Maternal Repression of the *P* Element Promoter in the Germline of *Drosophila melanogaster*: A Model for the P Cytotype

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ABSTRACT

The transposition of *P* elements in *Drosophila melanogaster* is regulated by products encoded by the *P* elements themselves. The P cytotype, which represses transposition and associated phenomena, exhibits both a maternal effect and maternal inheritance. The genetic and molecular mechanisms of this regulation are complex and not yet fully understood. In a previous study, using *P-lacZ* fusion genes, we have shown that *P* element regulatory products were able to inhibit the activity of the *P* promoter in somatic tissues. However, the repression observed did not exhibit the maternal effect characteristic of the P cytotype. With a similar approach, we have assayed *in vivo* the effect of *P* element regulatory products in the germline. We show that the P cytotype is able to repress the *P* promoter in the germline as well as in the soma. Furthermore, this repression exhibits a maternal effect restricted to the germline. On the basis of these new observations, we propose a model for the mechanism of P cytotype repression and its maternal inheritance.

THE transposition of the *Drosophila melanogaster* *P* element is genetically regulated. It occurs at high frequencies in the progeny of a cross in which males from a *P* element-containing strain (P strain) are mated to females devoid of *P* elements (M strain). The progeny of both sexes resulting from this type of cross display the P-M hybrid dysgenesis syndrome as a consequence of *P* element mobilization (reviewed in ENGELS 1989; RIO 1990). This syndrome includes high rates of mutations, chromosome rearrangements, male recombination and a thermosensitive agametic sterility (GD sterility). The virtual absence of these dysgenic traits in the progeny of the reciprocal cross (P females crossed to M males) demonstrates maternal repression of *P*. This maternal effect is attributed to a cytoplasmic condition not permissive for *P* element transposition called the P cytotype. Moreover, this P cytotype state can be maternally inherited for several generations. Both the maternal effect and the maternal inheritance are specified by the genomic *P* elements themselves (reviewed in ENGELS 1989; RIO 1990).

P element transposition is normally restricted to the germline. This germline specificity is regulated at the level of pre-mRNA splicing of the third *P* element intron between ORFs (open reading frames) 2 and 3 (LASKI, RIO and RUBIN 1986). In germline tissues, the full length 2.9-kb *P* element expresses a trans-acting protein of 87 kDa, demonstrated to be the transposase (RIO, LASKI and RUBIN 1986). In somatic tissues, the retention of the third intron results in the production of a smaller protein of 66 kDa specified by the first three ORFs. This truncated protein was postulated to be the repressor of transposition involved in the P cytotype (RIO, LASKI and RUBIN 1986).

To test this hypothesis, *in vitro* modified *P* elements have been studied regarding their ability to specify the P cytotype (ROBERTSON and ENGELS 1989; MISRA and RIO 1990). Elements having lesions affecting the fourth transposase exon but leaving intact the first three exons have the ability to engender many aspects of the P cytotype. However, none of the lines carrying these *in vitro* modified *P* elements exhibits the maternal effect characteristic of the P cytotype, and they are unable to suppress totally the GD sterility induced by a P strain. The authors have interpreted this observation as resulting from an insufficient amount of repressor in the appropriate tissue (*i.e.*, the germline)(ROBERTSON and ENGELS 1989; MISRA and RIO 1990). This could be due either to a position effect or to the nature of the repressor produced by the element tested or to both. In addition, although the 66-kDa protein clearly has regulatory properties, the molecular mechanism of its repression is still unknown. Competition at the DNA binding site between the transposase and the repressor, transcriptional repression, action on RNA splicing or RNA stability and protein-protein interactions are all considered possible mechanisms (reviewed by RIO 1990).

In a previous study, we have shown that *P*-encoded regulatory products, including the 66-kDa protein synthesized by a modified *P* element, inhibit *in vivo* the expression of the *P* promoter (LEMAITRE and

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COEN 1991). These results were obtained by using "enhancer-trap" insertions (*P-lacZ* fusion genes). In these constructs, the *Escherichia coli lacZ* gene is fused in frame with the *P* element transposase gene (for a review on "enhancer trap" see WILSON, BELLEN and GERHING 1990). Therefore, they function as reporter genes of *P* promoter activity. LEMAITRE and COEN (1991) assayed the repression by *P* regulatory products on *P-lacZ* expression only in the somatic tissues. It was noted that in these tissues the repression did not show the maternal effect characteristic of P cytotype.

Here we have extended our investigation to the germline by using *P-lacZ* insertions that are expressed in this tissue. We show that P cytotype repression of *P-lacZ* expression is also observed in the germline. The intensity of this repression is consistently stronger in this tissue than in the somatic tissues. Furthermore, a maternal effect of the repression was clearly detected and appeared to be restricted to the germline. These observations allow us to propose a model for the mechanism of P cytotypic repression and its maternal inheritance.

MATERIALS AND METHODS

***P-lacZ* fusion genes:** *P[lac, ry*$^+$*]*A contains an in-frame translational fusion of the *E. coli* β-galactosidase gene (*lacZ*) to the second exon of the *P* transposase gene and also contains the *rosy*$^+$ gene as a marker for transformation (O'KANE and GEHRING 1987).

*P[lw*B] is a similar *P-lacZ* construction except that the marker for transformation is the mini-*white* gene (KLEMENZ, WEBER and GEHRING 1987) and that it contains bacterial plasmid sequences allowing rapid cloning by plasmid rescue (BELLEN *et al.* 1989).

Drosophila stocks: Canton (KIDWELL, KIDWELL and SVED 1977), Gruta (ANXOLABÉHÈRE *et al.* 1987): typical M strains containing no *P* element sequences.

Harwich (KIDWELL, KIDWELL and SVED 1977): a reference P strain.

HS2-25: this P strain was derived from the Gruta M stock by germline transformation with complete *P* element DNA (ANXOLABÉHÈRE *et al.* 1987) and this stock is therefore essentially isogenic to the Gruta strain (LEMAITRE and COEN 1991).

ry^{506} *P[ry*$^+$Δ2-3*]*(99B), hereafter designated Δ2-3(99B), contains an essentially immobile *P* element insertion that lacks the last intron and produces a high level of transposition in the soma as well as in the germline (ROBERTSON *et al.* 1988).

R20a is a line harboring an insertion of a *P-lacZ* element on the third chromosome at cytological site 100CD. It shows ubiquitous *lacZ* expression (LEMAITRE and COEN 1991).

BG07, BQ16, BC69, BA37, ABOO, LJ25, BP76, BA34, BA32, BP73, BL54 are 11 lines harboring an autosomal insertion of a *P-lacZ* element (*P[lac, ry*$^+$*]*A) balanced by *T(2;3),CyO; TM6*. They were isolated in a screen for female sterile mutations and express the *P-lacZ* transgene in ovaries and testes (J. L. COUDERC and F. LASKI, unpublished data). These strains are described in Table 1 and in the text.

KP-D, KP-F2, KP-U are three lines bearing one autosomal *KP* insertion and displaying some regulatory properties (W. R. ENGELS, G. GLOOR and C. PRESTON, unpublished data; see DISCUSSION for description). The U line contains a nested element composed of two KPs, one inserted in the 3′ end of the other (W. R. ENGELS, G. GLOOR and C. PRESTON, unpublished data).

The Lk-P(1A), *P[ry+, SalI]*(89D), ν_6 and WY113 strains are described in the text.

