Cytotype Control of *Drosophila melanogaster P* Element Transposition: Genomic Position Determines Maternal Repression

Sima Misra,* Robin M. Buratowski,† Taro Ohkawa†,[1] and Donald C. Rio*

**Department of Molecular and Cell Biology, Division of Genetics and Division of Biochemistry and Molecular Biology, University of California, Berkeley, California 94720 and †Whitehead Institute for Biomedical Research, Cambridge, Massachusetts 02142*

Manuscript received April 14, 1993
Accepted for publication July 8, 1993

ABSTRACT

P element transposition in Drosophila is controlled by the cytotype regulatory state: in P cytotype, transposition is repressed, whereas in M cytotype, transposition can occur. P cytotype is determined by a combination of maternally inherited factors and chromosomal *P* elements in the zygote. Transformant strains containing single elements that encoded the 66-kD *P* element protein zygotically repressed transposition, but did not display the maternal repression characteristic of P cytotype. Upon mobilization to new genomic positions, some of these repressor elements showed significant maternal repression of transposition in genetic assays, involving a true maternal effect. Thus, the genomic position of repressor elements can determine the maternal *vs.* zygotic inheritance of P cytotype. Immunoblotting experiments indicate that this genomic position effect does not operate solely by controlling the expression level of the 66-kD repressor protein during oogenesis. Likewise, *P* element derivatives containing the *hsp26* maternal regulator sequence expressed high levels of the 66-kD protein during oogenesis, but showed no detectable maternal repression. These data suggest that the location of a repressor element in the genome may determine maternal inheritance of P cytotype by a mechanism involving more than the overall level of expression of the 66-kD protein in the ovary.

THE *P* transposable element family, often used as a molecular genetic tool, is one of the best characterized transposable elements in *Drosophila melanogaster*. Movement of these elements is regulated in two ways: first, transposition is restricted to germline cells (tissue specificity); and second, transposition only occurs when a P strain male carrying *P* elements is mated to an M strain female lacking *P* elements and not in the reciprocal cross (genetic regulation; for reviews see RIO 1990; ENGELS 1989). The tissue specificity of transposition is due at least in part to inhibition of *P* element pre-mRNA splicing in somatic tissues (CHAIN *et al.* 1991; SIEBEL, FRESCO and RIO 1992; SIEBEL and RIO 1990), but the molecular basis for the genetic regulation of transposition remains largely unknown.

Genetic repression of *P* element movement is initially maternally inherited, but after a few generations repression potential is determined by chromosomal *P* elements in the zygote (ENGELS 1979a; KIDWELL 1981). This regulatory state is defined as cytotype, with both maternal and zygotic components: in M cytotype *P* elements are mobile and can cause a syndrome of genetic disorders called hybrid dysgenesis, whereas in P cytotype *P* elements are stable (ENGELS 1979a). P strain females (carrying *P* elements) show P cytotype and are therefore able to prevent dysgenesis in their progeny, but M strain females (lacking *P* elements) show M cytotype and cannot prevent hybrid dysgenesis in their progeny.

Molecular analysis has shown that the typical P strain contains a heterogeneous collection of 40–50 *P* elements, 10–15 of which are full length (O'HARE and RUBIN 1983; O'HARE *et al.* 1992). The complete *P* element contains four open reading frames, which in the germline pre-mRNA are spliced to encode the 87-kD transposase protein (RIO, LASKI and RUBIN 1986; KARESS and RUBIN 1984). However, when the last intron is not spliced, the mRNA encodes a truncated 66-kD protein that represses the genetic effects of transposase in both the soma and germline (MISRA and RIO 1990; ROBERTSON and ENGELS 1989).

We previously observed that single elements encoding the 66-kD *P* element protein could repress transposition zygotically, but not maternally (MISRA and RIO 1990). Such a transformant strain expressed only a low level of 66-kD repressor protein during oogenesis, whereas a P strain that exhibited true P cytotype expressed a high level. This led to a model in which the maternal aspect of P cytotype is due to the production of repressor protein (or mRNA encoding repressor) during oogenesis. The second part of the model proposed that genomic position might be a determinant of maternal repression. "Enhancer-trap" experiments with *P* element-*lacZ* fusion genes have indicated that the temporal and spatial pattern of

[1] Present address: Department of Entomology, University of California, Davis, California 95616.

expression depends on the location of the element in the genome (O'KANE and GEHRING 1987; GROSSNIKLAUS *et al.* 1989; BELLEN *et al.* 1989; BOWNES 1990). Due to these position effects, the single elements encoding the 66-kD protein that we tested may not have expressed the repressor at the appropriate time or level to show maternal inheritance (MISRA and RIO 1990). Moreover, P strains that exhibit maternal P cytotype may have undergone a selection for repressor elements in positions that allow high level repressor expression during oogenesis.

We describe here experiments that directly test both aspects of this model. If genomic position is the basis for the maternal component of cytotype, then the original 66-kD protein-encoding elements would be predicted to show maternal repression in a subset of locations in the genome. We show that repressor elements mobilized to new positions in the genome can show substantial maternal effect repression in the germline, indicating that genomic position is an important determinant of maternal P cytotype. Significantly, immunoblot analysis of these transformant strains showed that the overall level of 66-kD protein expressed during oogenesis did not correlate with maternal effect repression. In addition, *P* element derivatives containing a regulatory sequence active during oogenesis expressed high levels of repressor protein in the ovary, yet transformants with such elements showed no detectable repression of *P* element movement in genetic assays. These results suggest that maternal repression requires a very specific time and location of repressor expression, or perhaps more interestingly, that genomic position of a repressor element may affect more than just repressor protein production.

MATERIALS AND METHODS

Recombinant DNA: Restriction enzymes were obtained from New England Biolabs or Boehringer Mannheim. The Klenow fragment of DNA polymerase, T4 DNA ligase and calf intestine alkaline phosphatase were from Boehringer Mannheim. All enzymes were used according to the manufacturer's specifications. Basic recombinant techniques were performed as described (MANIATIS, FRITSCH and SAMBROOK 1982).

The maternal regulator constructs are diagramed in Figure 1. *P*[*ry*$^+$; *hsp26* 66K *CycB*] was a derivative of 26gZS (CHEUNG, SERANO and COHEN 1992; FRANK, CHEUNG and COHEN 1992) and contained a 388-bp *hsp26* nurse cell regulator fragment (*Eco*RI to *Xba*I) and 150-bp *sgs3* promoter fragment (*Xba*I at nucleotide −127 to *Nhe*I at nucleotide 33) inserted into Carnegie 20 (RUBIN and SPRADLING 1983) at the *Eco*RI and *Nhe*I sites of the polylinker. The polymerase chain reaction was used to isolate (SCHARF 1990) the 800-bp 3′ untranslated region (UTR) of the *Cyclin B* (*CycB*) gene and polyadenylation site (RAFF, WHITFIELD and GLOVER 1990) with synthetic *Bam*HI and *Xba*I sites at the ends. *P* element nucleotides (nts) 36–2078 were isolated from *P*[*ry*$^+$;66K] with *Apa*LI and *Xba*I, and ligated with the 800-bp *CycB* fragment and linearized 26gZS plasmid to produce *P*[*ry*$^+$; *hsp26* 66K *CycB*].

P[*ry*$^+$; *hsp26* 66K *K10*] was a derivative of 26gZK, which is identical to 26gZS except that a 2.2-kb fragment containing the 3′ UTR and polyadenylation site of the *fs(1)K10* gene is inserted into the *Sal*I site of Carnegie 20 (CHEUNG, SERANO and COHEN 1992). *P* element nts 36 to 2078 (as above) were ligated into *Not*I-cleaved 26gZK to make *P*[*ry*$^+$; *hsp26* 66K *K10*].

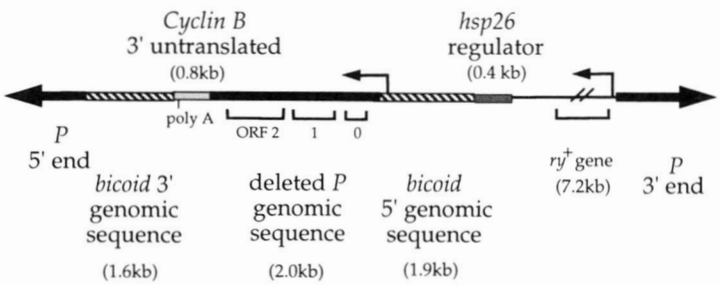
The *P*[*ry*$^+$; *hsp26 bcd* 66K *CycB*] element was made from a derivative of pCaSpeR*bcdBgl*II, which contained genomic sequences from the *bicoid* (*bcd*) gene from nt −660 to +1244, and nt +4292 to nt +5900, with respect to the *Sal*I site upstream of the gene (nt 0) and a *Bgl*II linker inserted into the deletion from nts 1244–4292 (DRIEVER, SIEGEL and NÜSSLEIN-VOLHARD 1990). A 2.8-kb fragment containing sequences from *P* element nts 36 to 2078 (as above) and the 800-bp *CycB* 3′ UTR was inserted into pCaSpeR*bcd-Bgl*II, then digested to release a 6.3-kb fragment containing the 5′ and 3′ *bcd* sequences, 66-kD coding region and *CycB* 3′ UTR. The 388-bp *hsp26* regulator fragment was removed from 26gZS and ligated to the 6.3-kb *bcd* 66K *CycB* fragment and inserted into the pDM30 vector (MISMER and RUBIN 1987) to generate *P*[*ry*$^+$; *hsp26 bcd* 66K *CycB*].

