Trans-Silencing by *P* Elements Inserted in Subtelomeric Heterochromatin Involves the Drosophila Polycomb Group Gene, *Enhancer of zeste*

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ABSTRACT

Drosophila *P*-element transposition is regulated by a maternally inherited state known as P cytotype. An important aspect of P cytotype is transcriptional repression of the *P*-element promoter. P cytotype can also repress non-*P*-element promoters within *P*-element ends, suggesting that P cytotype repression might involve chromatin-based transcriptional silencing. To learn more about the role of chromatin in P cytotype repression, we have been studying the P strain *Lk-P*(1A). This strain contains two full-length *P* elements inserted in the heterochromatic telomere-associated sequences (*TAS* elements) at cytological location 1A. Mutations in the Polycomb group gene (Pc-G gene), *Enhancer of zeste* (*E(z)*), whose protein product binds at 1A, resulted in a loss of *Lk-P*(1A) cytotype control. *E(z)* mutations also affected the *trans*-silencing of heterologous promoters between *P*-element termini by *P*-element transgenes inserted in the *TAS* repeats. These data suggest that pairing interactions between *P* elements, resulting in exchange of chromatin structures, may be a mechanism for controlling the expression and activity of *P* elements.

P ELEMENTS are a group of mobile DNA elements found in *Drosophila melanogaster*. They transpose by a nonreplicative cut and paste mechanism that is controlled by a regulatory state known as P cytotype (for reviews see Engels 1983, 1989; Rio 1991). The existence of a state prohibitive for *P*-element transposition was initially recognized when reciprocal crosses were performed between strains that contain *P* elements (P strains) and those that lack *P* elements (M strains). If a P strain male is crossed to an M strain female, the *P* elements are mobilized in the germ line of the progeny, resulting in a series of abnormalities called hybrid dysgenesis. However, the progeny of a cross between an M strain male and a P strain female are normal, indicating that P strain females are able to repress *P*-element transposition in the germ line of their offspring. These reciprocal cross experiments led to the definition of a repressive state for *P*-element transposition, called P cytotype, and a permissive state for transposition, called M cytotype. Genetic experiments also indicated that P cytotype has both a maternal effect and is maternally inherited. Maternally derived cytoplasm is sufficient to confer the repressive state to offspring for one generation but maternal inheritance of chromosomal *P* elements is required for the maintenance of P cytotype through multiple generations (Engels 1983, 1989, 1996; Ronsseray *et al.* 1993).

Molecular characterization of P strains revealed the existence of two types of *P* elements: full-length 2.9-kb elements and internally deleted *P* elements. A typical P strain contains 40–50 *P* elements and only approximately one-third of these elements are full-length (O'Hare *et al.* 1992; O'Hare and Rubin 1983). Full-length *P* elements encode two polypeptides, an 87-kD transposase protein and a truncated 66-kD repressor protein (Rio *et al.* 1986). Transposase production, and hence transposition, occurs only in the germ line due to the restricted splicing of the *P*-element third intron (IVS3) to this tissue (Laski *et al.* 1986). Retention of IVS3 in the germ line and the soma results in the production of the 66-kD repressor protein (Misra and Rio 1990). Additional proteins that repress transposition *in vivo* are encoded by internally deleted elements, such as the *KP* element (Black *et al.* 1987; Rasmusson *et al.* 1993). The maternal component of P cytotype is thought to arise from a deposition of *P*-element repressor proteins into unfertilized oocytes, as observed for the P strains Harwich and π2 (Misra and Rio 1990; and S. E. Roche and D. C. Rio, unpublished data). The zygotic component of P cytotype could be due to a requirement for continuous repressor production by chromosomal *P* elements inherited by subsequent generations.

An important component of P cytotype is transcriptional repression of the *P*-element promoter. Both the 66-kD and KP repressor proteins bind to a site within the *P*-element termini that overlaps the *P*-element promoter TATA element and the transposase binding site (Kaufman *et al.* 1989; and C. C. Lee and D. C. Rio, unpublished data), suggesting that these proteins might affect *P*-element transcription. Indeed, transcriptional repression of *P-lacZ* enhancer trap elements in the germ line and the soma occurs in a P cytotype-dependent manner

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(Lemaitre and Coen 1991; Lemaitre *et al.* 1993). In addition, the transposase protein inhibits the binding of TFIID to the *P*-element promoter TATA element, which results in transcriptional repression of the *P*-element promoter *in vitro* (Kaufman and Rio 1991). Other mechanisms of P cytotype regulation may involve antisense *P* element RNA (Simmons *et al.* 1996), the formation of inactive transposase-repressor protein heteromultimers, or a competition between transposase and repressor proteins for binding to their common site at the *P*-element termini (Rio 1990).