For complete description of marker genes and balancer chromosomes, see LINDSLEY and GRELL (1968).

β-Galactosidase localization: Ovaries and testes were fixed in 0.5% glutaraldehyde, 1 mM $MgCl_2$ in PBS pH 7.5 for 4 min, washed in PBS buffer, then submerged in 0.2% 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal), 3.5 mM $K_4Fe(CN)_6$, 3.5 mM $K_3Fe(CN)_6$, 1 mM $MgCl_2$, 150 mM NaCl, 10 mM Na_2HPO_4, 10 mM NaH_2PO_4 and incubated overnight at 37° (HIROMI, KUROIWA and GEHRING 1985). They were then mounted in glycerol.

Quantitative measurement of β-galactosidase activity: Three ovaries were homogenized in Z buffer (MILLER 1972) and centrifuged for 10 min at 10,000 rpm (4°) to remove debris. β-Galactosidase activity was measured as described in MILLER (1972). The protein concentration was determined in each sample by the Bradford assay (BioRad), using BSA as a standard. Results are given in nM/min/mg of proteins.

Statistical analysis: Comparisons of means were performed with the Fisher-Student *t*-test when the data analyzed fitted the conditions of validity. The use of the *t*-test implies two conditions. (1) The data must be normally distributed. We have checked this point by testing (with a χ^2 test) the distribution among intervals of equiprobability of a large number of preliminary measurements of the BC69 *P-lacZ* activity in various backgrounds. The distribution of *P-lacZ* activity measurements was clearly consistent with the hypothesis of normality. In fact, this result is not surprising since biological characters are often normally distributed. (2) The variances must be identical throughout the groups compared. The *t*-test needs then the calculation of a common weighted SD value calculated with the whole set of data. For the results in Tables 2 and 4, the SD values obtained for the different sets of measurements are consistent with the hypothesis of homoscedasticity when tested with the Hartley-test. Consequently, within each of these two tables, the comparisons of means were performed with the *t*-test using a common weighted SD calculated from all the data in the table. This common SD is given with its degree of freedom (*df*) in the legend of each table. In Table 3, some sets of measurements produced unexpectedly high SD values. So in this table, comparisons of means were performed with the nonparametric Mann-Whitney test, which does not imply homoscedasticity.

RESULTS

P cytotype represses *P-lacZ* expression in the germline: We have studied the effect of P cytotype on the promoter of the *P* element in the germline in the same way as we previously studied it in somatic tissues (LEMAITRE and COEN 1991). For these studies we used 11 lines harboring an autosomal *P-lacZ* insertion that causes recessive female sterility (J. L. COUDERC and F. LASKI, unpublished data) and the R20a *P-lacZ* line (LEMAITRE and COEN 1991). All these *P-lacZ* insertion lines showed β-galactosidase expression in ovaries and testes (see Table 1).

