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Genetics

P regulatory products repress *in vivo* the *P* promoter activity in *P–lacZ* fusion genes

(*Drosophila melanogaster*/enhancer-trap/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 molecular mechanisms of this regulation are complex and still unclear. We have assayed *in vivo* the effects of *P* regulatory products on the *P* promoter itself by using *P–lacZ* fusion genes. We have found that all the *P–lacZ* insertions are repressed in a P background. This repression occurs in all the tissues observed and at all the developmental stages. The amount of transcripts specific for *P–lacZ* is substantially reduced in a P background. These results suggest that *P* trans-acting products can exert a direct repression on the *P* promoter transcription.**

The transposition of the *Drosophila melanogaster P* element is genetically regulated: it occurs at high frequencies only 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* elements mobilization. This syndrome includes high rates of mutations, chromosomal rearrangements, male recombination, and a thermosensitive agametic sterility. The absence of these traits in the reciprocal cross is due to a cytoplasmic condition nonpermissive for *P* element transposition. This state, called the P cytotype, was shown to depend on both genomic *P* elements and maternal inheritance (for review, see ref. 1).

P element transposition is also normally restricted to the germ line. This germ-line specificity is regulated at the level of pre-mRNA splicing of the third *P* element intron (2). In germ-line tissues, the full-length 2.9-kilobase *P* element expresses a trans-acting protein of 87 kDa, demonstrated to be the transposase (3). 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 open reading frames. This truncated protein was postulated to be the repressor of transposition involved in the P cytotype (3). To test this hypothesis, *in vitro*-modified *P* elements have been studied with regard to their ability to specify P cytotype (4). Elements having lesions affecting the fourth transposase exon but leaving intact the first three exons have the ability to mimic P cytotype in many aspects. However, the lack of reciprocal cross effects and the inability of the repressor to suppress thermosensitive agametic sterility induced by a P strain indicate that the repressor encoded by this type of element differs quantitatively or qualitatively from the P cytotype (4).

We have decided to investigate directly the *in vivo* effect of *P* regulatory products on the expression of the *P* promoter itself, controlling a reporter gene. Tools for this study already existed and are in fact widely used in the study of *Drosophila* development: *P–lacZ* constructs, in which the *P* element transposase gene is fused in-frame with the *lacZ* gene of *Escherichia coli* (which codes for β-galactosidase) were designed for the detection of genomic regulatory elements (5). They have allowed the demonstration that *P* promoter expression is highly dependent on sequences nearby the integration site. This property has been extensively used to generate cell- or tissue-specific markers and in the recovery of genes displaying this same specific regulation (5–9).

We have examined the effects of the P cytotype on various insertions of *P–lacZ* transgenes. We have found that all the *P–lacZ* insertions tested have their expression repressed by the P cytotype: no position effect was detected. This repression occurs in all the tissues observed and at all the developmental stages. P repression results in a decrease of the amount of the specific *P–lacZ* RNA detected. These results suggest that *P* trans-acting products can exert a direct repression on the *P* promoter. This provides a powerful quantitative assay for the study of *P* regulation.

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 the rosy$^+$ gene as a marker for transformation (5). *P*[*lw*B] (H. Bellen, personal communication) is a similar *P–lacZ* construction except that the marker for transformation is the mini-white gene (10) and that it contains bacterial plasmid sequences allowing rapid cloning by plasmid rescue. *P*[*l*Δ*r*B] is similar to *P*[*lw*B] (9) but has rosy$^+$ as a transformation marker and contains in addition the *Adh*$^+$ gene.

Other Transgenes. The HZ50, DHS, and HZ elements are described in the text.

***Drosophila* Stocks.** Canton (11) and Gruta (12) are typical M strains containing no *P* element sequences. The genetic homogeneity of our Gruta stock was tested 3 years ago by C. Petit (personal communication), using the method described in ref. 13. She found that this stock can be considered as genetically homogeneous. Harwich (11) and π_2 (14) are standard P reference strains. *C(1)DX, y w f/w v l(1)44/Y*, is an M strain and *C(1)DX, y w f/w v l(1)44/Y*; Harwich is derived from it by contamination of the X chromosomes by *P* elements and replacement of the autosomes by Harwich autosomes (15). These two stocks will be hereafter called *C(1)DX*(M) and *C(1)DX*(P), respectively. w^{1118} is a line with a null white allele (16). HS2-20, HS2-25, and HS1 are P lines that were derived from the Gruta M stock by germ-line transformation with *P* DNA (12). Stocks were started from mixed populations of several transformants, diminishing the risk of clonal variations between them and the Gruta stock. These stocks are, therefore, essentially isogenic to the Gruta strain and between themselves. *M-5* Birmingham is an M′ stock. It contains numerous deleted *P* elements but has an M cytotype (17). ry^{506} *P*[*ry*$^+$Δ2-3](99B), hereafter designated Δ2-3(99B), contains an essentially immobile *P* element that produces a high level of transposase in the soma and in the