Drosophila strains: The Drosophila M strains Canton-S *ry*^{506} (referred to as *ry*^{506}), *y sn*^{3}*v*/*y*$^+$ Y and *y sn*^{w}; *ry*^{506}, as well as the second chromosome balancer stock *CyO/Sco; ry*^{506} and the third chromosome balancer stock *TM3, Sb ry*^{RK}/*ry*^{506} are described in KARESS and RUBIN (1984) and LINDSLEY and ZIMM (1992). The standard P strain Harwich is described in RONSSERAY (1986), and the Q strain ν_6 and *C(1)DX, y f; ry*^{506} *P*[*ry* $^+$;Δ2–3](99B) stock are described in ROBERTSON and ENGELS (1989), as is the *FM7, y sn*^{31d} *sn*^{x2} *B* strain, except that the *y sn*^{w} chromosome was substituted into the balancer strain to make it *FM7, y sn*^{31d} *sn*^{x2} *B*/*y sn*^{w}. The *CyO/Sp; ry*^{506} *Sb P*[*ry* $^+$;Δ2–3](99B)/*TM6B, Ubx* strain is derived from a strain described in ROBERTSON *et al.* (1988) and is described in BELLEN *et al.* (1989). Flies were reared on standard cornmeal, molasses and yeast medium at 25°. The *sn P*[*lacZ*] strain was obtained from K. O'HARE and contains a *P* promoter-*lacZ* element inserted into the *singed* gene that causes an extreme singed (sn^e) bristle phenotype (K. O'HARE, unpublished data).

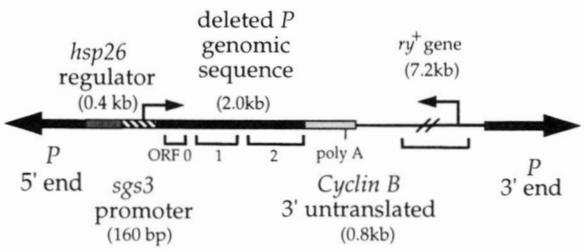
***P* element transformation, mobilization and molecular analysis:** Transformation of *ry*^{506} was by standard procedures (SPRADLING 1986), except that the *P* element transposase fraction TdT 0.3 M was used as described in KAUFMAN and RIO (1991) at a ratio of ~0.4–2 transposase molecules to plasmid DNA molecules. Isolines were established from individual independent transformants. Flies from these isolines were also outcrossed to *ry*^{506} flies and subsequently crossed to *CyO/Sco; ry*^{506} or *TM3 ry*^{RK}/*ry*^{506} balancer strains to establish balanced stocks (LINDSLEY and ZIMM 1992) and then to *y sn*^{w}; *ry*^{506} to make them homozygous for the *y sn*^{w} chromosome. Isolines established after mobilization (Figure 2) of the *P*[*ry*$^+$;66K] or *P*[*ry*$^+$;hs66K] elements were similarly outcrossed.

DNA blot hybridization was used to confirm that each strain contained a single insertion and that the transposon was not deleted or rearranged; other strains were discarded. Genomic DNA extraction and DNA blot hybridization with a *P* element probe were performed as described (MISRA and RIO 1990) using standard procedures (MANIATIS, FRITSCH and SAMBROOK 1982). DNA was digested with *Eco*RI, and DNA blots were hybridized with random hexamer ^{32}P-labeled *P* element cDNA probe (pHSX-*P*c3; MISRA and RIO 1990). An internal 4.8-kb band was expected and observed

A. *P[ry⁺; hsp26 bcd 66K CycB]*



B. *P[ry⁺; hsp26 66K CycB]*



C. *P[ry⁺; hsp26 66K K10]*

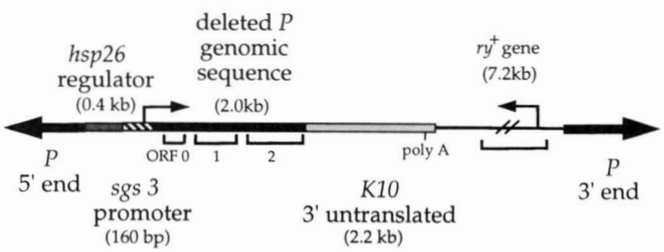


FIGURE 1.—Maternal regulator *P* element constructs. (A) *P[ry⁺;hsp26bcd*66K *CycB]*contains a 380-bp *hsp26* regulator sequence that stimulates germline expression in the ovary (FRANK, CHEUNG and Cohen 1992) as well as 5′ and 3′ genomic sequences from the maternal effect gene *bicoid*(*bcd*), which specifies pattern along the anterior-posterior axis of the embryo (DRIEVER and NÜSSLEIN-VOLHARD 1988). A fragment containing 800 bp of the 3′ untranslated region (UTR) of *Cyclin B* (*CycB*) is downstream of *P* element nucleotide 2078; this sequence localizes transcripts to the germline during embryogenesis (DALBY and GLOVER 1992). (B) *P[ry⁺;hsp2*66K *CycB*]contains the *hsp26* regulator sequence and the *CycB* 3′ UTR as above, but the basal *sgs3* promoter is used instead of the *bcd* promoter. (C) *P[ry⁺;hsp2*66K *K10*] contains the *hsp26* regulator sequence and the *sgs3* promoter, but the *fs(1)K10* gene 3′ UTR is included to localize transcripts to the oocyte during oogenesis (CHEUNG, SERANO and COHEN 1992). The *fs(1)K10* gene is a maternal effect gene responsible for dorsal-ventral patterning of the oocyte and eggshell (WIESCHAUS, MARSH and GEHRING 1978).

in *P[ry⁺*;66K] strains and an internal 4.0-kb band in *P[ry⁺*; hs66K] strains.

Eleven of the 21 *P[ry⁺*;66K] strains and four of the 14 *P[ry⁺*;hs66K] strains contained insertions on the *CyO* balancer chromosome (Table 1). Because of the homozygous lethal mutations on this chromosome, only flies heterozygous for the element could be obtained. Several third chromosomes with insertions were homozygous lethal as well (data not shown). *In situ* hybridization analysis of 66-kD element strains was performed by preparation and hybridization of larval salivary gland chromosomes (ASHBURNER 1989) to a biotinylated DNA probe synthesized from linearized pHSX-*P*c3 plasmid.

Germline P cytotype assays: Standard procedures were used to test the transformant strains for gonadal dysgenic (GD) sterility. Ten balanced *P[ry⁺*;66K] or *P[ry⁺*;hs66K] heterozygous virgin females from each strain were mated individually to Harwich males at 29° and progeny were collected on day 11, transferred to fresh vials with yeast paste, aged 3 days at 25° and squashed between glass plates against a dark background. If no eggs were extruded from a female, she was scored as sterile. For simplicity, results are presented pooled over all female progeny tested in a particular class, rather than separately for each parent. Significance levels were calculated using the chi-square test as in RONSSERAY (1986).

Tests for sn^w destabilization were performed as outlined in Figure 5, using males and females from the *C(1)DX, y f; ry*^{506} *P[ry⁺*;Δ2-3](99B) stock. Ten G_1 males from each reciprocal cross were singly mated to *y* sn^3v females and excision frequency from the *y* sn^w chromosome was estimated by scoring only sn^e G_2 female progeny from both reciprocal crosses. Although somatic mosaicism was caused by the *P[ry⁺*;Δ2-3] element in the sn^w G_2 progeny, the G_2 progeny resulting from a germline excision event showed no mosaicism, since one of the elements permanently excised from the genome, whereas mosaic progeny had, in addition to sn^e bristles, sn^+ and sn^w bristles that could be easily identified. Therefore, sn^e G_2 progeny could be accurately and reproducibly scored. Standard statistical procedures were used (ENGELS 1979c); mutation rate was estimated by an unweighted average because of premeiotic clustering, and its precision was estimated by an unweighted variance. Strains were compared to the M strain control by the rank sum test (ENGELS 1979c).

Tests for singed sterility were performed as in ROBERTSON and ENGELS (1989), except that heterozygous sn^{X2}/y sn^w daughters were collected after 2 days and transferred to fresh vials, where they were aged for 3 days before examination of eggs; females with the *P[ry⁺*;66K] or *P[ry⁺*;hs66K] were scored, but siblings lacking the elements were compared as internal controls and showed normal fertility. In

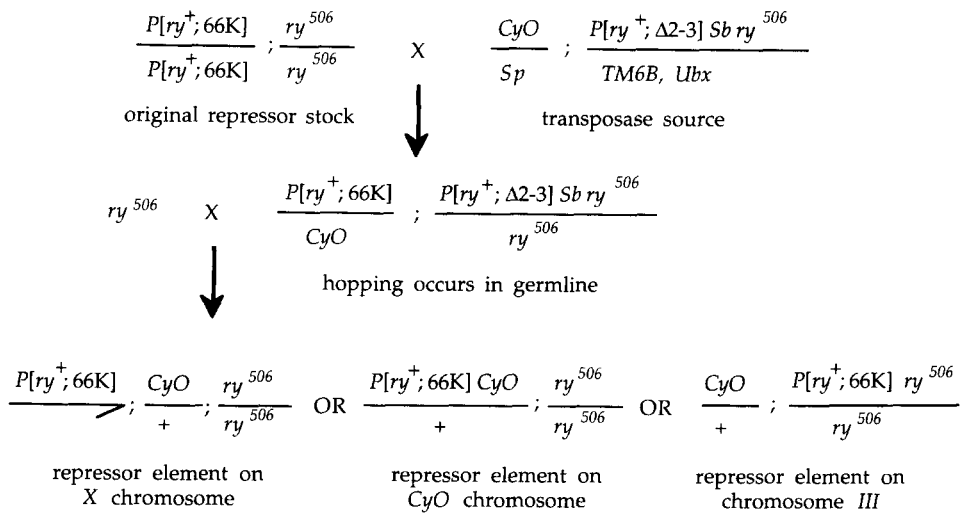


FIGURE 2.—Mobilization of the 66-kD protein-encoding *P* elements. The *P*[*ry*⁺;66K]-C1or *P*[*ry*⁺;hs66K]-C2strains provided the starting elements because each expresses the 66-kD protein, is homozygous viable and contains the insert on the second chromosome (MISRA and RIO 1990); the *P*[*ry*⁺;Δ2-3] (99B) strain contained the stable transposase source. The *P*[*ry*⁺;66K] element contains the *P* element promoter, whereas the *P*[*ry*⁺;hs66K] element uses the *hsp70* promoter to drive expression of the 66-kD protein (MISRA and RIO 1990). Flies heterozygous for repressor and transposase elements were isolated and crossed to the M strain *ry*^{506}. New insertions were identified by the presence of *ry*⁺ and the absence of the original *P*[*ry*⁺;66K]-C1 or *P*[*ry*⁺;hs66K]-C2 chromosome (and therefore the presence of the *CyO* balancer marked with Curly wings) and the absence of *P*[*ry*⁺;Δ2-3] (and therefore the absence of the Stubble bristle dominant marker).

some cases, fertility was also assessed by crossing single heterozygous *sn*^{X2}/*y sn*^{w} daughters to sibling males at 18°, 25° and 29°. Females scored as showing strong P cytotype were completely sterile at all temperatures and produced sn^{c} eggs, which were very short and bullet-shaped at both ends, with very short dorsal appendages. Those scored as partially sterile showed 10–50% fertility of the M strain control on average and produced slightly shorter, bullet-shaped eggs and shorter dorsal appendages. Those scored as fertile showed very little difference in egg morphology from wild type and 50–100% fertility compared with the M strain controls.