Males containing these insertions were crossed with

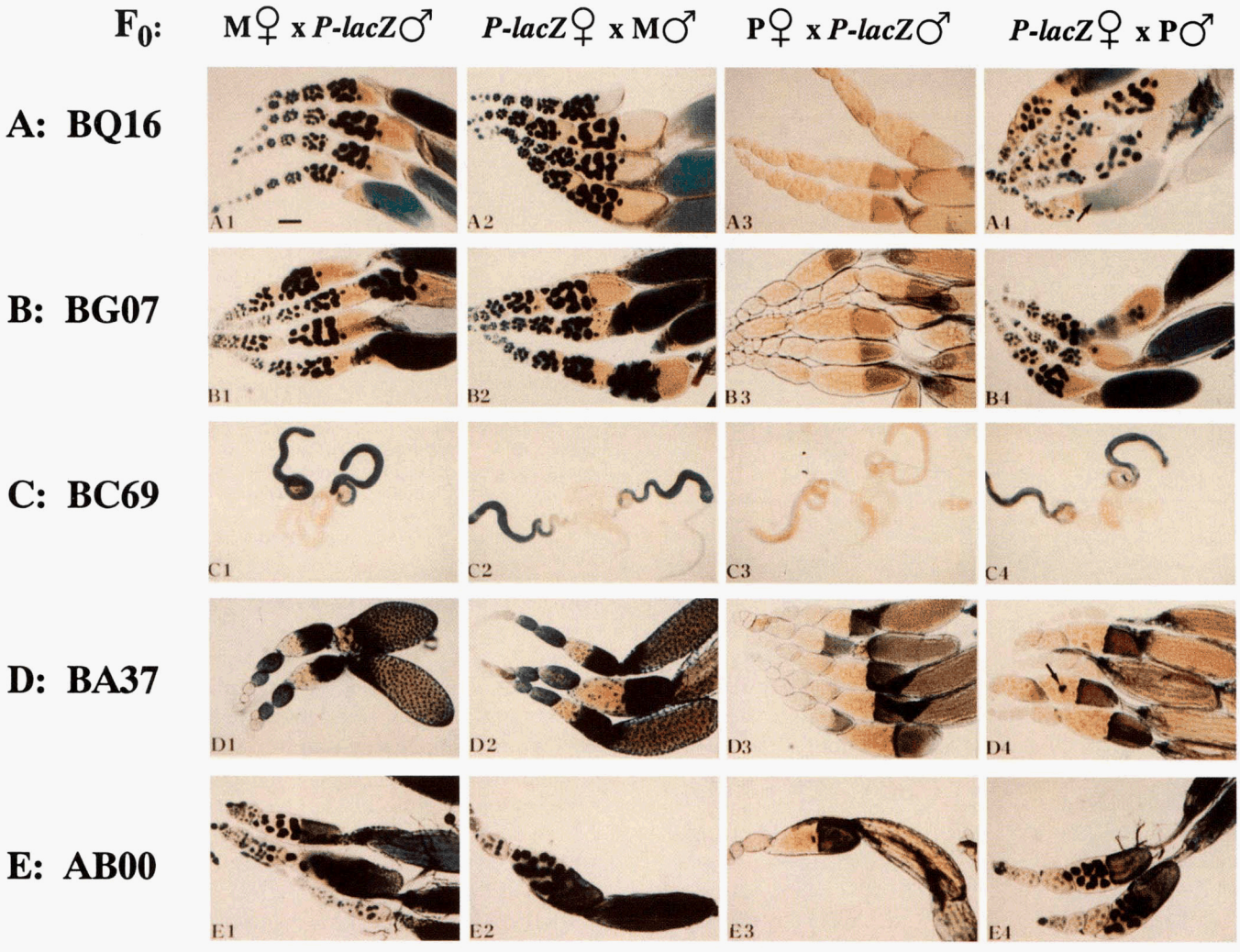

FIGURE 1.—Restriction of the maternal effect of P cytotype repression to germline tissues. Each row shows the staining of a *P-lacZ* insertion in the ovaries or testes of F_1 progeny derived from the crosses indicated at the top. Staining was performed under identical conditions (media, duration of reaction) to allow a visual comparison. The β-galactosidase enzyme is restricted to the nuclei since *P-lacZ* constructions carry the *P* element first exon (BELLEN *et al.* 1989), which encodes a signal for nuclear import. In the first four rows, the M strain was Canton and the P strain was Harwich (BAR = 100 μ). (Row A) The BQ16 line expresses β-galactosidase only in the ovarian germline tissues. No difference in the level of *P-lacZ* expression is observed in an M background when the insertion is derived either paternally (A1) or maternally (A2). However, complete repression is observed in a P background when the insertion is derived paternally (A3). A mosaic pattern of staining is observed when the *P-lacZ* insertion is derived maternally and *P* element is derived paternally (A4, unstained cells; see arrow for an example). (Row B) The BG07 *P-lacZ* insertion is another insertion that is expressed only in ovarian germline tissues. (Row C) BC69 is expressed only in the male and female germlines (J. L. COUDERC and F. LASKI, unpublished data). Stainings of the sexual apparatus of F_1 male progeny of crosses involving this line are displayed. Staining is clearly restricted to the germline cells of the testes. As can be seen in the four panels, a maternal effect of P cytotype repression on *P-lacZ* expression is also observed in the male germline. (Row D) The BA37 *P-lacZ* insertion used in this row expresses β-galactosidase only in the somatic tissues (follicle and border cells). This line was used as a control to observe P cytotype repression of *P-lacZ* in the somatic tissues of the ovaries. Strong but incomplete repression is seen in these stainings whatever the origin (maternal or paternal) of the regulatory *P* elements (see panels D3 and D4). A single stained a nurse cell (panel D4 (arrow)) may be the result of a transposition that relocated the *P-lacZ* element to a site allowing its expression in the germline. (Row E) For the crosses in this row, the M strain was Canton and the P strain was Lk-P(1A). These strains were crossed with the ABOO insertion which expresses *P-lacZ* in both the germline and somatic tissues of the ovaries. As can be seen in panels E3 and E4, Lk-P(1A) is able to repress *P-lacZ* expression in the germline completely but only when it is used as the female parent. However, it did not repress *P-lacZ* expression in the somatic tissues of the ovary.

females of M or P cytotype (Canton and Harwich, respectively) at 18°. The ovaries and testes of the F_1 progeny were stained for β-galactosidase activity and compared. For all the insertion lines studied, strong repression of the β-galactosidase activity was observed in both the somatic and germline tissues of ovaries and in the testes of the progeny derived from the P-cytotype females. As can be seen in Figure 1 (compare A1 and A3 (BQ16), B1 and B3 (BG07)), there was apparently complete repression of *P-lacZ* activity in the female germline, although there was weak β-galactosidase activity in the somatic tissues of the ovaries (Figure 1, compare panels D1 and D3 showing line BA37). All 11 lines showing expression in the

TABLE 1

β-Galactosidase staining pattern in the ovaries and testes of *P-lacZ* insertion strains

Line	Chromosomal location	Ovaries		Testes
		Germline	Soma	
BG07	*II*	+++	−	++
BC69	*II*	+++	−	+++
BQ16	*III*	+++	−	+
BA32	*III*	++	−	+++
BL54	*A*	++	−	++
BA37	*II*	−	+++	+
ABOO	*II*	++	++	++
LJ25	*II*	++	++	+
BA34	*II*	+	+	+
BP76	*II*	+	+	+
BP73	*A*	++	+	+
R20a	*III*	++	++	+

−, +, ++ and +++: no, low, medium and high level of *lacZ* expression.

A: autosome.

TABLE 2

***P* repression of *P-lacZ* expression in ovaries**

	P-lacZ insertion	Parental crosses			
		M[a] ♀ × *P-LacZ* ♂	*P-lacZ* ♀ × M[a] ♂	P[b] ♀ × *P-LacZ* ♂	*P-lacZ* ♀ × P[b] ♂
Germline	BQ16	3.5 (0.7)	4.2 (1.0)	0.8 (0.2)	2.0 (0.5)
	BG07	4.9 (0.3)	4.7 (0.4)	0.9 (0.3)	3.5 (0.5)
		5.2 (0.7)[c]	5.0 (0.3)[c]	1.1 (0.5)[c]	3.4 (0.9)[c]
	BC69	5.7 (0.5)	4.7 (0.8)	1.0 (0.4)	4.8 (0.4)
Soma	BA37	5.7 (0.5)	4.7 (0.4)	1.6 (0.3)	1.5 (0.1)

Three measurements were done for each β-galactosidase activity determination. Means are given in nmol/min/mg of proteins, with the standard deviations in parentheses. Cultures were reared at 18°. Measurement of the *lacZ* activities in the ovaries of a strain devoid of a *P-lacZ* insertion (Canton) was used as a control for endogenous β-galactosidase activity. The results are: 0.4 (0.2).

The overall weighted standard deviation calculated for this table is equal to 0.524, $df = 42$.

[a] Canton and [b] Harwich were used as M and P strains, respectively.

[c] For BG07, similar measurements were performed using the co-isogenic M (Gruta) and P (HS2-25) strains.

germline gave similar result: very strong if not complete repression in the germline. The repression was less efficient in the somatic tissues for the seven lines showing expression in the somatic ovarian tissues (data not shown). Such results in the somatic tissues are consistent with our previous observations (LEMAITRE and COEN 1991).

The BC69 line was used to assess repression of *P-lacZ* in the male germline. BC69 expresses β-galactosidase only in the germline cells (J. L. COUDERC and F. LASKI, unpublished data). With this line, repression in the male germline is readily observed in P background (compare panels C1 and C3 in Figure 1).

With the aim of quantifying P-cytotype repression, males from three lines exhibiting strong germline restricted β-galactosidase expression (BQ16, BG07, BC69) and from another line exhibiting somatic ovarian expression (BA37) were crossed in parallel with M and with P females. Crude extracts from dissected ovaries of the F_1 females were assayed for their β-galactosidase activity. As can be seen in Table 2, there is a very strong repression of β-galactosidase activity for the *P-lacZ* insertions expressed in the germline. In each case, the M level of activity (first column) is significantly higher than the P level (third column; the least significant t value was obtained for BQ16, $P < 0.001$). In the P background, the levels found, although slightly higher, do not differ significantly (by a t-test) from the level of endogenous activity. The repression appeared less pronounced in somatic ovarian tissues when assayed with the BA37 line. In this case, the P level (third column) is significantly higher than the endogenous level ($P < 0.01$).

P-cytotype repression of *P-lacZ* expression exhibits a maternal effect restricted to the germline: To test the existence of a maternal effect of P cytotype on *P-lacZ* expression in the germline, the reciprocal crosses to those described in the previous section were performed. Females containing *P-lacZ* insertions were crossed with M and with P males (Canton and Harwich, respectively). These crosses were performed at 18° to avoid gonadal dysgenesis in the F_1. The ovaries and the testes were dissected and stained under the same conditions as previously.

In the M cytotype background, for each line the level of *P-lacZ* expression was not influenced by the direction of the cross (*i.e.*, there was no reciprocal cross effect). This was true for germline as well as for somatic gonadal expression (for examples, compare in Figure 1, panels A1, B1, C1 and D1 to panels A2, B2, C2 and D2, respectively). Therefore, the paternal or maternal origin of the *P-lacZ* insertion has no influence on its level of expression.