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germ line (18). R20a, K20a, R30b, L44, Q39b, A1421F1, and *P*[*lw*B]56B are lines harboring an insertion of a *P–lacZ* element (see Table 1 for description).

For complete description of marker genes and balancer chromosomes see Lindsley and Grell (19).

Mobilization of *P*[*lw*B] to Autosomal Sites. Autosomal insertions of *P*[*lw*B] were generated by the following scheme (7): females harboring an X chromosome-linked insertion of *P*[*lw*B] were crossed to ry^{506} *Sb P*[ry^{+}Δ2-3](99B)/*TM6 Ubx* males. Sb F_1 males were individually crossed to w^{1118} virgin females. Jumps to autosomal locations result in exceptional $white^{+}$ F_2 males. Independent $w^{+}Sb^{+}$ F_2 males were mated individually with w^{1118} females to establish lines.

β-Galactosidase Localization. Larvae and adults were fixed in 4% (vol/vol) glutaraldehyde/1 mM $MgCl_2$ in phosphate-buffered saline (PBS, pH 7.5) for 15 min, washed in PBS, then submerged in 0.2% 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-Gal)/4 mM $K_4Fe(CN)_6$/4 mM $K_3Fe(CN)_6$/1 mM $MgCl_2$ in PBS (pH 7.5), and incubated overnight at 37°C (20). They were then mounted in 90% (vol/vol) glycerol in PBS. Localization of β-galactosidase in embryos was performed as described in ref. 20.

Quantitative Measurement of β-Galactosidase Activity. The procedure described in ref. 21 was applied to homogenates of ≈200 embryos or of three individuals of the third instar larval, pupal, or adult stages. Results are given as nmol per min per mg of protein.

RNA Preparation and Analysis. Crosses were performed at 25°C and third instar larvae were collected, frozen in liquid nitrogen, and stored at −80°C until extraction. Extraction, electrophoresis, transfer, and hybridization were performed as described in ref. 22. Probes were synthesized from clones carried in BlueScript vectors with T3 RNA polymerase (Stratagene) for the *lacZ* probe and T7 RNA polymerase (Promega Biotec) for the ribosomal protein 49 probe (rp49) (23), as recommended by the manufacturer.

RESULTS

Generation of Random Insertions of a *P–lacZ* Fusion Transgene. An X chromosome-linked insertion of the *P–lacZ* fusion gene *P*[*lw*B] (H. Bellen, personal communication) was mobilized using Δ2-3(99B) as a transposase source. Fifteen lines named D1 to D15, each containing one autosomal insertion of *P*[*lw*B], were established in an M cytotype background. They were examined for their pattern of β-galactosidase activity at the larval stage, and all but one strain showed a tissue-specific activity besides the endogenous β-galactosidase staining (Table 1). Such a high frequency of specific staining was also observed in studies performed at the embryonic stage (5–9).

***P–lacZ* Expression Is Repressed in a P Background.** To study P cytotype effects on the *P* element promoter, males containing the above mentioned autosomal insertions of *P*[*lw*B] were crossed with *C(1)DX*(M) and *C(1)DX*(P) females. F_1 larvae from both crosses (i.e., in M or in P background) were stained for β-galactosidase activity. Experiments were performed under the same conditions (medium, reagents, and incubation time), allowing a comparison of staining pattern and intensity for each *P–lacZ* insertion in M or P cytotype. For all the 14 strains displaying a *P–lacZ*-dependent β-galactosidase activity, the staining intensity was lower in the P cytotype than in the M cytotype (see examples in Fig. 1). This P repression occurs in all the organs displaying a specific expression of β-galactosidase.

To confirm these results, seven other *P–lacZ* lines from various origins were tested according to the same scheme (Table 1). In all cases the β-galactosidase staining was less intense in the P cytotype as compared to the M cytotype.