Finally, a role for chromatin structure in P cytotype transcriptional repression was proposed because heterologous promoters contained within *P*-element ends are repressed by P cytotype (Roche *et al.* 1995). For example, germ-line-expressed *hsp83* or *vasa*-IVS3-β-*geo* reporter transgenes are transcriptionally repressed in a P cytotype-dependent manner. Neither the *hsp83* nor *vasa* promoter contains binding sites for *P*-element protein products, suggesting that P cytotype transcriptional repression may occur through a chromatin-based transcriptional silencing mechanism. Repression of a *P[white]* transgene by *zeste*^{1} is enhanced in a *P*-element background (Coen 1990) and may be explained by an influence of *P*-element products on chromatin organization. Alterations of chromatin structure might also be responsible for the suppression of the phenotype of cytotype-dependent *vestigial* (*vg*) alleles in the presence of P cytotype (Williams *et al.* 1988). Therefore, P cytotype transcriptional repression may not occur solely by a simple repressor-operator interaction.

The ability of a strain containing *P* elements to exhibit P cytotype is strongly determined by the genomic position of the repressor-producing elements (Robertson and Engels 1989; Misra and Rio 1990; Misra *et al.* 1993). A study in which 17 inbred *P*-element-containing fly lines were examined for P cytotype over 100 generations of inbreeding, resulted in the identification of only three lines that could repress hybrid dysgenesis (Biemont *et al.* 1990). All three lines contained at least one complete *P* element that was located at cytological position 1A, at the tip of the *X* chromosome. Other studies of the distribution of *P* elements in natural Drosophila populations (Ajioka and Eanes 1989), and in local transposition experiments using a minichromosome (Karpen and Spradling 1992), demonstrated that 1A is a hotspot for *P*-element insertion. By outcrossing and recombination, a strain was created from one of the inbred *P* lines that contained only two *P* elements, both of which were located at cytological position 1A (Ronsseray *et al.* 1991). This strain, *Lk-P*(1A), completely represses transposition in the germ line and does not have a strong ability to induce hybrid dysgenesis even though both elements are complete.

Here, we report that the two *P* elements of the *Lk-P*(1A) P strain are inserted into subtelomeric heterochromatic repeated sequences, known as *TAS* repeats (see also Ronsseray *et al.* 1996). To investigate the influence of host factors known to associate with the 1A location on *Lk-P*(1A) P cytotype, we tested the effects of mutations in the Polycomb group (Pc-G) genes. The Pc-G genes are known to play a role in chromatin-based transcriptional repression of homeotic genes, such as *Ubx*, in Drosophila (Paro 1993; Pirrotta 1997). Several Pc-G protein products bind to cytological location 1A, as demonstrated by immunostainings of larval polytene chromosomes (Rastelli *et al.* 1993; Carrington and Jones 1996). We show that mutations in the Pc-G gene, *E(z)*, abolish repression of *P*-element transposition, and transcriptional repression of an *hsp83*-IVS3-β-*geo* reporter transgene by the *Lk-P*(1A) strain. Mutations in the gene that encodes Heterochromatin protein 1, *Su(var)205* (James and Elgin 1986; Eissenberg *et al.* 1992), also abolish *Lk-P*(1A) P cytotype (Ronsseray *et al.* 1996). We found that the loss of P cytotype in the presence of mutations in *E(z)* was not due to effects on expression of the 1A *P* elements. However, *E(z)* is involved in the silencing of β-*geo* reporter transgenes by recombinant *P*-element transgenes inserted within the *TAS* repeats at 1A or the *TAS*-related sequences at 100F (*trans*-silencing). We propose that *trans*-silencing may involve pairing interactions between *P* elements at different cytological locations that allow for the spread of a repressive chromatin structure from the heterochromatic *TAS* repeats to the site of the euchromatic reporter transgene. E(Z) protein may mediate interactions between *P* elements or may be a component of the repressive chromatin structure.

MATERIALS AND METHODS

Drosophila strains: The *Lk-P*(1A) strain is described in Ronsseray *et al.* (1991). The different Pc-G mutants tested are described in Table 1. The β-*geo* reporter transgene line B54.4 contains an X-linked insertion of a *P[ry*$^{+}$*;hsp83*-IVS3-β-*geo]* transgene, as described in Roche *et al.* (1995). The transgene maps to cytological location 5A. The reporter transgene line D27.1 contains a third chromosome insertion of a *P[ry*$^{+}$*;vasa*-IVS3-β-*geo]* reporter transgene (Roche *et al.* 1995). The standard P strain Harwich is described in Ronsseray (1986). All balancer chromosomes used are described in Lindsley and Zimm (1992). The different Pc-G mutant alleles were crossed into the *Lk-P*(1A) background as follows: alleles that map to the third chromosome and that were balanced by *TM3, Ser* were crossed to the double balancer stock *FM6;TM3, ry*^{RK}. Male progeny of the genotype *FM6*;mutant/*TM3, ry*^{RK} were crossed to *P*(1A);*TM3*/*CxD* females, to generate *P*(1A)/*FM6*;mutant/*TM3* stocks. The *E(z)* alleles that are on chromosomes marked with *ebony* [*E(z)*^{61}, *E(z)*^{63}, *E(z)*^{son2} and *E(z)*^{son3}] were crossed to *P*(1A);+/*TM3* females and progeny with ebony bodies were selected. Pc-G alleles that map to the second chromosome were crossed to *Binsinscy;SM6*β females. *Binsinscy*;mutant/*SM6*β male progeny were crossed to *P*(1A);+/*CyO* females, to generate the *P*(1A)/*Binsinscy*;mutant/*CyO* stocks. The *P*(1A)/*FM6*;+/*TM3* and *P*(1A)/*Binsinscy*;+/*CyO* control females were created by crossing doubly balanced males to *P*(1A);+/*TM3* or *P*(1A);+/*CyO* females, respectively. Crosses to the P strain *Lk-P*(1A) were performed at 18° while all other