In striking contrast, in offspring of crosses involving P strains, we observed a strong difference in germline β-galactosidase activity depending upon the parental origin of the regulatory *P* elements. Although considerable repression was observed when the regulatory *P* elements were matroclinous, a high level of β-galactosidase activity was detected in the germline of the F_1 progeny that had received the regulatory *P* elements from their father (*e.g.*, Figure 1, compare panels A3 and A4, B3 and B4). This difference can only be explained if the repressing capabilities of the introduced *P* elements depend on their parental origin, since the *P-lacZ* element expression *per se* is not influenced by its parental origin (Figure 1, columns 1 and 2). This maternal effect is apparently restricted to the germline. Indeed, the BA37 line, which showed specific expression in somatic ovarian cells, exhibited

P-cytotype repression regardless of the parental origin of regulatory *P* elements (compare Figure 1, panels D3 and D4). The maternal effect was observed with the five lines that show *P-lacZ* expression only in the germline. All seven lines that displayed *P-lacZ* expression in both somatic and germline ovarian tissues showed the maternal effect of *P* repression only with regard to germline expression (data not shown).

In BC69 males, β-galactosidase expression is restricted to the germline in the testes. Repression was observed only when the regulatory *P* elements were maternal in origin, demonstrating a maternal effect (Figure 1, compare panels C3 and C4).

Quantitation of β-galactosidase levels agrees with visual observations. The dysgenic F_1 (derived from the M♀ × P♂ crosses) females from the lines with germinally expressed insertions have significantly higher levels of β-galactosidase activity than the comparable F_1 from the reciprocal crosses (Table 2, compare columns 3 and 4; the least significant *t*-test was obtained with BQ16, $P < 0.01$). However, the levels of activity of these females seem to be lower than when the same *P-lacZ* insertions are in an M background (Table 2, compare column 4 to column 2). In each case, except for BC69, the difference was statistically significant by a *t*-test. This diminution of the β-galactosidase correlates with the mosaic pattern of staining observed in the ovaries of the F_1 dysgenic ovaries: some of germline cells showed a diminution or an absence of histochemical staining (see Figure 1, panels A4 and B4 and compare to panels A2 and B2). Dysgenic individuals bearing the BC69 *P-lacZ* insertion displayed extremely rarely a mosaic pattern of staining (*e.g.*, Figure 1, panel C4). This line gave an essentially identical level of β-galactosidase expression regardless of whether it was placed in a dysgenic or in an M background (Table 2, Figure 1 panels C2 and C4). Yet it is sensitive to maternal repression (Table 2, Figuare 1 panels C3 and C4).

To test the possibility of an effect due to the genetic background, the same types of crosses were carried out with the line BG07 and M and P lines that are effectively isogenic (Gruta and HS2-25, respectively). β-Galactosidase levels in the F_1 of these crosses paralleled those just described (Table 2, third row). Therefore, the observed repression can only be due to the maternal introduction of regulatory *P* elements.

Various lines differ in tissue-specificity and maternal effect of the repression: We have used the BC69 line, which exhibited strong β-galactosidase expression in the ovarian germline, and the BA37 line, which strongly expresses β-galactosidase in the somatic ovarian tissues, to assess the capacity of various *P* element containing strains to repress *P-lacZ* expression.

The Lk-P(1A) line contains only two full-length *P* elements, both at cytological location 1A, and has the P cytotype. Genetic analysis of this line has shown that its *P* repression capacity is complete in the germline but, atypically weak in the soma (RONSSERAY, LEHMANN and ANXOLABÉHÈRE 1991). Our results were in agreement with this analysis. Table 3 shows that Lk-P(1A) is strongly able to repress the germline *P-lacZ* expression only if the *P* regulators are inherited maternally (compare columns 1 and 2, the difference is significant by a Mann-Whitney test with $P < 0.01$). No repression was detected in the somatic tissues when assayed with the BA37 insertion. Lk-P(1A) has also been crossed to ABOO, a line containing a *P-lacZ* insertion expressed both in the somatic and germline tissues (see Figure 1, row E). No repression in the F_1 progeny could be detected in the somatic tissues in either reciprocal cross, but maximal repression occurred in the germline only when the Lk-P(1A) elements were maternally derived.

ν_6 and WY113 are two Q strains (ENGELS and PRESTON 1981; NITASAKA, MUKAI and YAMAZAKI 1987, respectively); these strains have the P cytotype but are not able to induce the dysgenic syndrome when males of these strains are crossed to M females. Table 3 shows that the two lines have the capacity to repress strongly the *P* promoter in the germline with a maternal effect (compare columns 1 and 2). Since there are

TABLE 3

Test of regulatory potential and maternal effect of various strains

Strain tested	Parental cross: Germline test: Tested ♀ × BC69 ♂	Parental cross: Germline test: BC69 ♀ × tested ♂	Parental cross: Somatic test: BA37 ♀ × tested ♂
Lk-P (1A)	0.5 (0.3; 6)	3.4 (0.6; 6)	5.8 (0.1; 3)
ν_6 (Q)	1.1 (0.5; 3)[a]	3.8 (0.2; 3)	5.3 (1.7; 3)[b]
WY113 (Q)	0.7 (0.2; 3)	4.2 (1.0; 3)	3.6 (0.4; 4)[b]
P[SalI] (89D)	5.5 (0.7; 3)	5.0 (0.5; 3)	1.8 (0.1; 3)
KP-D	2.8 (0.6; 9)	2.7 (0.6; 9)	2.0 (0.2; 3)
KP-F2	3.0 (0.6; 8)	2.9 (0.5; 14)	2.4 (0.3; 8)
KP-U	2.8 (0.2; 6)	2.5 (0.6; 11)	1.5 (0.2; 3)
Δ2-3 (99B)	4.4 (0.7; 9)	5.1 (1.5; 12)	N.T.
Canton (M)	3.8 (0.8; 5)	3.8 (0.5; 6)	4.3 (0.3; 6)
Harwich (P)	0.4 (0.3; 5)	N.T.	1.2 (0.1; 2)

Means are given in nmol/min/mg of proteins, with standard deviations and the numbers of measurements in parentheses. Cultures were reared at 25°.

N.T.: not tested.

[a] This value was determined by using the BG07 *P-lacZ* insertion line instead of BC69.

[b] The somatic repressing capacities of both Q strains were determined by using the R20a *P-lacZ* insertion instead of BA37. The activity in the somatic tissues was measured in F_1 third instar larvae carrying the R20a insertion. The levels of activity of the BG07 and R20a *P-lacZ* insertions in M cytotype are similar to those of BC69 or BA37, respectively.

only three measurements in each group, the Mann-Whitney test is not powerful enough to compare these means. However the differences appear clear: for ν_6, the values range from 0.7–1.7 in the P background whereas in the M background they range from 3.6–3.9. For WY113 they range from 0.49–0.72 and from 3.2–5.2 in the P and M backgrounds, respectively. As with Lk-P(1A), in the somatic tissues (column 3) no clear decrease of β-galactosidase expression was detected with these two lines.

P[*Sal*I](89D) is a line containing an unique *in vitro* modified *P* element bearing a frameshift mutation in the fourth transposase exon (KARESS and RUBIN 1984). It is known to exhibit some repression properties in the somatic tissues (ROBERTSON and ENGELS 1989; COEN 1990; LEMAITRE and COEN 1991) but if at all displays only a very weak repression of the dysgenic sterility induced by a strong P strain like π2 or Harwich (ROBERTSON and ENGELS 1989; our unpublished data), although it is able to repress partially the dysgenic sterility induced by a strain harboring a single P chromosome (RASMUSSON, RAYMOND and SIMMONS 1993). We have assayed the ability of *P*[*Sal*I](89D) to repress *P-lacZ* expression in the germline. In contrast to the results obtained in the somatic tissues, where there is a significant decrease of the *P-lacZ* expression (Table 3: $P < 0.05$ by the Mann-Whitney test; Lemaitre and Coen 1991), no diminution of β-galactosidase activity could be detected in the germline (Table 3).