Tests for β-galactosidase expression were performed by crossing *P*[*ry*⁺;66K] or *P*[*ry*⁺;hs66K] heterozygous virgin females (with the element on or over a dominantly marked balancer chromosome) to hemizygous *sn P*[*lacZ*] males, collecting the female progeny carrying the repressor element, aging for 2–3 days on yeast paste, dissecting ovaries and using the procedure described in GROSSNIKLAUS *et al.* (1989).

Nuclear extracts and immunoblot analysis: Ovary nuclear extracts were prepared as described (MISRA and RIO 1990), except that after centrifugation of the homogenate, the supernatant was saved as a cytoplasmic extract at the same time as the nuclei in the pellet were collected. Analysis of proteins on denaturing SDS-polyacrylamide gels, silver-staining and immunoblotting were performed as described, using the monoclonal antibody supernatant αR/H3-RD6 (MISRA and RIO 1990).

RNA purification and analysis: Total RNA extractions were performed as described by BARKER *et al.* (1992), except scaled down for ~100 μl embryos in an Eppendorf tube. Single-stranded probes for ribonuclease (RNase) protection experiments were synthesized as described using the pBSKS(+)-N/P582 *P* element probe (MISRA and RIO 1990) or a *tubulin* probe (HEDLEY and MANIATIS 1991). RNase protection analysis was performed essentially as described (MISRA and RIO 1990), except 2–5 μg total RNA was used and incubated with probe overnight to hybridize.

RESULTS

Mobilization of 66-kD protein-encoding elements: Previously, transformant strains carrying single *P*[*ry*⁺;66K] and *P*[*ry*⁺;hs66K] *P* elements encoding the 66-kD protein (referred to as 66-kD elements) did not maternally repress transposase activity, although they did show zygotic repression (MISRA and RIO 1990). In addition, these transformants showed significant position-dependence of expression and repressor activity. To test whether new sites in the genome might promote maternal P cytotype, transposase was used to mobilize the repressor element and produce transformant strains that each contained a single 66-kD element in a new genomic position (Figure 2). A list of the resulting strains is shown in Table 1. These flies lack the repressor element in its original location and carry a new insertion of *P*[*ry*⁺;66K] or *P*[*ry*⁺;hs66K] on the *X*, second (the *CyO* balancer), or third chromosome (Figure 2). Mobilization of the 66-kD elements could have altered these repressor elements: internal deletions and mutations are not uncommon during *P* element transposition (ENGELS 1989). Therefore, DNA blot hybridization of genomic DNA with *P* element and *rosy* gene probes was used to confirm that the *P* element insert in each of the new transformants was intact and in single copy (data not shown). Within the resolution of this analysis, no obvious changes in the structure of the 66-kD elements carried by the transformant strains were observed.

Single 66-kD elements show maternal effect repression of gonadal dysgenic sterility: Assays used to measure P cytotype may measure different aspects of transposase or repressor function or may be sensi-

TABLE 1

Transformant 66-kD element strains and results of sterility assays

Strain	Chr.	Cytol. loc.	No. G0 ♀[a]	GD sterility % F♀ (total)[b]	%Fertile P[66K] progeny	%Fertile sibling progeny	Singed sterility
ry^{506} (M)			10	2.3% (218)	NA[c]	NA	Fertile
Harwich (P)			11	100% (226)	NA	NA	Sterile
ν_6 (Q)[d]			8	94% (125)	NA	NA	ND[e]
P[ry^+;66K]-S	*III*		11	2.3% (43)	4.5% (22)	0% (21)	ND
P[ry^+;66K]-C1	*II*		8	7.1% (99)	4.3% (46)	9.4% (53)	ND
P[ry^+;66K]-3	*II*		9	16% (103)	20% (49)	13% (54)	Fertile
P[ry^+;66K]-5	*II*		10	29% (149)*	34% (61)	26% (88)	Fertile
P[ry^+;66K]-7	*III*		11	4.1% (220)	4.0% (125)	4.2% (95)	Fertile
P[ry^+;66K]-8	*III*		11	4.1% (269)	6.8% (146)	0.8% (123)	Fertile
P[ry^+;66K]-9	*II*	57A 5-6	12	13% (84)	17% (34)	10% (50)	Partially sterile
P[ry^+;66K]-11	*II*		10	34% (110)**	45% (53)	25% (57)	Partially sterile
P[ry^+;66K]-13	*III*		4	0% (12)	0% (7)	0% (5)	Partially sterile
P[ry^+;66K]-36	*III*		4	1.9% (105)	1.7% (59)	2.2% (46)	Fertile
P[ry^+;66K]-37	*II*		12	33% (369)**	36% (176)	29% (193)	Partially sterile
P[ry^+;66K]-38	*II*		12	23% (227)	21% (107)	25% (120)	Sterile
P[ry^+;66K]-44	*II*		12	37% (136)**	35% (66)	39% (70)	Sterile
P[ry^+;66K]-45	*III*		7	5.9% (51)	3.3% (30)	9.5% (21)	Fertile
P[ry^+;66K]-46	*III*		8	5.0% (99)	5.0% (60)	5.1% (39)	Fertile
P[ry^+;66K]-47	*III*		8	6.1% (99)	5.6% (54)	6.7% (45)	Fertile
P[ry^+;66K]-51	*III*		11	4.8% (104)	7.4% (54)	2.0% (50)	Partially sterile
P[ry^+;66K]-67	*II*	59F 1-2	10	34% (183)**	35% (84)	34% (99)	Sterile
P[ry^+;66K]-68	*II*	54C 1-4	10	49% (150)***	53% (70)	45% (80)	Sterile
P[ry^+;66K]-69	*II*	34A 1-2	14	19% (267)	19% (127)	19% (140)	Sterile
P[ry^+;66K]-70	*III*		10	4.7% (190)	7.8% (102)	1.1% (88)	Partially sterile
P[ry^+;66K]-71	*II*		10	34% (232)**	33% (100)	34% (132)	Fertile
P[ry^+;66K]-76	*III*		14	2.5% (81)	4.3% (46)	0% (35)	Partially sterile
P[ry^+;hs66K]-7	*X*		10	28% (312)[f]*	NA	NA	Fertile
P[ry^+;hs66K]-17	*III*		6	1.7% (59)	2.6% (38)	0% (21)	Fertile
P[ry^+;hs66K]-21	*III*		6	0% (89)	0% (44)	0% (45)	Fertile
P[ry^+;hs66K]-24	*III*		12	6.5% (108)	5.4% (56)	7.7% (52)	Fertile
P[ry^+;hs66K]-26	*II*	28E 1-2	9	34% (76)**	39% (23)	32% (53)	Sterile
P[ry^+;hs66K]-34	*II*		6	32% (28)**	33% (3)	32% (25)	Sterile
P[ry^+;hs66K]-48	*X*		11	28% (153)[f]*	NA	NA	Fertile
P[ry^+;hs66K]-69	*III*		10	9.6% (83)	14% (51)	3.1% (32)	Fertile
P[ry^+;hs66K]-70	*X*		9	12% (136)[f]	NA	NA	Fertile[f]
P[ry^+;hs66K]-71	*II*		0	ND	ND	ND	Partially sterile
P[ry^+;hs66K]-72	*X*		7	38% (135)[f]***	NA	NA	Partially sterile
P[ry^+;hs66K]-74	*III*		10	33% (138)[f]**	NA	NA	ND
P[ry^+;hs66K]-74	*III*		10	12% (200)	21% (108)	2.2% (92)	Fertile
P[ry^+;hs66K]-75	*II*	25A 1-2	9	20% (66)	13% (23)	23% (43)	Sterile
P[ry^+;hs66K]-91	*III*		0	ND	ND	ND	Fertile[f]

The chi-square test was used to determine if the percentage of GD sterility was significantly less than that of the ry^{506} M strain control, with results pooled over all female progeny tested in a class. Asterisks indicate conventional levels of significance.

[a] No. of G_0 ♀ = number of G_0 heterozygous females assayed in the GD sterility test.

[b] F ♀ = fertile G_1 females.

[c] NA = not applicable.

[d] Q (quiescent or neutral) strains show strong P cytotype like P strains, but lack significant transposase activity.

[e] ND = not determined.

[f] G_0 females tested as homozygotes.

tive to different levels of transposase activity. In addition, the time and tissue in which repression is assayed, as well as spatial and temporal differences in repressor and transposase expression, probably influence the level of repression observed. For example, a repressor element may work better in somatic assays (ROBERTSON and ENGELS 1989) or germline assays (RONSSERAY, LEHMANN and ANXOLABÉHÈRE 1991) due to differences in the expression pattern of repressor. Since hybrid dysgenesis occurs only in the germline, we used genetic tests to assay repressor activity in this tissue.