Table 1. Larval β-galactosidase staining pattern of *P–lacZ* insertion strains

Line	Chromosomal location	β-Galactosidase staining pattern
		Experiment A
D1[a]	II	Br(+), Mt(++)
D2[a]	III	Br(+), Mt(+)
D3[a]	A	Br(++), Mt(++), Sg(+)
D4[a]	II	Br(++), Cu(−), Fb(+), Sg(+)
D5[a]	III	Mt(+), Cu(++), Fb(+)
D6[a]	III	Br(+)
D7[a]	A	Br(−)
D8[a]	A	No staining
D9[a]	A	Br(+), Mi(++), Mt(+), P
D10[a]	A	Mt(+), Cu(+), Fb(+)
D11[a]	A	Br(+), Mt(+), Sg(+)
D12[a]	A	Br(+), Hi(+), Mt(+)
D13[a]	A	Br(+), Mt(+)
D14[a]	A	Br(−), Mt(+)
D15[a]	A	Mt(+)
		Experiment B
K20a[b]	A	Br(++), Hi(+), Mt(+)
L44[b]	A	Br(+), Fb(++), Hi(+), Mi(+)
Q39B[b]	A	Br(+), Id(+), Mt(+)
R20a[b]	III(100CD)	All except parts of Id
R30b[b]	A	Br(++), Cu(++), Id(++), Mi(++)
A1421F1[c]	X	Fb(+)
P[*lw*B]56B[a]	X	Br(++), Cu(++), Fb(++), Mi(+), Pr(+)

Origin of the tested lines: D1 to D15, the present study; K20a, L44, Q39B, R20a, and R30b, L. Fasano and S. Kerridge (personal communication); A1421F1 and *P*[*lw*B]56B, H. Bellen (personal communication). *P–lacZ* element harbored by the lines are indicated by the following letters as superscripts. *a*, *P*[*lw*B]; *b*, *P*[*lac*,ry^{+}]A; *c*, *P*[*l*Δ*r*B]. Chromosomal locations: A, autosome (II, second; III, third); X, X chromosome. β-Galactosidase staining patterns: Br, brain; Cu, cuticule; Fb, fat body; Hi, hindintestine; Id, imaginal discs; Mi, midintestine; Mt, malpighian tubules; Pr, proventriculus and oesophagus; Sg, salivary glands; −, +, and ++, low, medium, and high level of *lacZ* expression, respectively.

R20a is a strain exhibiting a ubiquitous *lacZ* expression (S. Kerridge, personal communication), due to the insertion of a single *P–lacZ* element in III(100CD). This allows us to confirm that the P repression of the *P–lacZ* expression is effective in all the larval organs (Fig. 1 *c* and *d*).

Six among 20 of these strains (D5, D6, D9, F70, L44, and R20a) express specific *lacZ* activity in the digestive or reproductive apparatus of the adult (easiest organs to observe). They all show lower *lacZ* activity when the *P–lacZ* gene is in a P background as compared to an M background.

P–lacZ expression is also reduced in P cytotype embryos as compared to M cytotype embryos harboring the R20a insertion (Fig. 1*e*). However, the small size of embryonic cells allow us to detect only a global difference and we cannot be sure that all the tissues display equal repression.

To quantify the P cytotype repression, measurements of the β-galactosidase activity were undertaken with R20a, L44, and D9, lines that were chosen for their relatively high levels of β-galactosidase expression. Males of these strains were crossed with females from two M strains [*C(1)DX*(M) and Canton] and from two P strains [*C(1)DX*(P) and Harwich]. The β-galactosidase activity of the F_1 larvae was measured (Table 2). As expected from larval stainings, the R20a and L44 insertions elicit a lower level of β-galactosidase activity for the progeny of crosses involving P strains as compared to those involving M strains. The D9 insertion line gives only a slightly higher level in an M background than in a P background. However, the difference is not significant, although we can observe a clear-cut repression on larval stainings (Fig. 1*b*) and on the RNA level (see below and Fig. 3). This is due