The *KP* element is a deleted *P* element (lacking nucleotides 809–2559). It is present in high copy number in natural populations and is thought to play a role in *P* regulation (BLACK *et al.* 1987; JACKSON, BLACK and DOVER 1988; SIMMONS *et al.* 1990). To determine the effect of *KP* elements on *P* promoter activity, three different lines bearing one autosomal *KP* insertion were tested (strains D, F2, and U, W. R. ENGELS and C. PRESTON, unpublished data). Table 3 shows that the three *KP* insertions were able to repress the BA37 *P-lacZ* activity in the somatic tissues (column 3). In each case, the difference with the level obtained with Canton is statistically significant: the least significant difference (with the Mann-Whitney test) was obtained for KP-D ($P < 0.05$). In the germline (columns 1 and 2) a repression of the BC69 *P-lacZ* activity is observed for all three *KP* insertions tested, regardless of the direction of the crosses. Four of the six differences with Canton-S are significant by the Mann-Whitney test ($P < 0.05$). If the comparison between *KP* × BC69 and Canton-S × BC69 crosses is performed after pooling the results from the two Canton-S × BC69 reciprocal crosses (which produce the same mean activity), the six differences are significant ($P < 0.01$).

The Δ2-3(99B) line produces relatively high rates of transposase in all tissues (ROBERTSON *et al.* 1988). To test the ability of the 87 kDa transposase protein to repress *P-lacZ* expression in the germline, crosses of the Δ2-3(99B) line and various *P-lacZ* strains were performed. Observations were made in the F_1 ovaries. In most of these lines, we observed a mosaic staining pattern due to excisions of the *P-lacZ* insertions by transposase activity. However, it was apparent that those stained nuclei (in which no excision occurred) exhibited a level of staining which is identical to that seen with the same insertion in an M background (data not shown). Moreover, the germline specific BC69 *P-lacZ* insertion displayed very few excisions and transpositions with Δ2-3(99B). Consequently, we were able to make quantitative comparisons. No repression by the Δ2-3(99B) insertion on *P-lacZ* expression was detected in the germline (Table 3).

TABLE 4

Thermosensitivity of repression of *P-lacZ* expression in the germline

Aging temperature of the F_1 ♀	Parental cross: Lk-P(1A) ♀ × BG07 ♂	Parental cross: BG07 ♀ × Lk-P(1A) ♂
18°	1.1 (0.4)	3.9 (0.3)
29°	0.9 (0.3)	1.6 (0.3)

Six measurements were done for each β-galactosidase activity determination. Means are given in nmol/min/mg of proteins, with the standard deviation in parentheses. Cultures were reared at 20°. F_1 females from the two crosses were aged for 15 days at 18° or 10 days at 29°. The overall weighted standard deviation calculated for this table is equal to 0.328, $df = 20$.

Thermosensitivity of the *P* repression of *P-lacZ* expression: A peculiar characteristic of cytotype is the thermosensitivity of its determination in F_1 females resulting from M × P crosses. During imaginal life, an 18° treatment results in a switch toward the M cytotype, whereas a 25° (or higher) treatment results in a switch toward the P cytotype (RONSSERAY, ANXOLABÉHÈRE and PERIQUET 1984; RONSSERAY 1986). We have tested the existence of such a thermal effect upon *P* repression of the *P-lacZ* reporter gene in the germline. Some young adult F_1 females from the two reciprocal crosses between the BG07 line and the Lk-P(1A) line were placed at two different temperatures: 10 days at 29° or 15 days at 18°. A temperature effect was observed (Table 4). F_1 dysgenic females showed significantly lower *P-lacZ* expression in their germline when they aged at the higher temperature ($P < 0.001$). This effect cannot be attributed to the induction of gonadal dysgenesis in the M♀ × P♂ cross since Lk-P(1A) does not induce gonadal dysgenesis. This result is in agreement with those previously obtained with Lk-P(1A) using GD sterility regulation assays (RONSSERAY, LEHMANN and

ANXOLABÉHÈRE 1991). Thus, thermosensitivity of *P-lacZ* repression paralleled thermosensitivity of the P cytotype measured with standard assays.

DISCUSSION

P repression of the P promoter is maximal in the germline: *P* transposition occurs normally in the germline. Thus, P cytotype must operate in this tissue to prevent it. Hence, we have studied *P* repression on *P-lacZ* insertions that are expressed specifically in the germline of males and females. As in the case of somatically expressed *P-lacZ* insertions (LEMAITRE and COEN 1991), all the *P-lacZ* lines tested are sensitive to *P* repression in the germline. Moreover, the repression in the germline appears consistently stronger than in the soma. For all the 11 lines tested, including those exhibiting a very high level of β-galactosidase activity, there is no or almost no germline staining in P cytotype after an overnight incubation at 37°. This is not the case for somatic tissues where a residual staining is always observed in P cytotype, except when the M level of activity is very low (28 lines tested, LEMAITRE and COEN 1991, this study). Quantitative measurements confirm this observation. The β-galactosidase activities measured in a P background from germinally expressed insertions were indistinguishable from (see Table 3 with Lk-P(1A) and Harwich at 25°) or slightly higher than the endogenous level (see Table 2 with Harwich at 18°). Conversely, in somatic tissues, the level observed in a P background was significantly higher than the endogenous level (see Table 1 with BA37 and Tables 2 and 4 in LEMAITRE and COEN 1991).

The maternal effect of the P cytotype is restricted to the germline: P cytotype repression exhibits a maternal effect: *P* element transposition and the associated dysgenic syndrome are observed mainly in the progeny from M females crossed to P males. This effect was observed for all the classic P strains using germline phenotypic assays: GD sterility and sn^w hypermutability (ENGELS 1979, 1981, 1989)

All the previous observations on somatic tissues have failed to detect a maternal P cytotype effect (ENGELS *et al.* 1987; WILLIAMS, PAPPU and BELL 1988; ROBERTSON and ENGELS 1989; COEN 1990; MISRA and RIO 1990; LEMAITRE and COEN 1991; RONSSERAY, LEHMANN and ANXOLABÉHÈRE 1991).

Here, we have observed a clear maternal effect of P cytotype with all the germline specific *P-lacZ* insertions tested. *P* repression is observed in the germline only when *P* elements from the P strain are maternally derived.

With some *P-lacZ* insertion lines, we observed a slight decrease in β-galactosidase levels in the germline of F_1 females when the *P* elements were paternally derived. In all cases staining the ovaries of the F_1 females from the same crosses showed concomitantly a mosaic pattern, with some nurse cells unstained or less stained, differing from one individual to the other.

Two hypotheses can be proposed to explain these observations: (1) This could be due to repression exerted in some of the germline cells by paternally inherited *P* elements. In this case, the mosaic pattern of staining would result from (random) differences in repression intensity among nurse cells. (2) Alternatively, because the females from this type of cross are dysgenic, transposase is produced in the germline. The decrease in β-galactosidase levels could therefore result from excisions of the *P-lacZ* element in some of the cells. Due to their polyteny, the nurse cell nuclei could contain different numbers of copies of the *P-lacZ* element, depending on when and how many excisions have occurred.