In general, assays for P cytotype measure (1) the negative regulation of transposase activity, or (2) transcriptional repression by *P* element repressor proteins, independent of transposase. Two assays of the first class, gonadal dysgenic (GD) sterility and *singed-weak* (sn^w) excision, measure the ability of P cytotype to prevent dysgenesis. Assays of the second class, such as cytotype-dependent singed sterility or negative con-

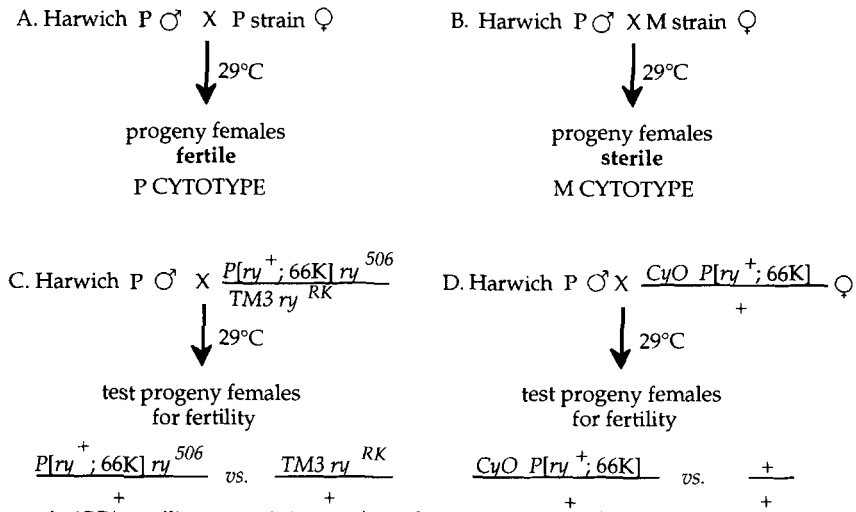


FIGURE 3.—Gonadal dysgenic (GD) sterility assay. (A) P strain males mated to P strain females produce normal, fertile progeny at 29°, due to the P cytotype regulatory state of the females. (B) P strain males mated to M strain females lacking *P* elements produce sterile female progeny when the progeny develop at 29°, due to the M cytotype regulatory state of the females. The gonads of the progeny females are agamous, due to germline cell death. (C) Transformant females containing a single *P*[*ry*$^+$;66K]or *P*[*ry*$^+$;hs66K] element on the third chromosome were crossed to P strain males, and the progeny raised at 29°. The female progeny with and without the element were examined for GD sterility. (D) Transformant females containing a single *P*[*ry*$^+$;66K] or *P*[*ry*$^+$;hs66K] element on the second chromosome *CyO* were tested as in (C).

trol of *P* promoter expression, may have an indirect relationship to P cytotype (see below).

GD sterility is thought to be due to chromosomal breaks at the sites of *P* elements in the developing germlines of hybrid dysgenic progeny, which causes cell lethality (ENGELS and PRESTON 1979; KIDWELL and NOVY 1979). Females that show true P cytotype should produce progeny that are fertile in this assay, due to repression of *P* element movement in their progeny's germlines (Figure 3). Thirteen of 35 single copy 66-kD elements showed significant repression of GD sterility ($P < 0.05$ by the chi-square test; Table 1 and Figure 4), and two strains showed strong repression ($P < 0.001$ by the chi-square test). The single-element strain *P*[*ry*$^+$;66K]-68 showed half as much repression as the P strain (Harwich) and Q strain (ν_6) controls that contain 30–50 *P* elements. Strains tested as homozygotes generally showed higher levels of repression in this assay in general, suggesting a dosage effect (*e.g.*, *P*[*ry*$^+$;hs66K]-74, Table 1).

As shown in Figure 4, 8 of the 10 strains exhibiting higher levels of repression ($P < 0.01$ by the chi-square test) carried repressor elements on the second chromosome (*e.g.*, *P*[*ry*$^+$;66K]-37, 44, 67 and 68), although not all insertions on this chromosome showed this effect (*e.g.*, *P*[*ry*$^+$;66K]-9). To determine whether the insertions on the second chromosome were in a single hotspot for *P* element insertion that fortuitously allowed strong maternal repression, larval salivary gland polytene chromosomes from six of these transformant strains were hybridized with *P* element DNA sequences to localize the insertion sites of the elements. The results in Table 1 indicate that each of the tested elements is in a different position, not in a single hotspot. An alternative explanation for the differences between strains with a 66-kD element on the second *vs.* third chromosomes could be modifiers on the chromosomes that affect the level of repression observed.

As shown in Figure 3C and 3D, heterozygous transformant females mated to Harwich P strain males produce progeny that carry the 66-kD protein-encoding element, as well as progeny lacking the repressor element. However, the level of GD sterility for the transformant strains was equivalent in sibling flies possessing or lacking the repressor element in their genome, indicating that repression was due to a true maternal effect (Table 1). This repression must be due to a maternal component, since progeny of the M strain control (ry^{506}) or the original transformant strain (*P*[*ry*$^+$;66K]-C1) showed no repression from the paternally inherited *P* elements. Zygotic expression of repressor from paternally inherited elements may occur, but if so requires an initial maternal contribution encoded by the *P*[*ry*$^+$;66K] or *P*[*ry*$^+$;hs66K] repressor element; similar results were reported with much smaller single *P* elements by RASMUSSON, RAYMOND and SIMMONS (1993).

A single 66-kD element can elicit maternal inheritance of repression of germline excision: The sn^w allele of the *singed* gene provides a sensitive measure of *P* element excision that can be used in reciprocal crosses to determine whether repression is maternally inherited. Transposase-catalyzed excision of the small *P* elements in sn^w changes the bristle phenotype from slightly bent (sn^w) to very short singed-extreme (sn^e) or to wild type (sn^+) (ENGELS 1979b, 1984; ROIHA, RUBIN and O'HARE 1988). Flies homozygous for sn^w

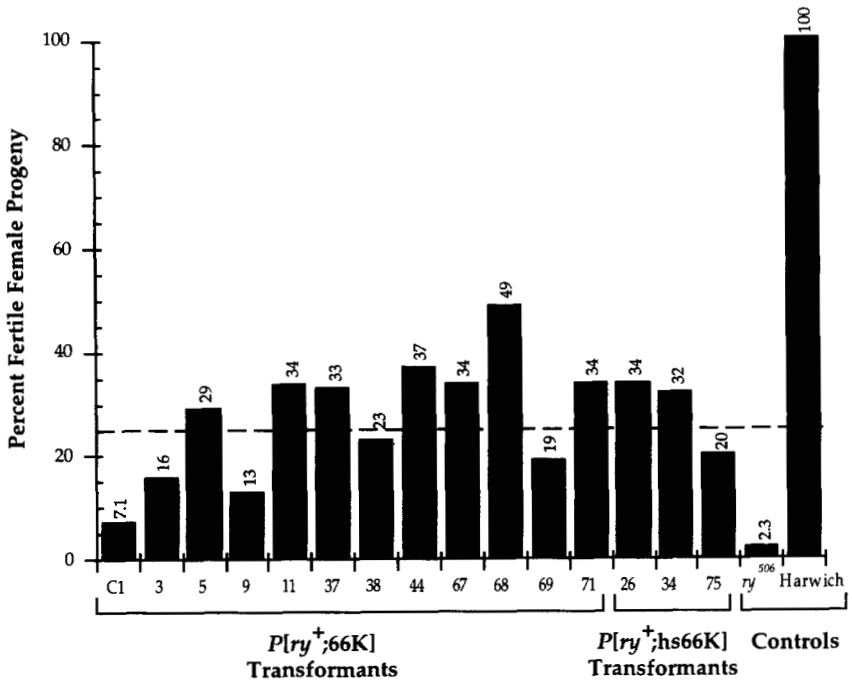


FIGURE 4.—GD sterility assay results with repressor elements on the second chromosome. The GD sterility assay described in Figure 2 was used to test the *P*[*ry*⁺;66K] and *P*[*ry*⁺;hs66K] transformant strains, and the results are shown in Table 1 and diagramed here as the percent fertile female progeny (value indicated above bars). The M strain (ry^{506}) and P strain (Harwich) controls are shown on the right, and the original transformant strain *P*[*ry*⁺;66K]-C1is shown on the left. Values above the dashed line are significantly more than that of the M strain control; see Table 1 for details.

and carrying a single *P*[*ry*⁺;66K] or *P*[*ry*⁺;hs66K] element on the *CyO* balancer chromosome (see legend of Figure 5) were mated in reciprocal crosses to flies homozygous for the stable source of transposase *P*[*ry*⁺;Δ2-3](99B), and germline excision events at the sn^w locus in the G_1 males were detected in the G_2 female progeny as sn^e bristles (Figure 5). If repression encoded by the 66-kD elements is maternal (like true P cytotype), the germline excision rate will be lower if the repressor element is inherited from the G_0 female (cross A, Figure 5) than from the G_0 male (cross B, Figure 5).