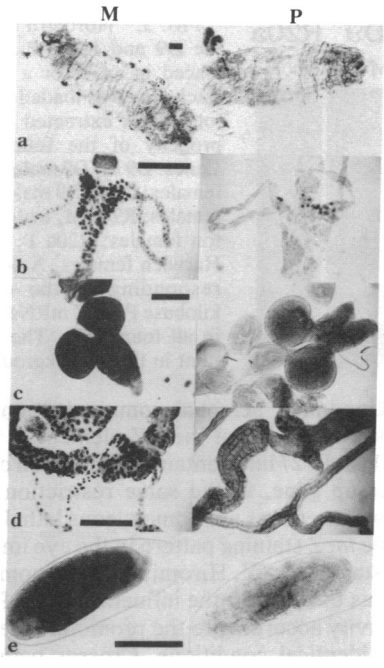

FIG. 1. β-Galactosidase staining of various *P–lacZ* insertion lines in M or P cytotype. Each pair displays the staining of a *P–lacZ* insertion placed in an M background (*Left*) or a P background (*Right*). Staining was performed under identical conditions (medium and coloration time) to allow a visual comparison. The β-galactosidase enzyme is restricted to the nuclei since *P–lacZ* constructions carry the *P* element first exon (5). (Bars = 250 μm.) (*a*) The D5 line displays a segmental pattern of the enzyme in the cuticule. Nearly no expression is detected in P background. (*b*) In the M background, the D9 line shows a *P–lacZ*-dependent staining in the proventriculus, in the anterior part of the midintestine, and in the gastric caeca. There is a gradient of staining intensity from the anterior part of the midintestine toward its posterior regions. In the P background, the only nuclei still stained are those located in the most anterior part of midintestine, which are the more intensely stained in the M background. (*c* and *d*) The R20A *P–lacZ* insertion expresses the enzyme in all tissues except parts of the imaginal discs at the larval stage. P repression apparently occurs in all tissues. The figure displays the P cytotype repression in the brain and imaginal disc (*c*) or in the malpighian tubules and the intestine (*d*). (*e*) A global P background repression in R20A embryos (stage 12) is shown.

to the high level of endogenous β-galactosidase activity (Table 2). We consequently used the R20A line for the following experiments.

The two reciprocal crosses between the Harwich (P) strain and the R20a line were performed: a similar repression of *P–lacZ* activity is found in F_1 larvae from both crosses (Table 3). The same was true for all reciprocal crosses performed. These results indicate the absence of maternal inheritance of the P repression exerted on *P–lacZ* expression. Thereafter, the measurements of *lacZ* activity from individuals from reciprocal crosses were pooled.

The crosses between *C(1)DX*(M) and *C(1)DX*(P) females and the R20a insertion males (carried out at 25°C) were repeated at a lower temperature (18°C). No effect of temperature on the F_1 larval β-galactosidase activity was noted whatever the cytotype (Table 3).

HS1, HS2-20, and HS2-25 are P strains derived from the Gruta M strain by transformation with complete *P* element DNA (12). They are, therefore, essentially isogenic to it. R20a individuals were crossed to individuals from these strains, and F_1 larvae were analyzed for their *lacZ* activity. The clear differences (Table 3) between the P strains and the M Gruta strain proved that the effect observed is due only to the presence of *P* elements on the chromosomes and not to an effect of differences in the genetic background.

P[R1](89a) and *P*[Sal](89D) are two lines containing a single *in vitro*-modified *P* element harboring a frameshift mutation in the third or fourth exon, respectively (24). The element present in *P*[Sal](89D) was shown to have repressor-producing capacities, unlike the *P*[R1](89A) element (4). These two lines were assayed for their ability to repress the R20a *P–lacZ* expression. Table 3 indicates that the *P*[Sal]-(89D) element but not the *P*[R1](89A) element is able to repress the *lacZ* activity like the P cytotype but with less intensity. Again, no reciprocal cross effect in the *P–lacZ* repression by *P*[Sal](89D) was detected (data not shown).

Finally the *M-5*-Birmingham M′ strain, which contains numerous internally deleted *P* elements, does not repress *lacZ* activity (Table 3).

P Repression Occurs at All Developmental Stages. The R20a *P–lacZ* insertion, being expressed at all stages, was used to study the P repression effect throughout development.

R20a males were mated to either *C(1)DX*(M) or *C(1)DX*(P) females at 20°C. F_1 synchronized embryos were collected. Measurements of their *lacZ* activity are displayed in Fig. 2, with measurements performed at larval, pupal, and adult stages. It appears clearly that *P–lacZ* expression is repressed by the P cytotype from its onset in embryos until the adult stage. The endogenous β-galactosidase activity was measured throughout development. Its low level relative to *P–lacZ* activity indicates that it could not account for the differences observed according to the cytotype.