We think that several observations favor the second explanation. The diminution of β-galactosidase activity was never observed with *P-lacZ* lines displaying no or a very low rate of excisions in dysgenic conditions (*e.g.*, BC69, Figure 1, panel C4). Neither was it observed when we used Q lines, which produce a very low or no transposase activity (Lk-P(1A), ν_6, WY1113, Figure 1, panel E4 and Table 3). In addition, staining mosaicism among various nuclei was only observed in the germline of dysgenic F_1. In somatic tissues, we never detected a difference in the pattern of staining when we compared M and P backgrounds, whatever the direction of the cross (LEMAITRE and COEN 1990; this study). In these tissues, transposase is not expressed while repression is effective and could have (as in nurse cells) resulted in cell to cell mosaicism.

Although the first explanation cannot be ruled out, we therefore think that the decrease of β-galactosidase activity when *P* elements are paternally introduced is mainly due to *P-LacZ* excision events.

Using the same assay, the maternal effect on P repression is not observed in the soma including somatic ovarian tissues. We suggest that indeed, the maternal effect of the P cytotype is restricted to the germline. Further, our conclusions are consistent with the observation that the maternal inheritance of P regulation is also restricted to the germline (RONSSERAY, LEHMANN and ANXOLABÉHÈRE 1991). The *P-lacZ* assay also allowed us to detect the temperature sensitivity of P cytotype determination in the germline described by RONSSERAY, ANXOLABÉHÈRE and PÉRIQUET (1984).

***KP* element represses the *P* promoter *in vivo*:** In some M′ strains, large numbers of a class of deleted elements named *KP* have been found. It has been suggested that these *KP* elements may possess some regulatory capacity that protects these M′ strains from *P*-induced hybrid dysgenesis. *KP* regulation differs from P cytotype because it does not exhibit maternal

inheritance (BLACK *et al.* 1987; JACKSON, BLACK and DOVER 1988; SIMMONS *et al.* 1990). It has been proposed that a truncated polypeptide possibly encoded by *KP* elements can inactivate the transposase by a "poisoning" mechanism (BLACK *et al.* 1987).

We have tested the ability of this kind of deleted element to repress the *P* promoter. Among the three lines tested, W. R. ENGELS and C. PRESTON (unpublished data) have observed that only the U, and to a lesser extent, the F2 strains were able to repress the gonadal dysgenesis induced by a P strain, demonstrating that they possess a regulatory capacity in the germline. They have also shown that the three lines were able to rescue the pupal lethality induced by Δ2-3(99B) (ENGELS *et al.* 1987), indicating that they all possess regulatory capacity in the somatic tissues. The results obtained with the somatic ovarian-specific BA37 *P-lacZ* insertion indicate clearly that these elements can alter the *in vivo* level of activity of the *P-lacZ* expression. The intensity of the repression is weaker than that exerted by a P strain.

All the *KP* insertions tested display a slight but significant ability to repress *P-lacZ* activity in the germline, regardless of the direction of the cross. This ability is uncorrelated with their ability to repress gonadal dysgenesis. To explain this discrepancy, we can imagine that the regulatory capacity of the *KP* elements that represses gonadal dysgenesis is effective at an earlier developmental stage of the germline and hence is not detected here. Indeed, the thermosensitive period for gonadal dysgenesis begins at the end of embryogenesis and gradually falls off during the second larval instar (ENGELS and PRESTON 1979; KIDWELL and NOVY 1979).

The same explanation could account for the lack of detection of *P-lacZ* repression by *P*[*Sal*I](89D), although this element is able to exert at least a partial repression of gonadal dysgenesis (RASMUSSON, RAYMOND and SIMMONS 1993).

The 87-kDa transposase protein has no detectable repressing capacity *in vivo*: The 87-kDa transposase has been shown to repress *P* transcription *in vitro* (KAUFMANN and RIO 1991). However, we do not observe any repression effect when the transposase is the sole *P* element product as in the Δ2-3(99B) line either in the germline (this study) or in the soma (LEMAITRE and COEN 1991). Moreover, as discussed above, we think that the transposase produced in the germline of dysgenic females (progeny of a cross of M females to males from a classic P strain, Harwich) does not affect significantly the *P* promoter activity (see BC69 in Table 2). Two explanations can account for this *in vitro-in vivo* discrepancy. It is possible that the quantity of transposase used in the *in vitro* assay is high compared with the level produced *in vivo* that is sufficient to induce a high rate of transposition. Alternatively, it is possible that some protein factor from the host prevents transposase repressor activity *in vivo*. Our observations parallel those made with the bacterial insertion sequence IS*1*: the expression of the *ins*A frame alone (coding for the repressor) exerts a 10-fold repression on the expression of an *ins*A-*lacZ* reporter gene, whereas the constitutive expression of the fusion *ins*AB′ (deriving from two consecutive open reading frames, *ins*A and *ins*B′ and coding for the transposase) leads to only a twofold repression of the same reporter gene (ESCOUBAS *et al.* 1991; M. CHANDLER, unpublished data).

Tissue-specific differences between repressor-producing *P* elements: The tissue-specificity of *P* repression on *P-lacZ* expression exhibited by the various lines has allowed us to distinguish different type of strains. Using this assay, there are lines that have their regulatory capacity mainly restricted to the somatic tissues (*P*[*Sal*I](89D)) or to the germline (ν_6, WY113, Lk-P(1A)). Other lines that repress in both type of tissues can be separated into two classes according to whether (Harwich) or not (the three *KP* strains) they exhibit a maternal effect and complete repression. It should be noted however, as mentioned above, that the *P-lacZ* repression level is not always correlated to the gonadal dysgenesis repression level.

The regulatory capacities of theses lines must depend on both the chromosomal location and the nature of the element(s) they contain. Strains from natural populations may contain all or several of these different types of repressor-producing *P* elements and therefore exhibit the different types of regulation. It is striking that all the lines described here that show maternal *P* repression also show a maximal repression in the germline tissues of the ovaries. All these lines contain at least one full length *P* element insertion (ENGELS 1984; NITASAKA and YAMASAKI 1991; RONSSERAY, LEHMANN and ANXOLABÉHÈRE 1991).

***P* cytotype acts through the repression of the *P* promoter activity:** P cytotype repression displays properties that differentiate it from other types of regulation: (1) a maternal effect (the dysgenic syndrome due to the mobilization of *P* elements is observed only in the progeny derived from M females crossed to P males), (2) a maternal inheritance of its repressing capacity through several generations and (3) a strong dependence on temperature.

Our results here show that P cytotype effects on *P-lacZ* expression parallel all the characteristics of P cytotype action on *P* element mobilization. In addition, the tissue-specificity of the *P-lacZ* regulation by the various *P* element lines studied here is similar to those already described using somatic or germline assays. Our previous study (LEMAITRE and COEN 1991) has shown that in the somatic tissues *P-lacZ* repression by *P* can be interpreted as an inhibition of

P promoter activity. It is very likely that in the germline, *P* repression is also due to a diminution of *P-lacZ* specific mRNA. We have found that all the *P-lacZ* insertions tested are sensitive to *P* repression, and Northern blot analysis shows that this transcription is indeed initiated at the *P* promoter (LEMAITRE and COEN 1991). The R20a line exhibits the *P-lacZ* repression in both somatic and germinal tissues. Northern blot analysis in the somatic tissues (whole instar larvae) has previously shown that with this line, the *P* repression is indeed correlated with a diminution in the level of *P-lacZ* specific transcript (LEMAITRE and COEN 1991). Although a complete demonstration of this effect would require an analysis of the *P-lacZ* RNA levels in ovaries, it is highly unlikely that for the same line (R20a) the mechanism of *P* repression differs between somatic tissues and the germline. J. PATERSON and K. O'HARE have independently obtained results that are in agreement with ours using a *P-lacZ* insertion at the *singed* locus (unpublished data). They have also demonstrated that the *P* repression in the germline results in a reduction of *P-lacZ* specific transcript.