TABLE 1

List of Pc-G alleles tested

Mutation	Comment	Reference
$E(z)^{61}$	Temperature sensitive Loss of function [$E(z)^{S2}$]	(Jones and Gelbart 1990)
$E(z)^{63}$	Null [$E(z)^{S4}$]	(Jones and Gelbart 1990)
$E(z)^{28}$	Temperature sensitive Loss of function [pco^{ox736}]	(Shearn *et al.* 1978)
$E(z)^{32}$	Temperature sensitive Loss of function [$pco^{pco25hs}$]	(Shearn *et al.* 1978)
$E(z)^{60}$	Gain of function [$E(z)^{S1}$]	(Wu *et al.* 1989)
$E(z)^{son2}$	Gain of function	(Pelegri and Lehmann 1994)
$E(z)^{son3}$	Gain of function	(Pelegri and Lehmann 1994)
Pc^{16}	Loss of function	(Struhl 1981; Kennison and Tamkun 1988)
esc^{2}	Null	(Struhl 1981)
$Su(z)2^{1}$	Gain of function	(Kalisch and Rasmuson 1974)
$Su(z)2^{5}$	Gain of function	(Wu *et al.* 1989)
$Su(z)2^{1.b7}$	Null	(Adler *et al.* 1989)
$Su(z)2D^{eos}$	Gain of function	(Wu and Howe 1995)
Psc^{1}	Gain of function	(Nusslein-Volhard 1984; Jurgens 1985)
Psc^{e22}	Gain of function	(Wu and Howe 1995)
Psc^{e25}	Loss of function	(Wu and Howe 1995)
Pcl^{D5}	Null	(Breen and Duncan 1986)

crosses were performed at 25° using standard fly culture medium.

The telomeric *P-lacZ*, *P[hsneo]* and *P[wA]* strains were obtained from the Drosophila stock center at Bloomington, Indiana. Most of the telomeric strains are referred to by their stock center names, except for AS1 and AS1079. AS refers to the source of these stocks, Allan Spradling (Carnegie Institution of Washington, Baltimore, MD), and the numbers correspond to their stock center numbers (P1 for AS1 and P1079 for AS1079). Descriptions of the telomeric strains can be found in Flybase. They are the same as those used by Ronsseray *et al.* (1998, accompanying article). The minichromosome lines are described in Karpen and Spradling (1992). The $E(z)^{32}$ allele was crossed into strains WG1103 and WG1152 as follows: *FM6;E(z)* / *TM3* males were mated to *P-lacZ;+* / *TM3, Ser* females. Serrated winged male offspring and serrated winged female offspring with semi-bar eyes were selected and mated to each other, to generate the *P-lacZ;E(z)* / *TM3, Ser* stocks. The *Dp8-152;*$E(z)^{32}$ / *TM3, Ser* stock was generated by crossing y^{-};$E(z)^{32}$ / *TM3* males to *Dp8-152*,y^{+};y^{-};+ / *TM3, Ser* females and selecting offspring that had wild-type body color and serrated wings. GR833 / $E(z)^{32}$ and GR833 / *TM3, Ser* females were created by crossing $E(z)^{32}$ / *TM3, Ser* males to GR833 females and selecting wild type (GR833 / $E(z)^{32}$) and serrated winged progeny ($E(z)^{32}$ / *TM3, Ser*).