Somatically Expressed Transposase Exerts No Repression Effect. To test the ability of the 87-kDa transposase protein to repress the *P–lacZ* expression, crosses involving the Δ2-3(99B) line, which produces high rates of transposase somatically (18), and various *P–lacZ* strains were performed. Observations were done at the larval stage.

Table 2. P repression of *P–lacZ* expression

P–lacZ insertion	β-Galactosidase activity, nmol per min per mg of protein			
	M background		P background	
	C(1)DX(M)	Canton	*C(1)DX*(P)	Harwich
R20A	4.8 (0.7)*	4.4 (0.5)	2.1 (0.5)*	1.6 (0.2)
L44	2.6 (0.1)	2.4 (0.1)	1.1 (0.1)	1.1 (0.1)
D9	1.6 (0.3)	1.3 (0.3)	0.8 (0.4)	1.0 (0.1)
Control	1.1 (0.3)	0.4 (0.2)	1.0 (0.2)	0.4 (0.1)

Results are the mean with the standard deviation in parentheses. Larvae were raised at 25°C. Three measurements were done for each β-galactosidase activity determination. Exceptions where six replicates were done are indicated by an *. Control were made on white sibs of the D9 strain, devoid of *P–lacZ* insertion.

Table 3. Test of R20A *P–lacZ* expression in various backgrounds

Test	Condition tested	β-Galactosidase activity
A	Harwich (paternal origin)	1.4 (0.2)
	Harwich (maternal origin)	1.6 (0.3)
B	*C(1)DX*(M) at 18°C	4.7 (0.4)
	C(1)DX(P) at 18°C	1.7 (0.2)
C	Gruta (M)	5.1 (0.7)*
	HS1 (P)	2.7 (0.4)
	HS2-20 (P)	1.8 (0.1)
	HS2-25 (P)	1.5 (0.5)
D	*P*[R1](86A)	4.2 (0.6)*
	P[Sal](89D)	2.6 (0.2)*
E	*M-5* Birmingham	4.9 (1.1)
F	Δ2-3(99B)	4.4 (0.6)*

Larvae were developed at 25°C except in test B. See Table 2.

With the L44 strain, which shows a specific *lacZ* expression in the fat body, a mosaic pattern of staining was observed due to somatic excisions of the *P–lacZ* insertion. But in all cases, cells displaying a staining (i.e., where no excision occurred) are colored with the same intensity as in an M cytotype. Four other strains tested in the same conditions showed also an absence of repression in cells where no excision had occurred.

Finally, it appears that the ubiquitously staining R20a strain showed very few, if any, somatic excisions with Δ2-3(99B). A quantitative measure of the β-galactosidase activity on F_1 larvae was thus undertaken, revealing no repression of the *lacZ* activity by Δ2-3(99B) (Table 3).

P Repression Reduces the Amount of *P–lacZ* Transcript. The effect of P repression on the transcription of two *P–lacZ* transgenes (D9 and R20a) was studied. Northern blots of total RNAs from third instar larvae were probed with a *lacZ*-specific probe (F. Schweisguth, personal communication) and with an rp49 probe (23) as a control of the amount of RNA loaded. The results (Fig. 3) show that the amount of the *P–lacZ* specific mRNA is strikingly lower in a P background than in an M background. Both insertions tested display this effect, although D9 is expressed less than R20a. However, the P repression does not lead to a total disappearance of the *P–lacZ* transcript: a faint band of the normal sized *P–lacZ* RNA is detected in a P background.

P Cytotype Effect on Other Promoters. As a control, lines carrying a *P* element vector containing the *lacZ* gene as a reporter of *Drosophila* gene promoters were assayed for the P repression. HZ50 is a *P* element vector containing an *hsp70–lacZ* fusion gene (25). The sequence from position −50 to the cap site of the *hsp70* gene is abutted to the *E. coli* β-galactosidase gene. The $rosy^+$ marker is located 5′ of the fusion gene. The insertion line tested expresses β-galactosidase in imaginal discs, brain, malpighian tubules, and salivary glands. DHS is a similar transgene except that the 1238-base-pair from the *P1* gene promoter has been inserted into the 5′ region of the *hsp70–lacZ* fusion gene (M. Laval, and J. Deutsch, personal communication). This results in a strong *lacZ* expression in fat body at the end of the third larval stage. The experimental scheme described above was used to generate and stain larvae from M and P cytotype harboring one of these two transgenes. In no case was a visual difference detected between the staining intensities in M or P backgrounds.