Altogether, these results strongly suggest that the P cytotype acts through a repression of *P* promoter activity. No *P-lacZ* expression is detected in the germline when the F_1 progeny receive their regulatory *P* elements from their mothers (P♀ × M♂). This repression of the *P* promoter in the germline would prevent the synthesis of *P* transcripts. In the reciprocal crosses (M♀ × P♂), the *P* promoter is not repressed. *P* transcripts can be synthesized from the paternally inherited *P* elements and spliced in the germline, resulting in the production of transposase and the associated dysgenic syndrome.

Such a mechanism of transcriptional repression is also consistent with the observation that the *Icarus* insertion line, which carries a modified *P*-element transposon that expresses transposase from the heat-inductible *hsp70* promoter, destabilizes sn^w in a P cytotype background and thus overcomes the *P* repression of a P strain ($\pi 2$) (STELLER and PIRROTTA 1986). Meanwhile, this element is partially sensitive to P cytotype. This result could seem paradoxical because the transcription of the *Icarus* element is initiated at the *hsp70* promoter. However, this could be explained by repression on the *P* promoter sequences that are still present (downstream of the *hsp70* promoter) in this construct or even by repression at a post-transcriptional level: such regulation of *P* element transposition at a post-transcriptional level cannot be excluded and could reinforce the transcriptional control. Experiments are underway to detect such regulation.

A model for the maternal inheritance of P cytotype: Maternal inheritance of the P cytotype is generally explained by the accumulation of the repressor in the developing eggs during oogenesis in P cytotype females. This results in preventing *P* element-induced hybrid dysgenesis in the progeny and in the maternal transmission of the P cytotype (ENGELS 1989; RIO 1990). However, our finding that the *P* promoter is almost completely repressed in the germline of P females and that hence *P* repressor should repress its own synthesis, leads us to reconsider and modify this model.

Several mechanisms taking this in account can be proposed:

1. By chromosomal position effect, some regulatory *P*-element insertions could be insensitive to P-cytotype repression. Therefore, they would still be active in a P background and thus be able to specify the P cytotype. It is probable that such insertions would receive strong positive selection because of the protection against hybrid dysgenesis that they confer. Although such an explanation cannot be excluded, we have found that all the *P-lacZ* insertions (more than 40) are sensitive to *P* repression. Furthermore, the existence of *P* elements insensitive to *P* repression cannot by itself account for the maternal inheritance.

2. Another possible explanation is that the repressor is synthesized in the follicle cells and is subsequently deposited into the eggs of P cytotype females. In follicle cells, *P* repression is not complete and IVS3 is not spliced (LASKI, RIO and RUBIN 1986; LASKI and RUBIN 1989). Hence, only the 66-kDa protein is made. However, we observed that the level of *P* promoter repression in the follicle cells is the same regardless of the parental origin of the *P* regulatory element. Therefore, follicle cell expression and transport cannot also account for the maternal transmission of the P cytotype.

3. Although *P* transcription is almost completely repressed in the germline of P females, a small quantity of repressor made during oogenesis would be sufficient to specify and transmit the P cytotype. This would require, as proposed by O'HARE and RUBIN (1983), that the repressor can exert a positive feedback on its own synthesis. This positive feedback restricted to the germline could be due to the incomplete splicing of the IVS3 from *P* pre-mRNAs. O'HARE *et al.* (1992) have proposed such a model. They have suggested that incomplete splicing of IVS3 could be the result of the presence in germline cells of a low concentration of the same repressor that inhibits IVS3 splicing in somatic cells (SIEBEL and RIO 1990; CHAIN *et al.* 1991) and that high levels of pre-mRNA would titrate out this repressor, making the IVS3 splicing possible. Alternatively, we propose that the *splicing efficiency* itself may depend upon the *P* pre-mRNA concentration. At low *P* pre-mRNA concentrations, there would be a low efficiency of the IVS3

splicing. This would result in a higher concentration of the 66-kDa regulatory protein translated from the unspliced *P* mRNA relative to the 87-kDa transposase translated from the spliced messenger. By contrast, at higher *P* pre-mRNA concentrations, the transposase/regulator ratio would be reversed, due to the higher efficiency of the IVS3 splicing.

We have shown that the autonomous *P* elements present in the Lk-P(1A) line are or are not able to specify the P cytotype, depending on maternally inherited factors (RONSSERAY, LEMAITRE and COEN 1993). We suspect that, in the P cytotype, these elements specify the 66-kDa protein, which is able to repress the *P* promoter (RONSSERAY, LEMAITRE and COEN 1993). Conversely, the 87-kDa transposase, which these elements are able to produce in dysgenic conditions, does not show any regulatory capacity *in vivo* (LEMAITRE and COEN 1991; this study). These results are in good agreement with the model proposed here. In the germline of a P cytotype female (which possesses numerous complete *P* elements), the *P* element promoter activity is strongly repressed. As a result, the small amount of newly synthesized *P* pre-mRNA is incompletely spliced. This will lead to the formation of an excess of the 66-kDa regulatory protein compared with the amount of transposase. This low level of transcription with preferential formation of the regulator over the transposase could be readily transmitted through succeeding generations because the regulator maintains this low transcriptional level over generations. Thus, even in the situation in which transposase-producing *P* elements are paternally derived, the level of maternally inherited regulator is sufficient to repress these paternally transmitted *P* elements and, hence, to maintain the P cytotype.

In the progeny of a dysgenic cross (an M female crossed with a P male), the paternally transmitted *P* elements are strongly transcribed and the transcripts would be efficiently spliced. This would lead to the production of high levels of transposase compared to the level of regulator in the germline, and thereby produce hybrid dysgenesis. Because the 87-kDa transposase lacks an *in vivo* regulatory capacity, this state of strong transcription will be maintained until there is a sufficient accumulation of repressor to establish a low steady-state level of *P* transcription and therefore switch the cytotype from M to P (this would take several generations).

The features of this model match most of the observations concerning P cytotype determination. It explains why most if not all strains eliciting the maternal effect and the maternal inheritance of P cytotype are the strains containing full-length *P* elements that can be differentially spliced. Most significantly, the Lk-P(1A) line displays the germline characteristics of P cytotype strains (maternal effect and inheritance) and contains only two full-length *P* elements (RONSSERAY, LEHMANN and ANXOLABÉHÈRE 1991) that are both autonomous (S. RONSSERAY, unpublished data). Therefore, because maternal inheritance is postulated to be determined by differential splicing efficiencies, it can only exist if there are regulatory *P* elements that can be differentially spliced.