Inverse PCR to clone the telomeric *P* elements in *Lk-P*(1A): Genomic DNA was isolated from 15 flies according to Misra and Rio (1990). The DNA was cleaved with *Nde*II and ligated for 4 hr at 15° with T4 DNA ligase. The ligation mix was PCR amplified with an annealing temperature of 55° and the following *P*-element primers: P_i1 5′-TCCAGTCACAGCTTTG CAGC-3′; P_i2 5′-GTGGGAGTACACAAACAGAG-3′. The PCR products were then reamplified at an annealing temperature of 51°, with a second set of *P*-element primers, P_o1 and P_o2, which contain restriction sites at the 5′ ends. However, the primer restriction sites were not used in cloning the PCR products. The sequences of the two primers are: P_o1 5′-CCG CTGCAGAAGTGTATACTTCGGTAAGC-3′; P_o2 5′-CCGCTC GAGAAATGCGTCGTTTAGAGCAG-3′. PCR products were analyzed by agarose gel electrophoresis. The two amplification products were gel isolated using NA45 paper (Maniatis *et al.* 1982) and treated with the Klenow fragment of DNA polymerase I and T4 polynucleotide kinase (Boehringer Mannheim, Indianapolis). The DNA fragments were subcloned into pBSIIKS(+) (Stratagene, La Jolla, CA) that was cleaved with *Eco*RV and treated with calf intestine alkaline phosphatase. Clones were screened by colony hybridization (Ausubel *et al.* 1987). DNA sequences flanking the *P* elements were obtained by chain termination sequencing with Sequenase 2.0 (United States Biochemical, Cleveland). The pBSIIKS(+) reverse primer and primers complementary to the *TAS* repeats were used. The flanking sequence obtained was aligned to the *TAS* repeat sequence (Karpen and Spradling 1992) by the Genetics Computer Group program Gap.

The distance between the *Lk-P*(1A) *P* elements was determined by DNA blot hybridization (Ausubel *et al.* 1987). *Lk-P*(1A) genomic DNA was cleaved with *Xho*I and *Spe*I or *Xho*I and *Xba*I and separated on a 0.8% agarose gel. The DNA was transferred to Hybond N membrane (Amersham, Arlington Heights, IL) and probed with a ^{32}P-labeled 5′ *P*-element probe, isolated from the plasmid pISP-2 / Km (Beall and Rio 1996), or a full-length *P*-element probe, isolated from the plasmid pUC18-*XbaP* (Misra and Rio 1990).

Histochemical *lacZ* assays: *lacZ* whole mount ovary stainings were performed on hand-dissected ovaries, as described (O'Kane and Gehring 1987), isolated from the progeny of the crosses outlined in Figure 2. The crosses were performed at 18°, 25° and 29° for temperature-sensitive *E(z)* alleles and

at 25° for all other Pc-G alleles assayed. The staining assay was repeated multiple times for each allele tested.

Quantitative *lacZ* assays were performed according to Ashburner (1989). Reporter males were crossed to *Lk-P*(1A) females, or to telomeric recombinant *P*-element-containing females, mutant or wild type for *E(z)*. Crosses assaying *Lk-P*(1A) repression were performed at 25° and 29°, while those with the recombinant *P* elements were performed at 25° only. Ten ovaries were dissected from 10 progeny females. One hundred microliters of assay buffer (50 mm potassium phosphate, 1 mm magnesium chloride pH 7.5) was added and the ovaries were homogenized with a plastic pestle (Kontes, Vineland, NJ). Extracts were spun for 10 min at 4° and 100 μl of supernatant was transferred to a fresh tube containing 900 μl of assay buffer. The extracts were vortexed for 30 sec to mix. Fifty microliters of ovary extract was added to 1.0 ml of 1 mm Chlorophenolred-β-D-galactopyranoside (CPRG; Boehringer Mannheim, Indianapolis) in assay buffer. The reactions were incubated at 37° and the optical absorbance at wavelength 574 nm was measured at 30 min, 1 hr, 1 hr 30 min and 2 hr. Both control and experimental samples were processed at the same time. The protein concentration of the ovary extracts was determined by the Bradford assay (Ausubel *et al.* 1987). The β-galactosidase activity of the ovary extracts was calculated as follows: OD readings between 0.1 and 0.6 were chosen for calculations and were divided by the amount of total protein in the extracts and by the amount of time the reactions were incubated at 37°. The β-galactosidase activity is expressed as OD units/gram of protein/min at 37° and was determined from the activities of at least three independent samples.

Gonadal sterility assays: *P*(1A)/*FM6* ;*E(z)* or +/*TM3* virgin females were crossed to Harwich males at 29°. Progeny females were collected and fattened with yeast for 2–3 days at 25°. Females were then squashed between two glass plates and scored against a dark background. A female was scored as fertile if she extruded at least one egg. At least 100 females were assayed for each cross. The percent fertility was calculated as (fertile females/total females scored) × 100. Each cross was repeated at least three times.