The HZ element is an "enhancer-trap" *P* element using a *hsp70–lacZ* fusion instead of the *P–lacZ* fusion (Y. Hiromi and C. S. Goodman, personal communication). The H162 line harbors an insertion of the HZ element in the seven-up gene (26). The AE127 line contains an insertion of *P*[*lw*B] also in the seven-up gene, in the same restriction fragment as H162; the two insertions are homozygous lethal and have the same specific *lacZ* staining pattern in the eye imaginal disc of late third instar larvae (Y. Hiromi, personal communication). This allows us to compare the influence of the P cytotype on the *lacZ* activity according to the promoter fused to the *lacZ* gene under identical conditions. Crosses between females from various M and P strains and AE127 or H162 males were performed. For convenience, *lacZ* activities were measured on adult heads (Table 4). The *P–lacZ* insertion (AE127) showed the typical P cytotype repression. However, the *hsp–lacZ* gene was not repressed in the P background, but it was in fact activated when placed in the P cytotype. The use of the isogenic strains, Gruta (M) and HS2-25 (P), proved that this increase was due to the presence of *P* elements and not to a difference in the genetic background.

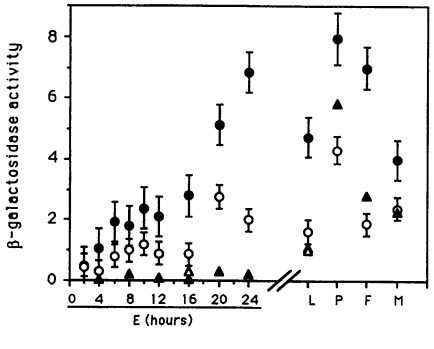

FIG. 2. R20a *P–lacZ* developmental expression in an M or a P background. The R20a *P–lacZ* expression levels in an M (solid circles) or a P (open circles) background are displayed with their confidence interval ($P < 0.05$). The endogenous activities of the *C(1)DX*(M) (solid triangles) or *C(1)DX*(P) (open triangles) strains are shown as controls. E, embryonic stages; L, third instar larvae; P, pupae; F, adult female; M, adult male. Embryos, larvae, and pupae were aged at 20°C; adults (both sexes) were aged at 25°C. Enzyme activity is expressed as nmol per min per mg of protein.

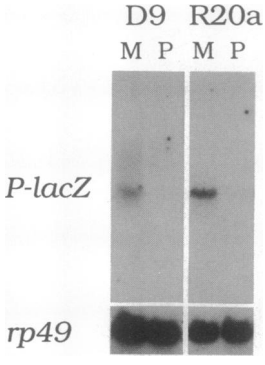

FIG. 3. Northern blot analysis of the D9 and R20a *P–lacZ* insertions placed in an M or a P background. Each lane was loaded with ≈10 μg of total RNA extracted from F_1 larvae progeny of the following crosses. Lanes: D9 M, D9 males × *C(1)DX*(M) females; D9 P, D9 males × *C(1)DX*(P) females; R20a M, males R20a × Canton females; R20a P, R20a males × Harwich females. A single band corresponding to the expected 3.9-kilobase P–lacZ mRNA was observed in all four lanes. The signal is very faint in the P background.

DISCUSSION

We have shown that the expression of *P–lacZ* fusion genes is repressed by *P* trans-acting regulatory products. Although the *P* promoter activity appears to depend greatly on the nature of sequences adjacent to the integration site (5), we were unable to detect any position effect for the sensitivity to P repression of the various *P–lacZ* insertions. This agrees with the observation that all the P strains and all the transposase-producing elements are sensitive to P cytotype repression.

