 Genetic and molecular analysis of the *Spm-dependent a-m2* alleles of the maize *a* locus. Genetics **117:** 117–137.

NIKI, Y., and S. I. CHIGUSA, 1986 Developmental analysis of the gonadal sterility of P-M hybrid dysgenesis in *Drosophila melanogaster*. Jpn. J. Genet. **61:** 147–156.

NITASAKA, E., T. MUKAI and T. YAMAZAKI, 1987 Repressor of *P* elements in *Drosophila melanogaster*: cytotype determination by a defective *P* element with only open reading frames 0 through 2. Proc. Natl. Acad. Sci. USA **84:** 7605–7608.

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.

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

PERRIMON, N., and M. GANS, 1983 Clonal analysis of the tissue specificity of recessive female-sterile mutations of *Drosophila melanogaster* using a dominant female-sterile mutation *Fs(1)K1237*. Dev. Biol. **100:** 365–373.

RIO, D. C., F. A. LASKI and G. M. RUBIN, 1986 Identification and immunochemical analysis of biologically active Drosophila *P* element transposase. Cell **44:** 21–32.

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.

SCHAEFER, R. E., M. G. KIDWELL and A. FAUSTO-STERLING, 1979 Hybrid dysgenesis in *Drosophila melanogaster*: morphological and cytological studies of ovarian dysgenesis. Genetics **92:** 1141–1152.

SIMMONS, M. J., and L. M. BUCHOLZ, 1985 Transposase titration in *Drosophila melanogaster*: a model of cytotype in the P-M system of hybrid dysgenesis. Proc. Natl. Acad. Sci. USA **82:** 8119–8123.

SIMMONS, M. J., J. D. RAYMOND, M. J. BOEDIGHEIMER and J. R. ZUNT, 1987 The influence of nonautonomous *P* elements on hybrid dysgenesis in *Drosophila melanogaster*. Genetics **117:** 671–685.

SVED, J. A., 1987 Hybrid dysgenesis in *Drosophila melanogaster*: evidence from sterility and Southern hybridization that P cytotype is not maintained in the absence of chromosomal *P* factors. Genetics **115:** 121–127.

WILLIAMS, J. A., S. S. PAPPU and J. B. BELL, 1988 Suppressible *P* element alleles of the *vestigial* locus in *Drosophila melanogaster*. Mol. Gen. Genet. **212:** 370–374.

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