RNA isolation and RNase protection analysis: Ovaries were dissected from females fattened at 29° for 3 days and total ovary RNA was isolated as described (Ausubel *et al.* 1987; Sambrook *et al.* 1989). RNase protection analyses were performed as described in Adams *et al.* (1997), with modifications. One hundred micrograms of total ovary RNA was incubated with 50,000 cpm of single-stranded ^{32}P-labeled IVS3 probe and 50,000–100,000 cpm of ^{32}P-labeled tubulin probe. RNA samples were digested with RNases A and T1 for 1 hr at 15° instead of 1 hr at room temperature. The IVS3 probe plasmid, pGEM2-R/X, is described in Misra and Rio (1990) and the α-tubulin probe plasmid, containing a 400 nt *Xba*I-*Hin*dIII fragment of *D. melanogaster* genomic sequence, is described in Hedley and Maniatis (1991). Single-stranded RNA probes were synthesized using 800 Ci/mmol [^{32}P]UTP (NEN-DuPont, Boston, MA) and T7 RNA polymerase, according to Yisraeli and Melton (1989), and were gel purified (Maniatis *et al.* 1982). Protected fragments were quantitated using a Fuji BAS-1500 imager (Fuji Photo Film Co., Stamford, CT) and were corrected for loading by comparison with the tubulin control. The corrected values are shown in Table 2 and were used to calculate the total number of counts in each lane and the percentage of IVS3 RNA that was spliced.

TABLE 2

Corrected values

	$E(z)^{+}$	$E(z)^{61}$	$E(z)^{28}$	$E(z)^{32}$
Unspliced IVS3	165.36	395	25.2	25.2
Spliced IVS3	71.76	70	35.7	51.6
Total IVS3 RNA	237.12	465	60.9	76.8
Percentage of spliced IVS3	30.2	15.0	58.6	67.2

RNase protection experiments were performed on three independent RNA preparations.

RESULTS

The *Lk-P*(1A) *P* elements are inserted within subtelomeric heterochromatin: Molecular characterization of the telomere of the minichromosome, *Dp1187*, revealed that it consists of a series of 1.8-kb repeated sequences termed *TAS* repeats (Karpen and Spradling 1992). *TAS* repeats have been identified, cytologically, at the ends of both the natural *X* chromosome and the autosomes. *P-lacZ* elements that are inserted within the *TAS* repeats are subject to variegated expression, a characteristic of genes inserted into heterochromatic sequences (Karpen and Spradling 1992). Due to the strong genomic position dependence of P cytotype, we were interested in determining whether the *Lk-P*(1A) *P* elements are inserted within the *TAS* repeats.

The genomic DNA flanking the *Lk-P*(1A) *P* elements was isolated by inverse PCR, using nested *P*-element primers. Two inverse PCR products of 1.0 kb and 1.6 kb were subcloned and sequenced. Analysis of the resulting sequence indicated that both *P* elements are inserted within the *TAS* repeats (Figure 1A). The exact insertion site of each element within the repeats differs and the two *P* elements are arranged in an inverted orientation (Figure 1B). These data agree with the previous mapping of the *Lk-P*(1A) *P* elements to the *TAS* repeats (Ronsseray *et al.* 1996). However, these data did not address whether both *Lk-P*(1A) *P* elements are inserted in the same *TAS* repeat.

To determine whether both *P* elements are inserted within the same *TAS* repeat, the distance between the two elements was determined. *Lk-P*(1A) genomic DNA was cleaved with restriction enzymes that lack recognition sequences within the *TAS* repeats, but contain sites within the *P*-element sequence (either *Xho*I and *Spe*I or *Xho*I and *Xba*I) (Figure 1B). The cleaved DNA was analyzed by DNA blot hybridization using either full-length or 5′ *P*-element probes. The 5′ *P*-element probe detected a single fragment of ~6.5 kb while the full-length probe detected two additional larger products (data not shown). The fact that the 5′ *P*-element probe only detected a single fragment indicates that the two *Lk-P*(1A) *P* elements are arranged in an inverted orientation. Since *Xho*I cleaves 727 nt from the 5′ end of the *P* element, the data indicate that the actual distance between the two *Lk-P*(1A) *P* elements is ~5.0 kb (6.5 kb minus 1.45 kb of *P*-element DNA). If the *TAS* repeats on normal *X* chromosomes are similar in length to those