The results of such reciprocal crosses are shown in Table 2. Eight transformant strains from the mobilization experiment, as well as two transformant strains tested previously (*P*[*ry*⁺;66K]-S and *P*[*ry*⁺;hs66K]-A1; MISRA and RIO 1990) were tested for sn^w mutability in reciprocal crosses (Table 2). Most of the 66-kD elements exhibited no detectable repression of transposase activity in this assay, even in transformant strains that showed strong repression in the GD sterility assay (*e.g.*, *P*[*ry*⁺;66K]-37 or 44). This suggests that the GD sterility assay may be more sensitive to repression than the germline sn^w excision assay, or measures repressor function at a different time in development (see DISCUSSION). However, the *P*[*ry*⁺;66K]-68 strain showed significant repression of germline excision when the repressor element was maternally inherited, but not when the element was paternally inherited ($P < 0.01$ by rank sum test; Table 2 and Figure 6). As noted previously, the strongest repression of GD sterility was observed in this strain (Table 1). Thus, a single element encoding the 66-kD protein is capable of eliciting maternal P cytotype characteristic of a P strain containing 50 elements, albeit at a lower level (Figure 6). These results also

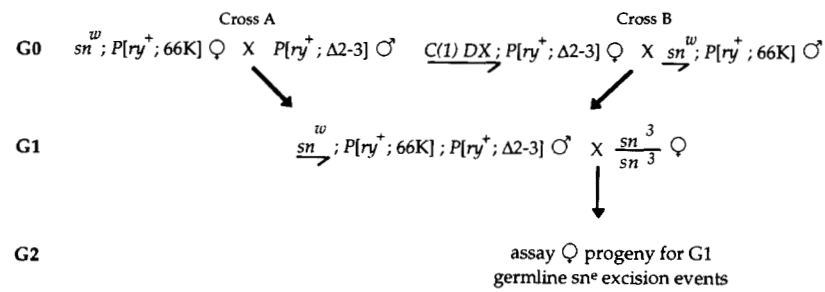


FIGURE 5.—Reciprocal crosses to assay germline excision by 66-kD elements. (Cross A) Females homozygous for sn^w and carrying a single *P*[*ry*⁺;66K]or *P*[*ry*⁺;hs66K]element on the *CyO* chromosome were mated to males homozygous for the *P*[*ry*⁺;Δ2-3] element, a source of transposase. Exceptions were the strain *P*[*ry*⁺;hs66K]-A1, which was homozygous for the element, and the *P*[*ry*⁺;66K]-S strain, which carried the element over a third chromosome balancer (see Table 2). Male G_1 germlines were assayed for sn^w destabilization by crosses to females homozygous for sn^3, a recessive extreme allele of *singed*, and G_2 female progeny with only sn^e bristles were scored. (Cross B) To test whether repression is decreased when the 66-kD element is inherited paternally, males hemizygous for sn^w and carrying a *P*[*ry*⁺;66K] or *P*[*ry*⁺;hs66K] element were crossed to attached *X* chromosome females carrying *P*[*ry*⁺;Δ2-3]. The males from such a cross inherit the *X* chromosome from their father.

TABLE 2

Reciprocal crosses testing maternal inheritance of germline excision repression

Strain	Transformant G_0 parent	Mean excision frequency ± SE[a] (Total G_2 ♀ progeny)	No. of G_1 ♂'s
ry^{506} M strain	*y sn*^{w}; *ry*^{506} ♂	21.3% ± 0.51% (496)	10
	y sn^w; *ry*^{506} ♀	29.1% ± 1.1% (415)	10
P strain	*sn*^{w}; *P* ♂	12.9% ± 0.29% (568)	10
	sn^w; *P* ♀	0% (430)	10
P[*ry*$^+$;hs66K]-A1	♂	17.7% ± 0.54% (406)	9
	♀	22.1% ± 0.60% (461)	10
P[*ry*$^+$;66K]-S/*TM3*	♂	17.5% ± 0.17% (602)	13
	♀	26.8% ± 0.05% (420)	12
P[*ry*$^+$;66K]-9 *CyO*/+	♂	31.0% ± 0.04% (586)	9
	♀	23.9% ± 0.89% (401)	10
P[*ry*$^+$;66K]-37 *CyO*/+	♂	22.0% ± 0.58% (472)	10
	♀	26.3% ± 0.12% (852)	10
P[*ry*$^+$;66K]-38 *CyO*/+	♂	23.0% ± 0.05% (680)	10
	♀	23.8% ± 0.16% (784)	10
P[*ry*$^+$;66K]-44 *CyO*/+	♂	30.5% ± 0.10% (738)	10
	♀	24.2% ± 0.08% (686)	10
***P*[*ry*$^+$;66K]-68 *CyO*/+**	♂	**19.6% ± 0.04%** (797)	10
	♀	**11.6% ± 0.02%** (924)	10
P[*ry*$^+$;66K]-69 *CyO*/+	♂	35.5% ± 0.09% (620)	9
	♀	29.8% ± 0.19% (764)	10
P[*ry*$^+$;hs66K]-34 *CyO*/+	♂	27.4% ± 0.02% (805)	10
	♀	18.6% ± 0.06% (849)	10
P[*ry*$^+$;hs66K]-75 *CyO*/+	♂	35.4% ± 0.19% (682)	10
	♀	42.5% ± 0.22% (247)	4

[a] Mean excision frequencies and standard errors were calculated as unweighted values as in ENGELS (1979c), counting sn^e G_2 progeny as mutation events.

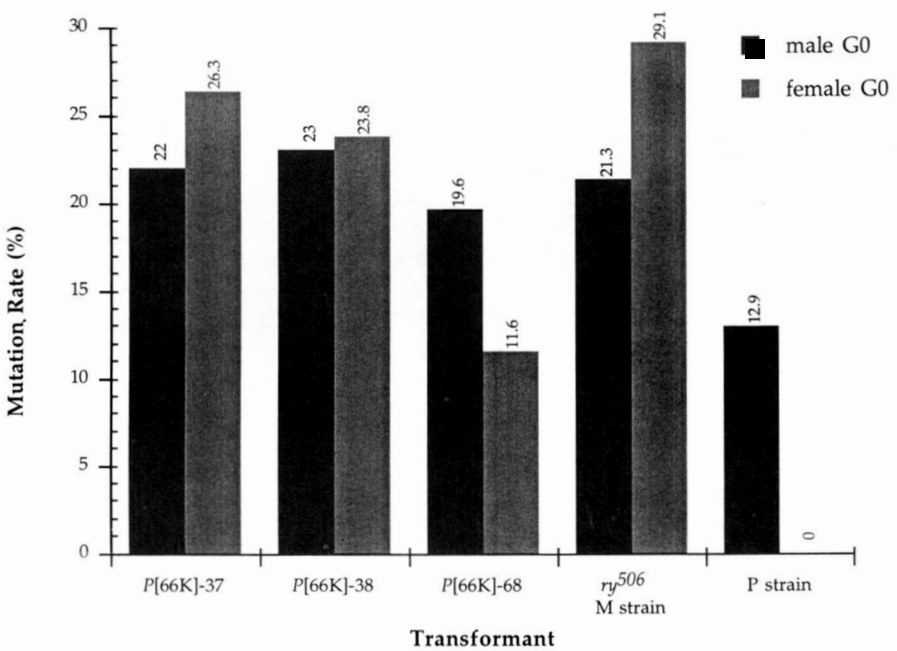


FIGURE 6.—Reciprocal crosses indicate the *P*[*ry*$^+$;66K]-68 transformant strain maternally represses germline excision. Results are shown as mutation rate, calculated from the frequency of sn^e progeny according to the method of ENGELS (1979c); values are indicated above the bars, and standard errors are shown in Table 2. The P strain and M strain (ry^{506}) controls are shown on the right. Dark bars indicate results when the 66-kD element is inherited from the male G_0; stippled bars indicate inheritance from the female G_0 (Figure 5). Results are shown for three *P*[*ry*$^+$;66K] elements on the second chromosome.

indicate that the genomic position of a repressor element determines its ability to show true P cytotype, since the strains tested contained identical repressor elements.

Cytotype-dependent assays measuring transcriptional repression: Two assays measuring P cytotype-dependent effects on transcription were also used to analyze the 66-kD element transformant strains. Wild-type *singed* product is required for female fertility and is provided by the *sn*^{w} allele in flies heterozygous for *sn*^{w} and a null allele of *singed* (*sn*^{X2}). However, in P cytotype these *sn*^{w}/*sn*^{X2} flies are sterile, an effect called "singed sterility" (ROBERTSON and ENGELS 1989). The mechanism of singed sterility is unknown, but a simple

interpretation is that expression from the sn^w allele can be shut off directly or indirectly by the action of *P* element repressor proteins on *P* elements inserted in the gene. Females heterozygous for sn^w, sn^{X2}, and *P*[*ry*$^+$;66K] or *P*[*ry*$^+$;hs66K] were tested for singed sterility, and several showed complete sterility, equivalent to that of the P strain control (Table 1). Repression potential of the transformant strains in the singed sterility assay showed a partial correlation with that in the GD sterility assay (Table 1), in that most strains showing strong repression of GD sterility showed some effect in the singed sterility assay. Exceptions to the correlation might be due to spatial or temporal differences between the assays (see DISCUSSION).

On the other hand, none of the 66-kD element strains showed P cytotype in an assay measuring the cytotype-dependence of expression from the *P* promoter (LEMAITRE and COEN 1991) using a *P* element-*lacZ* fusion construct (*P*[*lacZ*]) inserted in the *singed* gene (data not shown). This assay takes advantage of the observation that expression from all *P*[*lacZ*] elements is repressed by P cytotype (LEMAITRE and COEN 1991). Almost all strains showed strong repression of β-galactosidase expression in somatic follicle cells, but no repression in germline cells of the ovary, unlike P or Q strain controls, which completely repressed transcription everywhere (data not shown and K. O'HARE, unpublished data). This suggests that the assay for β-galactosidase activity may be less sensitive than the sterility assays to low levels of germline 66-kD repressor. It is also possible that P cytotype repression of transcription of the sn^w allele in singed sterility and the *P* element promoter in *P*[*lacZ*] elements may be mechanistically distinct, since it is not known how either phenomenon occurs.

Maternal regulator-containing elements do not generate P cytotype: Certain positions in the genome might influence maternal repression by placing repressor elements adjacent to maternal enhancer sequences, which could subsequently stimulate expression of repressor during oogenesis. Therefore, we constructed 66-kD repressor-encoding elements that contained the *hsp26* gene's regulator sequence (referred to as maternal regulator elements), hypothesizing that such elements might show strong maternal P cytotype in any position in the genome, since they include sequences sufficient for high level expression during oogenesis (FRANK, CHEUNG and COHEN 1992). These modified *P* element derivatives are diagramed in Figure 1.

The first construct, *P*[*ry*$^+$; *hsp26 bcd* 66K *CycB*] (Figure 1A), utilized genomic sequences from the *bicoid* (*bcd*) gene (DRIEVER, SIEGEL and NÜSSLEIN-VOLHARD 1990), a maternal effect gene that is expressed during oogenesis (DRIEVER and NÜSSLEIN-VOLHARD 1988), as well as the *hsp26* regulator element (FRANK, CHEUNG and COHEN 1992) to stimulate expression during oogenesis from the promoter. These sequences have been shown to be sufficient for expression of *bcd* mRNA during oogenesis and rescue of a strong *bcd* mutation (DRIEVER, SIEGEL and NÜSSLEIN-VOLHARD 1990) and therefore were expected to include sequences to activate expression in the ovary. The 3′ untranslated region of the *Cyclin B* (*CycB*) gene was included to localize repressor transcripts to the germline precursor (pole) cells during embryogenesis (DALBY and GLOVER 1992; WHITFIELD *et al.* 1989; RAFF, WHITFIELD and GLOVER 1990).