In the somatic tissues, irrespective of the direction of the cross, complete *P* elements code for the 66-kDa regulatory protein, due to the lack of splicing of the IVS3 in these tissues (LASKI, RIO and RUBIN 1986). This could account for the lack of maternal effect in these tissues. Similarly, the strains that carry only deleted *P* elements (*e.g.*, *KP*, *P[SalI](89D)*, or *P[ry+, 66kd]*) would display biparental repression in the germline as well as in the soma (BLACK *et al.* 1987; ROBERTSON and ENGELS 1989; MISRA and RIO 1990; our study). Indeed, these elements can only code for the same regulatory products in the somatic and germline tissues, regardless of the direction of the cross producing the F_1.

In this model, the thermosensitivity of the P cytotype could be explained by an effect of temperature on IVS3 splicing efficiency.

Finally, the observation that the repression of *P-lacZ* expression is stronger in the germline than in the soma could be simply explained because the P and Q lines tested have been selected for their ability to repress *P* element transposition in the germline. Therefore, these lines could be more effective to repress in this tissue. An alternative explanation could be the existence of a germline factor that enhances the repression of the *P* promoter. This factor can either be an host encoded factor or the transposase itself, the only germline-specific *P* element product. Either would have to be able to interact synergistically with the regulator to repress the *P* element promoter. Experiments are currently under way to test these hypotheses.

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LITERATURE CITED

ANXOLABÉHÈRE, D., H. BENES, D. NOUAUD and G. PERIQUET, 1987 Evolutionary steps and transposable elements in *Drosophila melanogaster*: the missing RP type obtained by genetic transformation. Evolution **41:** 846–853.

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

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

CHAIN, A. C., ZOLLMAN, S., TSENG, J. C. and F. A. LASKI, 1991 Identification of a *cis*-acting sequence required form germ line-specific splicing of the *P* element ORF2-ORF3 intron. Mol. Cell. Biol. **11:** 1538–1546.

COEN, D., 1990 *P* Element regulatory products enhance *zeste*^{1} repression of a *P*[*white* ^{duplicated}] transgene in *Drosophila melanogaster*. Genetics **126:** 949–960.

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

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 in *Drosophila*. Science **226:** 1194–1196.

ENGELS, W. R., 1989 *P* elements in *Drosophila melanogaster*, 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, 1981 Characteristics of a "neutral" strain in the P-M system of hybrid dysgenesis. *Drosophila* Inform. Serv. **56:** 35–37.

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.

ESCOUBAS, J. M., M. F. PRÈRE, O. FAYET, I. SALVIGNOL, D. GALAS, D. ZERBIB and M. CHANDLER, 1991 Translational control of transposition activity of the bacterial insertion sequence IS*1*. EMBO J. **10:** 705–712.

HIROMI, Y., A. KUROIWA and W. J. GEHRING, 1985 Control elements of the *Drosophila* segmentation gene *fushi tarazu*. Cell **43:** 603–613.

JACKSON, M. S., D. M. BLACK and G. A. DOVER, 1988 Amplifications 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 *P* transposable element functions in *Drosophila*. Cell **38:** 135–146.

KAUFMAN, P. D., and D. C. RIO, 1991 *Drosophila P* element transposase acts as a transcriptional repressors *in vitro*. Proc. Natl. Acad. Sci. USA **88:** 2613–2617.

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.

KIDWELL, M. G., and J. B. NOVY, 1979 Hybrid dysgenesis in *Drosophila melanogaster*: sterility resulting from gonadal dysgenesis in the P-M system. Genetics **92:** 1127–1140.

KLEMENZ, R., U. WEBER and W. J. GEHRING, 1987 The *white* gene as a marker in a new *P*-element vector for gene transfer in *Drosophila*. Nucleic Acids Res. **15:** 3947–3959.

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.

LASKI, F. A., and G. M. RUBIN, 1989 Analysis of cis-acting requirement for germ-line-specific splicing of *P* element ORF2-ORF3 intron. Genes Dev. **3:** 720–728.

LEMAITRE, B., and D. COEN, 1991 *P* regulatory products repress *in vivo* the *P* promoter activity in *P-lacZ* fusion genes. Proc. Natl. Acad. Sci. USA **88:** 4419–4423.

LINDSLEY, D. L., and E. H. GRELL, 1968 *Genetic Variations of Drosophila melanogaster*. Carnegie Institute, Washington D.C.

MILLER, J. H. 1972 *Experiments in Molecular Genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., pp. 352–355.

MISRA, S., and D. C. RIO, 1990 Cytotype control of *P* element transposition: the 66kd protein is a repressor of transposase activity. Cell **62:** 269–284.

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

NITASAKA, E., and T. YAMAZAKI, 1991 Genetic variation and regulation of *P* element in *Drosophila melanogaster*, pp. 263–276 in *New Aspects of the Genetics of Molecular Evolution*, edited by Japan. Sci. Soc. Press. Tokyo/Springer-Verlag, Berlin.

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

O'HARE, K., A. DRIVER, S. MCGRATH and D. M. JOHNSON-SCHLITZ, 1992 Distribution and structure of *P* elements from the *Drosophila melanogaster* P strain π2. Genet. Res. **60:** 33–41.

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

RASMUSSON, K. E., J. D. RAYMOND and M. J. SIMMONS 1993 Repression of hybrid dysgenesis in *Drosophila melanogaster* by individual naturally occurring *P* elements. Genetics **133:** 605–622

RIO, D. C., 1990 Molecular mechanisms regulating *Drosophila P* element transposition. Annu. Rev. Genet. **24:** 543–578.

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., and W. R. ENGELS, 1989 Modified *P* elements that mimic the P cytotype in *Drosophila melanogaster*. Genetics **123:** 815–824.

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.

RONSSERAY, S., 1986 P-M system of hybrid dysgenesis in *Drosophila melanogaster*: thermic modifications of the cytotype can be detected for several generations. Mol. Gen. Genet. **205:** 23–27.

RONSSERAY, S., D. ANXOLABÉHÈRE and G. PERIQUET, 1984 Hybrid dysgenesis in *Drosophila melanogaster*: influence of temperature on cytotype determination in the P-M system. Mol. Gen. Genet. **196:** 17–23.

RONSSERAY, S., M. LEHMANN and D. ANXOLABÉHÈRE, 1991 The maternally inherited regulation of *P* elements in *Drosophila melanogaster* can be elicited by two *P* copies at cytological site 1A on the *X* chromosome. Genetics **129:** 501–512.

RONSSERAY, S., B. LEMAITRE and D. COEN, 1993 Maternal inheritance of P cytotype in *D. melanogaster*: a "pre-P cytotype" is strictly extra-chromosomally transmitted. Mol. Gen. Genet. (in press).

SIEBEL, C. W., and D. C. RIO, 1990 Regulated splicing of the *Drosophila P* transposable element third intron *in vitro*: somatic repression. Science **248:** 1200–1248.

SIMMONS, M. J., J. D. RAYMOND, K. E. RASMUSSON, L. M. MILLER,

C. F. MCLARNON and J. R. ZUNT, 1990 Repression of *P* element mediated hybrid dysgenesis in *Drosophila melanogaster*. Genetics **124:** 663–676.

STELLER, H., and V. PIRROTTA, 1986 *P* transposons controlled by the heat shock promoter. Mol. Cell. Biol. **6:** 1640–1649.

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

WILSON, C., H. J. BELLEN and W. J. GERHING, 1990 Position effect on eucaryotic gene expression. Annu. Rev. Cell. Biol. **6:** 679–714.

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