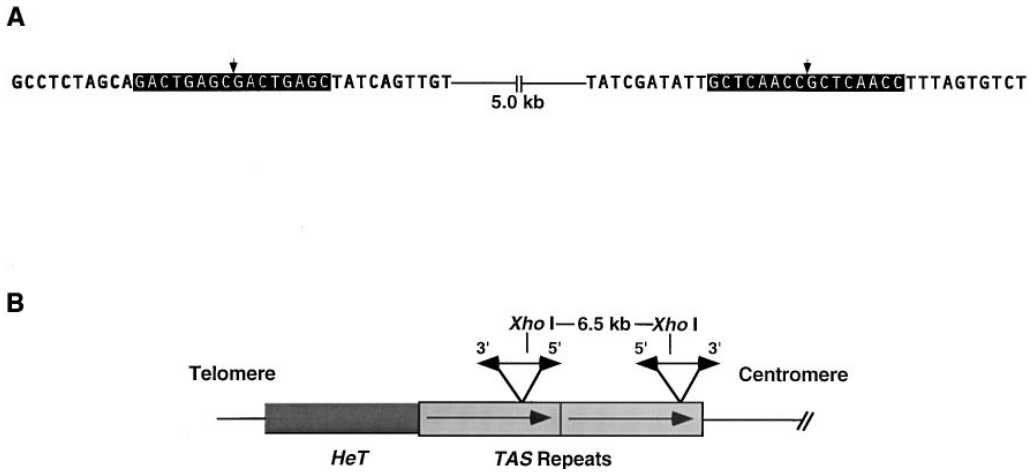

Figure 1.—The *Lk-P*(1A) *P* elements are inserted into the subtelomeric *TAS* repeats. (A) DNA sequence flanking the *Lk-P*(1A) *P* elements. The 8-bp target site duplications of *TAS* element DNA are indicated by the black boxes, while arrows indicate the exact insertion sites of the *P* elements. Genomic DNA (5.0 kb) lies between the two *P* elements. (B) Schematic drawing showing the location and orientation of the *Lk-P*(1A) *P* elements. The *TAS* repeats are depicted by the light gray boxes containing arrows. The dark gray box depicts *HeT* element DNA. *P* elements are drawn as double-headed arrows with the numbers above each arrow head referring to the orientation of the *P* elements. The position of the *Xho*I restriction sites used for determining the distance between and the relative orientation of the two *P* elements is indicated, as is the distance between these sites.

on *Dp1187*, then the two *P* elements of *Lk-P*(1A) must be in separate *TAS* repeats.

Mutations in the Polycomb group gene, *E(z)*, abolish *Lk-P*(1A) P cytotype: Because the *Lk-P*(1A) *P* elements are inserted within sequences that can silence gene expression, we wanted to test the effects of the 1A heterochromatin on the P cytotype of *Lk-P*(1A). We decided to study the effects of mutations in the Pc-G genes, whose products have been shown cytologically to bind at 1A (Rastelli *et al.* 1993; Carrington and Jones 1996). One role of the Pc-G genes is to maintain homeotic genes in a silent state possibly through the formation of a transcriptionally inactive chromatin state (Paro 1993; Pirrotta 1997).

Mutant alleles of several Pc-G genes were crossed into *Lk-P*(1A) (Table 1 and materials and methods). Two assays were used to test the effects of Pc-G gene mutations on *Lk-P*(1A) P cytotype. In the first assay, transcriptional repression of a germ-line-expressed *P[ry*$^{+}$*;hsp83*-IVS3-β-*geo]* reporter transgene was tested (Roche *et al.* 1995). This reporter transgene is transcriptionally repressed when the reporter transgene-containing strain, B54.4, is crossed to a P strain female, such as *Lk-P*(1A), but not when crossed to a P strain male. Transcriptional repression is assayed by histochemical staining of dissected ovaries isolated from the progeny. It has previously been shown that a reduction in β-galactosidase activity directly reflects a reduction in mRNA levels (Roche *et al.* 1995). *Lk-P*(1A) females heterozygous for mutations in the Pc-G genes were crossed to males containing the reporter transgene and the ovaries from the progeny were assayed for *lacZ* expression (Figure 2). If a mutation in a Pc-G gene affects the ability of *Lk-P*(1A) to repress transcription of the reporter transgene, then an increase in the expression of the reporter transgene should be observed. Only mutations in the Pc-G member, *E(z)*, resulted in a loss of transcriptional repression of the reporter transgene (Figure 2A). Mutations in *Psc*, *Pc*, *esc*, *Su(z)2*, *Su(z)2D*, and *Pcl* did not affect *Lk-P*(1A) P cytotype by this assay (Figure 2B and data not shown).

Seven different mutant alleles of *E(z)* were tested for transcriptional derepression of the reporter transgene (Table 1). When *Lk-P*(1A) females containing the $E(z)^{61}$, $E(z)^{28}$, $E(z)^{32}$, or $E(z)^{60}$ alleles were crossed to strain B54.4, the reporter transgene was transcriptionally derepressed in the ovaries of the progeny (Figure 2A, right panels; Table 3; and data not shown). As expected, control females containing two wild-type copies of *E(z)* repressed the reporter transgene when the progeny were assayed for *lacZ* expression (Figure 2A, left panels; Table 3). It is unknown why a low level of staining was observed in the control female ovaries when the *TM3*, and not the *Cy0*, balancer chromosome was present in the *Lk-P*(1A) background (Figure 2, A and B, left panels).