Two other elements also contained the *hsp26* regulator element, but used the basal *sgs3* gene promoter to express the 66-kD protein (Figure 1, B and C). *P*[*ry*$^+$; *hsp26* 66K *CycB*] contained the 3′ untranslated region of *CycB* to localize 66-kD protein-encoding transcripts to the presumptive germline of the embryo (Figure 1B). *P*[*ry*$^+$; *hsp26* 66K *K10*] contained the 3′ untranslated region of the *fs(1)K10* gene (WIESCHAUS, MARSH and GEHRING 1978; WIESCHAUS 1979), which is sufficient to specifically translocate transcripts from the nurse cells to the oocyte during oogenesis (CHEUNG, SERANO and COHEN 1992). Several independent insertions of each construct were obtained by germline transformation and verified by DNA hybridization blot (data not shown).

The maternal regulator transformant strains were tested for their ability to repress transposase activity in the germline using the genetic tests described above. Surprisingly, unlike the *P*[*ry*$^+$;66K] elements tested above, none of the transformants showed significant repression of GD sterility (Figure 7). In addition, none of the transformants showed any repression of sn^w excision when the element was inherited maternally or paternally (Table 3), or repression of germline β-galactosidase expression from a *P*[*lacZ*] element (data not shown). Similar results were obtained with elements containing the *hsp83* enhancer element, which constitutively drives expression during oogenesis and embryogenesis (S. ROCHE and D. RIO, unpublished data). Immunoblotting of ovary nuclear extracts was used to determine whether the *hsp26* maternal regulator elements expressed high levels of the 66-kD protein during oogenesis (see below).

Levels of 66-kD repressor protein and mRNA encoding 66-kD protein do not correlate with P cytotype: We had previously observed that a true P strain that exhibited maternal P cytotype expressed more 66-kD protein in ovaries and unfertilized oocytes than a strain that showed no maternal but only zygotic repression of transposase activity in the germline (MISRA and RIO 1990). Thus, we wished to test whether transformant strains that elicited strong maternal P cytotype in the genetic assays would show

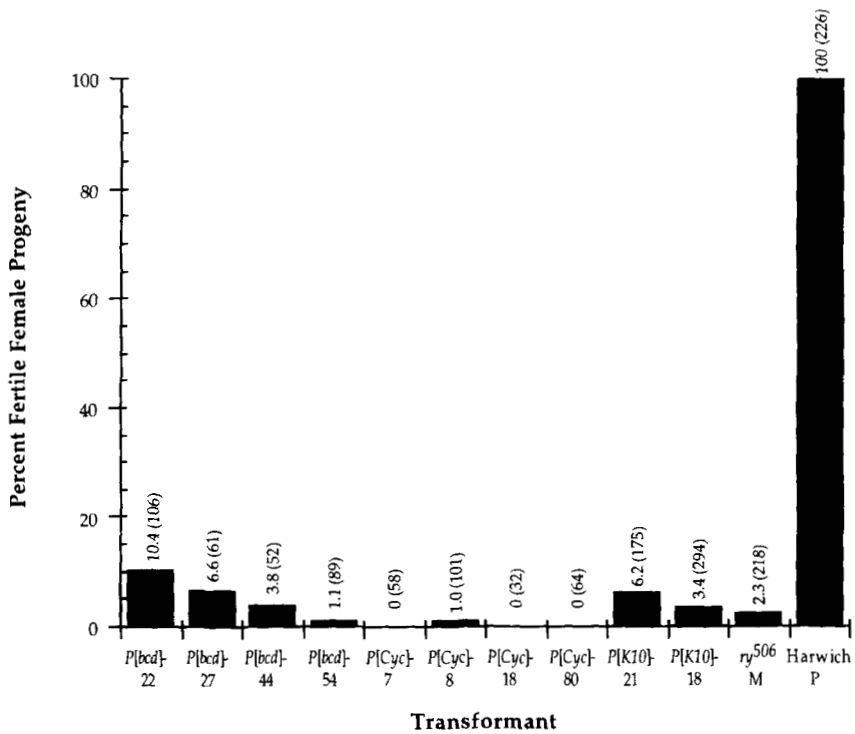


FIGURE 7.—Maternal regulator elements do not repress GD sterility. The assay described in Figure 2 was used to test the *P*[*ry*$^+$;*hsp26bcd*66K *CycB*], indicated "*P*[*bcd*]," *P*[*ry*$^+$;*hsp2666K CycB*], indicated "*P*[*Cyc*]," and *P*[*ry*$^+$;*hsp2666K K10*], indicated "*P*[*K10*]" transformant strains. The results of the assay are diagramed here as the percent fertile female progeny; values are indicated above the bars, and the number of total female progeny tested for each strain is shown in parentheses. The M and P strain controls are shown on the right. For simplicity, results are pooled over all female progeny tested from each transformant strain.

TABLE 3

Maternal regulator elements do not repress germline excision

Strain	Transformant G_0 parent	Mean excision frequency ± SE[a] (Total G_2 ♀ progeny)	No. of G_1 ♂'s
ry^{506} M strain	*y sn*^{w}; *ry*^{506} ♂	21.3% ± 0.51% (496)	10
	y sn^w; *ry*^{506} ♀	29.1% ± 1.1% (415)	10
P strain	*sn*^{w}; *P* ♂	12.9% ± 0.29% (568)	10
	sn^w; *P* ♀	0% (430)	10
P[*ry*$^+$;*hsp26 bcd 66K CycB*]22-1	♂	29.2% ± 0.07% (641)	10
	♀	21.7% ± 0.09% (666)	10
P[*ry*$^+$; *hsp26 bcd 66K CycB*]27-1	♂	26.6% ± 0.06% (692)	10
	♀	27.7% ± 0.08% (590)	10
P[*ry*$^+$; *hsp26 bcd 66K CycB*]44-1/*CyO*	♂	19.7% ± 0.05% (397)	10
	♀	24.1% ± 0.08% (514)	10
P[*ry*$^+$;*hsp26 bcd 66K CycB*]54-1/*TM3*	♂	31.0% ± 0.13% (621)	10
	♀	24.0% ± 0.64% (666)	10
P[*ry*$^+$;hsp26 66K *CycB*]8-1	♂	25.0% ± 0.14% (439)	7
	♀	20.6% ± 0.04% (699)	10
P[*ry*$^+$;*hsp26 66K CycB*]7-2	♂	25.9% ± 0.20% (636)	10
	♀	18.3% ± 0.05% (562)	10
P[*ry*$^+$;*hsp26 66K CycB*]18-3	♂	28.5% ± 0.19% (698)	10
	♀	30.3% ± 0.07% (713)	10
P[*ry*$^+$;*hsp26 66K CycB*]80-3	♂	30.4% ± 0.18% (523)	10
	♀	31.5% ± 0.18% (424)	10
P[*ry*$^+$;*hsp26 66K K10*]21-1	♂	33.6% ± 0.10% (492)	10
	♀	31.9% ± 0.46% (352)	7
P[*ry*$^+$;*hsp26 66K K10*]18-3	♂	25.1% ± 0.04% (649)	10
	♀	24.0% ± 0.59% (301)	6

[a] Mean excision frequencies and standard errors were calculated as unweighted values as in Engels (1979c), counting sn^c G_2 progeny as mutation events.

high 66-kD protein expression during oogenesis, whereas those that failed to repress would show low 66-kD protein expression.

Proteins in nuclear extracts from transformant females' ovaries were immunoblotted with a monoclonal antibody that recognizes an epitope near the carboxyl terminus of the 66-kD protein (αR/H3-RD6; MISRA and RIO 1990). The results, shown in Figure 8, sug-

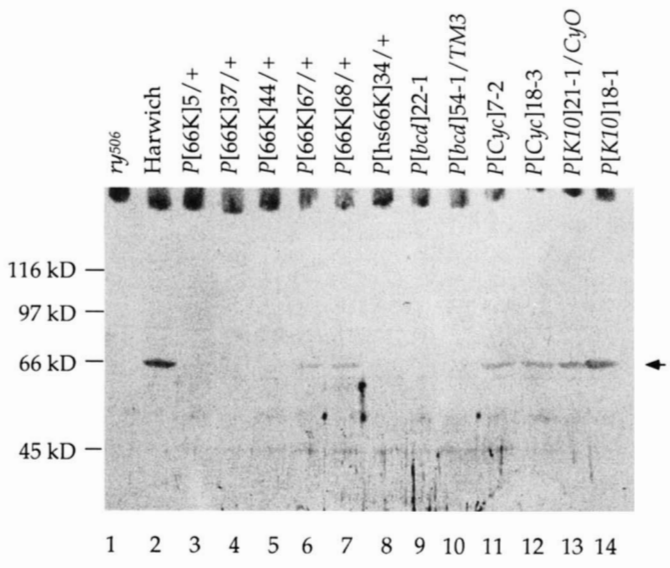


FIGURE 8.—Immunoblot analysis of transformant strain ovary extracts. Equal amounts of protein from nuclear extracts made from ovaries were immunoblotted with a monoclonal antibody recognizing an epitope in the carboxyl terminus of the 66-kD protein. Extracts were from the strains indicated above the gel. *P*[*ry*$^+$;66K]*CyO*/+ transformants are denoted "*P*[66K]," the *P*[*ry*$^+$;hs66K]*CyO*/+ transformant is denoted "*P*[hs66K]," *P*[*ry*$^+$;*hsp26bcd*66K *CycB*] is denoted "*P*[*bcd*]," *P*[*ry*$^+$;*hsp26*66K *CycB*] is denoted "*P*[*Cyc*]" and *P*[*ry*$^+$;*hsp26*66K *K10*] is denoted "*P*[*K10*]." Strains were homozygous unless the genotype is otherwise noted. Marker sizes in kilodaltons are indicated at left, and the arrow at right indicates 66-kD protein.

gest that not all *P*[*ry*$^+$;66K] and *P*[*ry*$^+$;hs66K] strains that showed repression in the GD sterility assay expressed detectable levels of the 66-kD protein in the ovary (lanes 1–8). However, the *P*[*ry*$^+$;66K]-67 and *P*[*ry*$^+$;66K]-68 females produced a detectable level of the 66-kD protein (Figure 8, lanes 6 and 7), but several-fold lower than the P strain Harwich (lane 2) or the Q strain ν_6 (data not shown).