The repression was also observed in all tissues or cell types in which the *P–lacZ* expression was detected. It is difficult, using only visual means, to assess whether the degree of repression is the same for all the insertions tested and for all the tissues observed. The level of expression varies greatly among the different lines and localizations. Repression can be virtually complete (e.g., line D5, Fig. 1*a*) or partial, with

Table 4. P repression is specific to the *P* promoter

	β-Galactosidase activity	
	AE127 (*P–lacZ*)	H162 (*hsp–lacZ*)
M background		
Canton	10.5 (1.0)	271.0 (76.9)
C(1)DX(M)	8.3 (1.6)	285.1 (27.3)
Gruta	NT	260.5 (39.3)
P background		
Harwich	4.1 (0.3)	459.1 (114.5)
C(1)DX(P)	3.6 (0.5)	497.5 (60.9)
HS2 25	NT	461.5 (101.3)

Results are given in nmol per min per mg of protein, with confidence interval in parentheses (t = 5%). Measurements of β-galactosidase activity were done on extracts from heads of adults raised at 25°C. NT, not tested.

some specific *lacZ* expression still detected in the P cytotype. This variation seems to depend on the level of expression in M cytotype, as it can be seen on line D9: in the P cytotype only a few nuclei are stained, corresponding to the most pigmented in the M cytotype (Fig. 1*b*). In the cases where quantitative measurements of β-galactosidase activity were performed, the P background level is close to the endogenous β-galactosidase activity.

Thus, these results lead to two conclusions: (*i*) *P* regulatory products are expressed in all cell types and tissues. (*ii*) *P* expression is sensitive to P repression in all tissues or cell types or, at least, the *P* promoter is accessible to *P* regulatory products as soon as it is expressed.

The *P–lacZ* elements provide a useful quantitative assay for the efficiency of P cytotype determinants. The P strains or *P* elements that we have shown to display an ability to repress *P–lacZ* expression are essentially the same as described (4, 15), notably, the *P*[Sal](89D), which can only encode the 66-kDa protein and has been shown to have regulatory effects.

Whatever the paternal or maternal inheritance of the *P* elements, the level of the *lacZ* repression was the same at the larval stage. However, it should be noted that all our observations were made on somatic tissues and that no maternal inheritance of the P cytotype has ever been detected in somatic tissues (4, 15).

The transposase produced by Δ2-3(99B) was unable to repress *P–lacZ* activity, indicating that the functional 87-kDa transposase could not act *in vivo* as a regulator at the transcription level. A similar, but qualitative, result has already been described for the cytotype dependent mutation sn^w (4). Nevertheless, the possibility cannot be excluded that, at the stage where we look at the *P–lacZ* expression, the transposase encoded by Δ2-3(99B) either is not expressed or is expressed at levels too low to have an effect.

P repression acts by reducing the quantity of the specific *P–lacZ* RNA. This precludes the possibility that this repression results from translational or post-translational events.

Three mechanisms explaining the P repression of *P–lacZ* expression can thus be considered: (*i*) a direct inhibition of the *P* promoter transcription by *P* trans-acting products, (*ii*) a mechanism involving a long range cis-effect, like disruption of enhancer–promoter interactions or local modification of chromatin structure, and (*iii*) a post-transcriptional effect affecting the mRNA stability (the *P–lacZ* fusion gene retains the 5′ untranslated sequence and 372 base pairs of the coding region of the *P* element).

No repression by the P cytotype was observed with any of the *P* insertions in which the *lacZ* gene is not driven by the *P* promoter tested here. Nevertheless, we have shown that the *P* repressor(s) is able to repress the expression of an unrelated gene (white) in a *P*[white] transgene by a long-range cis-effect (15). However, this effect is strongly position-dependent and acts specifically in the eye, through the enhancement of $zeste^1$ repression (15). Thus it is unlikely that this kind of effect is responsible for the general P repression seen on all the *P–lacZ* insertions, whatever their site of insertion and the tissue in which they are expressed.

The 87-kDa *P* transposase has been shown to be a site-specific DNA-binding protein (27): it interacts *in vitro* with sequences internal to the *P* element ends. At the 5′ end, these sequences overlap sequences necessary for *in vitro* transcription from the *P* promoter. The 66-kDa *P* protein shares with the transposase its putative DNA-binding domain (3) and may thus have conserved a similar DNA binding specificity (27). This observation and our results argue strongly for a direct effect of *P* regulatory products on the transcription from the *P* promoter. Yet, post-transcriptional effects affecting the stability of P–lacZ mRNAs cannot be totally excluded. However, converse to this last hypothesis, direct repression of the *P* promoter may also explain the enhancement of *lacZ* activity found for the HZ element in the P cytotype. This construct still contains the *P* promoter. In an M background, its activity may thus interfere with the transcription from the *hsp* promoter (28, 29). Whereas in the P cytotype, repression of the *P* promoter, by preventing such transcriptional interference, would permit optimal transcription from the *hsp* promoter.

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