Transcriptional derepression of the reporter transgene by mutations in *E(z)* exhibited a maternal effect. Specifically, transcriptional derepression was observed in the progeny that inherited either the *TM3* balancer chromosome or the mutant *E(z)* allele. Reporter transgene derepression occurred even though the female parent contained a wild-type copy of *E(z)* on the *TM3* balancer chromosome, suggesting that the observed effect was either due to a decreased dosage of the wild-type *E(z)* product or to a dominant effect of the mutant *E(z)* allele. A null allele of *E(z)* had no effect on *Lk-P*(1A) P cytotype by this assay (Table 3, $E(z)^{63}$), indicating that the *E(z)* alleles that affected *Lk-P*(1A) P cyto-

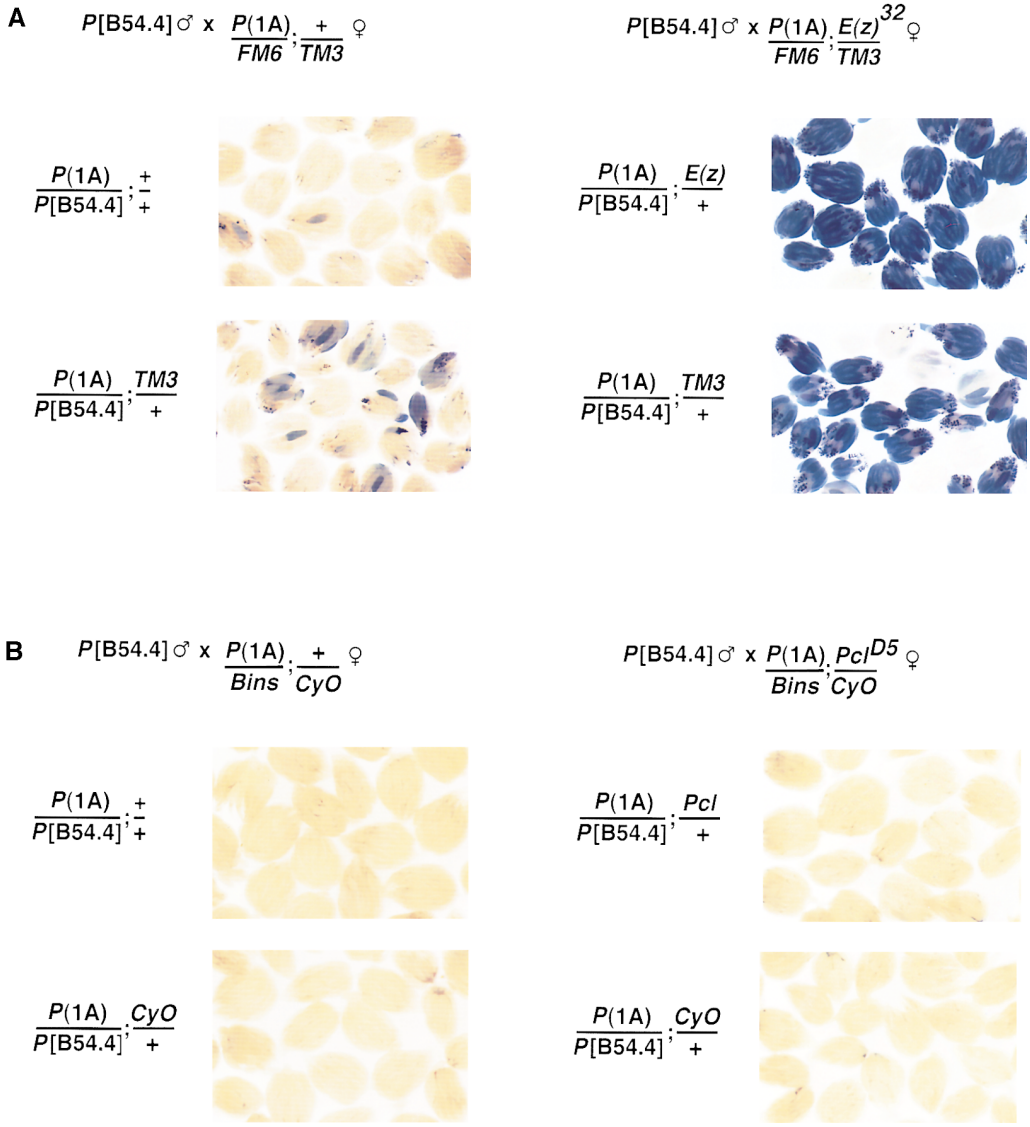

Figure 2.—Mutations in *E(z)* cause transcriptional derepression of an *hsp83*-IVS3-β-*geo* reporter transgene. Each panel shows *lacZ* histochemical staining of ovaries isolated from the progeny of the indicated crosses (see materials and methods for details). The genotype of the females from which the ovaries were isolated is indicated beside each panel. *P*(1A) refers to the *Lk-P*(1A) *P* elements. (A) The left set of panels shows repressed ovaries derived from the progeny of males containing the *hsp83*-IVS3-β-*geo* reporter transgene (B54.4) mated to control *P*(1A)/*FM6*;+/*TM3* females. The right set of panels shows the ovaries isolated from the progeny of reporter transgene males mated to *P*(1A)/*FM6*; $E(z)^{32}$/*TM3* females. The progeny were separated according to whether they inherited the *E(z)* mutant allele (*P*(1A)/B54.4;*E(z)*/+ progeny) or the *TM3* balancer chromosome (*P*(1A)/B54.4; *TM3*/+ progeny). The reporter transgene is derepressed in the ovaries of both sets of progeny. The data shown are from crosses performed at 29°. Control and experimental ovaries were stained side by side. Similar staining patterns were observed when *Lk-P*(1A) females containing $E(z)^{52}$, $E(z)^{28}$ or $E(z)^{60}$ alleles were tested. (B) Mutations in other Polycomb group genes do not cause derepression of the reporter transgene. The left set of panels shows repressed ovaries isolated from the progeny of a mating between reporter transgene males and *P*(1A)/*Bins*;+/*CyO* control females. The right set of panels shows ovaries isolated from the progeny of a cross between reporter transgene males and *P*(1A)/*Bins*; Pcl^{D5}/*CyO* females. The reporter transgene is still repressed in both classes of females. Similar staining patterns were observed when *Lk-P*(1A) females containing mutations in the other Polycomb group genes were tested.