As predicted from the inclusion of the *hsp26* regulator, the *P*[*ry*$^+$; *hsp26* 66K *CycB*] and *P*[*ry*$^+$; *hsp26* 66K *K10*] transformant strain females produced a high level of 66-kD protein in the ovary nuclei (Figure 8, lanes 11–14) and in the cytoplasm (data not shown), and homozygous *P*[*ry*$^+$; *hsp26* 66K *K10*] females produced as much 66-kD protein as the P strain. Strikingly, although each was located in a different position in the genome, each strain produced a similar level of 66-kD protein during oogenesis, as predicted if 66-kD protein expression were controlled by the *hsp26* regulator within the element, rather than by flanking genomic enhancers (Figure 8 and data not shown). However, the *P*[*ry*$^+$; *hsp26 bcd* 66K *CycB*] transformant strains produced a very low level of 66-kD protein in the ovary (Figure 8, lanes 9 and 10).

We postulated that the level of maternally provided mRNA transcripts encoding the protein might more closely match the level of repression assayed in the zygote. Therefore, the level of mRNA transcripts encoding the 66-kD protein in 0–4 hr embryos was analyzed using an RNase protection assay. We reasoned that at this early time, before most zygotic transcription has begun, most of the mRNA would be maternally donated. The results, shown in Figure 9, suggest that the amount of maternally donated mRNA encoding the 66-kD protein does not correlate with the level of repression observed in the genetic assays of P cytotype.

These results are inconsistent with a model for maternal P cytotype in which expression of repressor at high levels during oogenesis is the sole determinant of repression potential. Repression was observed in the GD sterility assay in 66-kD element-containing strains that showed no detectable 66-kD protein expression during oogenesis. In contrast, the maternal regulator elements that expressed the 66-kD protein and mRNA encoding it at high levels during oogenesis, *P*[*ry*$^+$; *hsp26* 66K *CycB*] and *P*[*ry*$^+$; *hsp26* 66K *K10*], did not show significant repression in the genetic assays.

DISCUSSION

The maternal component of P cytotype is determined by genomic position: One of the fundamental questions underlying P cytotype regulation of transposition is why repression can be maternally inherited in some cases, but can depend solely on the presence of *P* elements in the zygote in other cases. It had been speculated that the position of elements in the genome might influence this phenomenon, given the significant effects of chromosomal location on *P* element expression (MISRA and RIO 1990; SIMMONS *et al.* 1990; ROBERTSON and ENGELS 1989; O'KANE and GEHRING 1987). This is the first study demonstrating that genomic position of an element encoding the 66-kD protein determines whether a repressor element is capable of maternally repressing transposase activity. In addition, a single element encoding this protein has been shown to exhibit significant maternal repression.

The range of repression potential observed in the transformant strains with 66-kD elements in different locations was similar to the range observed in pseudo M (M′) strains using the GD sterility assay (HEATH and SIMMONS 1991; RAYMOND *et al.* 1991; SIMMONS *et al.* 1990; RASMUSSON *et al.* 1990). M′ strains contain *P* elements, but usually show M cytotype in dysgenic crosses. However, almost all M′ strains can show some repression of GD sterility or *sn*^{w} hypermutability, and some M′ strains even show low levels of transposase activity (KIDWELL 1985; BLACK *et al.* 1987; JACKSON, BLACK and DOVER 1988; HAGIWARA *et al.* 1987; SIMMONS *et al.* 1990; HEATH and SIMMONS 1991). Experiments conducted with strains carrying many *P* elements are complex because the number, structure and position of the *P* elements vary. To simplify this system, we have conducted experiments with single elements of defined structure. We find that a single

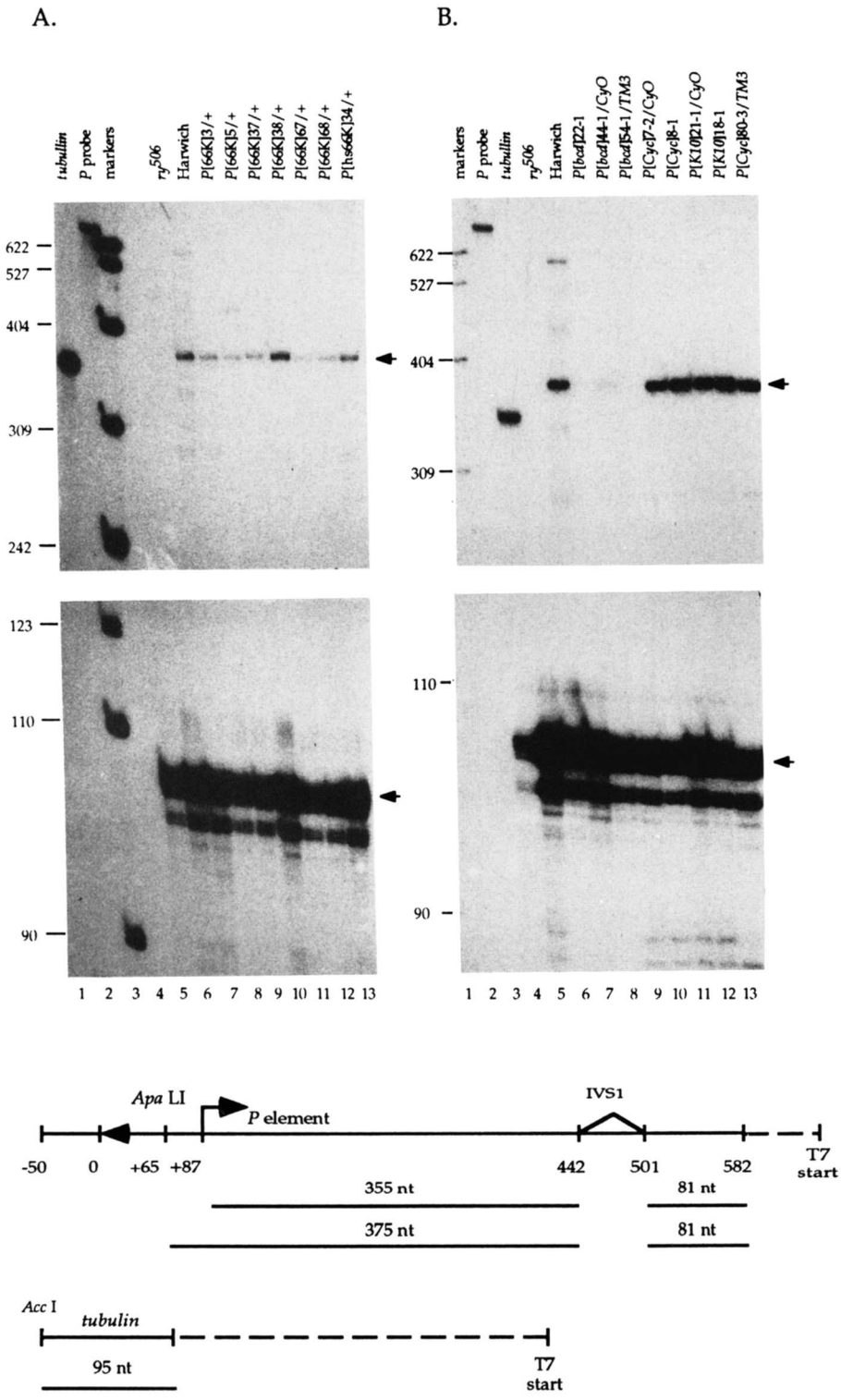


FIGURE 9.—Ribonuclease protection analysis of 66-kD transformant mRNA transcripts from early embryos. Total RNA was annealed to the ^{32}P-labeled *P* element and *tubulin* probes shown below. After digestion with ribonuclease, protected fragments were resolved on 8% denaturing polyacrylamide gels. ^{32}P-labeled *Msp*I pBR322 DNA marker sizes are indicated in nucleotides at the left of each gel, and arrows at right indicate expected sized fragments. Top and bottom panels are taken from the same exposure of the same gel. (A) An RNA probe complementary to the first 582 nt of the *P* element and a *tubulin* probe as a control for total RNA were annealed to transcripts from 0–4 hr 66-kD element transformant embryos, to examine maternally donated RNA. Samples are indicated above the gel and include *tubulin* probe (lane 1), *P* element probe (lane 2), markers (lane 3), M strain control (ry^{506}), P strain control (Harwich), "*P*[66K]" for *P*[ry^+;hs66K]*CyO*/+ transformants and "*P*[hs66K]" for the *P*[ry^+;hs66K]*CyO*/+ transformant. The expected 355-nt and 95-nt protected fragments are observed in all transformants, but the levels do not correlate with the genetic results. The P strain Harwich shows many protected fragments, since many different *P* elements are present. (B) Experiment as in (A), but with maternal regulator element transformant embryos: *P*[ry^+;*hsp26bcd*66K *CycB*] is denoted "*P*[*bcd*]," *P*[ry^+;*hsp26*66K *CycB*] is denoted "*P*[*Cyc*]," and *P*[ry^+;*hsp26*66K *K10*] is denoted "*P*[*K10*]." Strains were homozygous unless otherwise specified. Note that although the expected 95-nt *tubulin* fragment is observed, rather than the expected 375-nt *P* element fragment, the 355-nt fragment from the original *P* element promoter downstream of the *bcd* or *sgs3* promoters is protected, and the levels correlate with the level of protein in the ovary observed in Figure 8. We believe that for some reason the natural *P* element promoter is used preferentially and is stimulated by the *hsp26* regulator sequence.

element in at least one genomic position can show maternal repression in more than one assay of P cytotype. Less repression was exhibited than a strain with two naturally occurring elements selected for repression, Lk-P(1A) (RONSSERAY, LEHMANN and ANXOLABÉHÈRE 1991), or P or Q strains that contain 30–50 elements, indicating that a combination of copy number and genomic position can affect the magnitude of maternal repression by *P* elements. A recent study of single, naturally occurring *P* elements by RASMUSSON, RAYMOND and SIMMONS (1993) suggested that elements encoding very short P element polypeptides show similar or even stronger repression of GD sterility (using a different transposase source than Harwich), but no maternal inheritance of repression of sn^w excision. The single defined element (*P*[ry^+;66K]-68) that we have found to maternally repress in both germline assays provides us with a

simple model for studying maternal repression in more detail.

True maternal effect P cytotype is exhibited by single 66-kD elements: Because most studies examining the maternal component of P cytotype have used strains with many *P* elements, it has been difficult to determine exactly which element(s) is responsible for the effects observed (ENGELS 1989). Since all sibling progeny from such crosses contain *P* elements, it is not possible to look for true maternal effects, and therefore reciprocal crosses are used to measure differences in what is termed "maternal inheritance," since backcrossing can show differences that are inherited through the female line several generations later. Reciprocal crosses compare differences between "cousins" that should be "chromosomally identical" (ENGELS 1979a; KIDWELL 1981). However, cousins are unlikely to be chromosomally identical in terms of their *P* element complement, since movement of *P* elements in the dysgenic cross may change the number and position of the *P* elements.

The use of single, genetically marked elements in this study allowed the comparison of progeny that carried the repressor element zygotically to sibling progeny lacking the element. Strikingly, all of the transformant females showed equal repression of GD sterility in both sets of their progeny, indicating that repression of P cytotype was due to a maternal effect. Similar results were reported in a study of repression of GD sterility by much shorter repressor elements, although some evidence for combined zygotic and maternal repression was also observed (RASMUSSON, RAYMOND and SIMMONS 1993).

P cytotype genetic tests may measure different aspects of repressor function: As discussed earlier, the results of P cytotype assays depend on the developmental time and tissue examined, the expression pattern of repressor and whether transposase activity or transcription is being repressed. We have chosen to focus on germline assays of cytotype, especially the GD sterility and sn^w excision assays, since these tests most closely monitor activities relevant to regulation of hybrid dysgenesis.

Single element transformant strains showed different levels of repression in different assays. Specifically, several strains showed maternal repression of GD sterility, but only one strain tested showed maternally inherited repression of sn^w hypermutability. This could be due to differential sensitivity of the assays to repressor levels, for example, because different transposase sources were used. Another possible difference is the developmental time period assayed in each test. The temperature sensitive period of GD sterility is from mid-embryogenesis through the second larval instar of the G_1 progeny (ENGELS and PRESTON 1979; KIDWELL and NOVY 1979), whereas repression of sn^w excision events might be required in the adult G_1 germline.

The repressor elements also showed different effects in the assays measuring transcriptional effects in the ovary, cytotype-dependent singed sterility and β-galactosidase expression. Whereas results in the singed sterility assay showed some correlation with results in the GD sterility assay, no strains showed P cytotype repression of transcription of a *P*[*lacZ*] reporter construct. It has been demonstrated that transcriptional control of *P* element expression does not account for the cytotype repression of transposase activity (STELLER and PIROTTA 1986). Therefore, although experiments with P strains suggest that direct or indirect association of P repressor proteins with *P* element sequences can affect transcription (LEMAITRE and COEN 1991), this repressor function may be distinct from P cytotype repression of hybrid dysgenesis. Our 66-kD element strain that maternally represses GD sterility and sn^w hypermutability (*P*[ry^+;66K]-68) mimics P cytotype functions that directly relate to *P* element regulation.

Models for maternal repression of transposition: The 66-kD element experiments indicate that genomic position determines maternal *vs.* zygotic P cytotype. One plausible explanation would be that position effects are mediated by proximity to enhancers that allow expression of repressor protein during oogenesis. However, immunoblotting and maternal regulator element experiments are inconsistent with a model in which maternal repression is due to repressor expression in the ovary.

The lack of correlation between repression and expression may have been due to our assays. Slight differences in location and timing of 66-kD protein expression may not have been detected by our immunoblots of ovary nuclear extracts, but could have been detected genetically. We were concerned that ovary extracts contain proteins from the nuclei of somatic follicle cells as well as germline nurse cells and oocytes, whereas our assays might measure only germline repressor function. However, immunoblotting of these extracts with antibodies against other proteins, for example, the germline *vasa* protein, indicates that the polyploid nurse cell nuclei contribute at least as much or more than the small follicle cell nuclei to the extracts (C. SIEBEL and D. RIO, unpublished data). Thus, the extracts appear to be useful in assaying germline oogenesis expression. But immunoblotting of whole ovaries does not assay the timing of expression during oogenesis, which might be crucial for maternal repression.

Strains that showed maternal effect P cytotype but no detectable ovary expression of the 66-kD protein might produce a low level of RNA encoding the protein that is sufficient to repress GD sterility in the

early embryo, after zygotic translation begins (*e.g.*, P[*ry*$^+$;66K]-37, Figures 8 and 9). Conversely, the maternal regulator elements might have mislocalized transcripts and protein, for example, since the *fs(1)K10* 3′ UTR localizes transcripts to the anterior of the oocyte (CHEUNG, SERANO and COHEN 1992), rather than the posterior, which will give rise to the germline during embryogenesis. On the other hand, the *P*[*ry*$^+$; *hsp26* 66K *CycB*] transformants would be expected to have transcripts and protein localized to the presumptive germline, but the hybrid transcripts might not have been efficiently translated in the germline, where they would be required for repression in our genetic assays. Immunohistochemical staining and *in situ* hybridization experiments might better indicate where and when during oogenesis and embryogenesis repressor expression is necessary for the strongest repression. Unfortunately, the amount of protein in embryos and ovaries from single element-containing transformants is below the level of detection by immunohistochemical staining with monoclonal and polyclonal antibodies that recognize the 66-kD protein (S. MISRA and D. RIO, unpublished data).

Genomic position of a repressor element may influence maternal repression in some way other than proximity to maternal enhancers. RASMUSSON, RAYMOND and SIMMONS (1993) recently reported that maternal repression can be observed, at least in one genomic position, by a very small *P* element predicted to encode only 14 amino acids, called the *SP* element. Because of the peptide's small size, these researchers suggest that RNA encoded by the element is more likely to provide the repressing activity. Thus, they proposed a model for repression of transposition, including maternal repression, based on the expression of antisense *P* RNA by repressor elements from downstream genomic promoters. Several aspects of this model are attractive. First, there is precedence for such regulation of transposition, since the prokaryotic transposon IS*10* utilizes a similar mechanism (SIMONS and KLECKNER 1988; CASE, SIMONS and SIMONS 1990; MA and SIMONS 1990). Second, the significant genomic position effects observed in our studies might be explained if expression of antisense RNA depended on proximity to promoters capable of expressing the proper antisense RNA maternally (RASMUSSON, RAYMOND and SIMMONS 1993). Third, the lack of correlation between the overall level of 66-kD protein in the ovary and the maternal repression we observed in the genetic assays might be explained by such a model. However, preliminary experiments with our transformants find no evidence for maternal antisense transcripts coded by the 5′ end of the *P* element (S. MISRA, unpublished data).

In a P strain, many elements probably contribute to P cytotype, including the *KP* element, predicted to encode a protein sharing only the first 199 amino acids of the 66-kD and transposase proteins (BLACK *et al.* 1987; JACKSON, BLACK and DOVER 1988) and the *SP* element (RASMUSSON, RAYMOND and SIMMONS 1993). Thus, by comparison to prokaryotic transposable elements (BERG and HOWE 1989), the *P* element may regulate transposition in a variety of ways simultaneously, for example, if a combination of different repressors functioned by more than one mechanism to repress transposase activity.

The data presented in this paper suggest that although genomic position is a determinant of maternal repression, this may not be mediated solely at the level of 66-kD repressor expression. This result is important in that it questions a simple and popular mechanism for *P* element regulation. However, if protein expression is not the key, there are a limited number of other repressing mechanisms that could operate. It is possible that antisense RNA expression from *P* elements is crucial to repression, but further experiments will be necessary to test this hypothesis.

Although the mechanism underlying the maternal inheritance of P cytotype remains elusive, our experiments have tested a straightforward model in which high-level expression of repressor protein during oogenesis provides the maternal component of P cytotype. These experiments highlight the important role of genomic position in maternal repression and support the idea that P strains may undergo a selection to carry repressor elements in special maternal repression-specific genomic positions. The complex nature of this regulation suggests that *P* elements might provide important insights into control of gene expression by expression of antisense RNA. Ultimately, the biochemical characterization of repression should provide a more complete picture of P cytotype repression and the inheritance of this regulatory state.

We would like to thank ROBERT COHEN for providing the *hsp26* regulator plasmids and KEVIN O'HARE for providing the ν_6 and *sn* P[*lacZ*] strains. We would also like to thank TODD LAVERTY and the Drosophila Genome Project for assistance in mapping *in situ* chromosome hybridizations, LUCILA LANCASTER for assistance with fly stocks and β-galactosidase staining experiments and SIOBHAN ROCHE and CHRIS SIEBEL for sharing unpublished results. We gratefully acknowledge KATHRYN ANDERSON, DONALD MORISATO, CHRIS SIEBEL and WAYNE WU for critical reading of the manuscript, and S.M. wishes to especially thank DONALD MORISATO for helpful discussions and CHRIS SIEBEL for enlightening debates and encouragement. This work was supported by National Institutes of Health grant GM48892 and the LUCILLE P. MARKEY charitable trust. D.C.R. is a LUCILLE P. MARKEY scholar.

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Communicating editor: T. SCHÜPBACH