type were acting in a dominant manner to derepress reporter transgene expression. Based on phenotypic analyses of homeotic transformations and effects on the *zeste-white* interaction, $E(z)^{60}$ was characterized as a gain-of-function allele (Wu *et al.* 1989), while $E(z)^{61}$, $E(z)^{28}$ and $E(z)^{32}$ were characterized as loss-of-function alleles (Shearn *et al.* 1978; Jones and Gelbart 1990; Phillips and Shearn 1990). Our data suggest that the latter three *E(z)* alleles may have both loss-of-function and gain-of-function characteristics. The observation that these alleles were acting in a dominant manner prompted us to test whether two other dominant alleles of *E(z)*, $E(z)^{son2}$ and $E(z)^{son3}$, affected repression of the reporter transgene. Neither of these alleles affected repression of the reporter by *Lk-P*(1A) (Table 3 and data not shown).

Since $E(z)^{61}$, $E(z)^{28}$ and $E(z)^{32}$ are temperature-sensitive

TABLE 3

β-Galactosidase activity of ovaries derepressed by *E(z)* mutations

♀ Parent[a]	Progeny[b]	β-Galactosidase activity[c] 29°	25°
P(1A); + / *TM3*	+ / +	30.9 ± 30.5	5.3 ± 1.1
	TM3 / +	11.3 ± 7.7	24.3 ± 16.6
P(1A);*E(z)*^{32} / *TM3*	*E(z)*^{32} / +	663.3 ± 239.7	363.3 ± 121.2
	TM3 / +	793.3 ± 362.3	265.6 ± 42.2
P(1A);*E(z)*^{28} / *TM3*	*E(z)*^{28} / +	621.0 ± 150.7	437.0 ± 73.6
	TM3 / +	373.3 ± 102.7	230.0 ± 85.2
P(1A);*E(z)*^{61} / *TM3*	*E(z)*^{61} / +	45.0 ± 46.0	9.0 ± 1.0
	TM3 / +	13.3 ± 8.8	30.7 ± 21.6
P(1A);*E(z)*^{63} / *TM3*	*E(z)*^{63} / +		13.1 ± 6.3
	TM3 / +		8.6 ± 2.7
P(1A);*E(z)*^{son2} / *TM3*	*E(z)*^{son2} / +		4.2 ± 1.0
	TM3 / +		0.7 ± 1.0
P(1A) / *FM6*;+ / *TM3*	+ / +	9.1 ± 5.1	2.7 ± 0.5
	TM3 / +	29.3 ± 25.2	24.3 ± 8.9
P(1A) / *FM6*;*E(z)*^{32} / *TM3*	*E(z)*^{32} / +	670.0 ± 108.0	386.0 ± 94.7
	TM3 / +	606.6 ± 54.4	375.0 ± 110.5
P(1A) / *FM6*;*R(z)*^{28} / *TM3*	*E(z)*^{28} / +	663.3 ± 144.3	546.3 ± 139.3
	TM3 / +	486.6 + 81.8	305.0 ± 180.6
P(1A) / *FM6*;*E(z)*^{61} / *TM3*	*E(z)*^{61} / +	616.7 ± 46.4	182.1 ± 50.8
	TM3 / +	403.3 ± 116.7	261.3 ± 61.9
P(1A) / *FM6*;*E(z)*^{63} / *TM3*	*E(z)*^{63} / +		8.7 ± 6.3
	TM3 / +		12.6 ± 11.1
P(1A) / *FM6*;*E(z)*^{son2} / *TM3*	*E(z)*^{son2} / +		6.43 ± 2.0
	TM3 / +		4.0 ± 1.0

[a] Genotype of female parent mated to the reporter line B54.4.
[b] Genotype of the progeny that were assayed for β-galactosidase activity. All progeny contained the *Lk-P*(1A) *P* elements and the β-*geo* reporter.
[c] β-Galactosidase activity ± SD is expressed as OD units/gram of total protein/minute. The data shown represent the average values obtained from three independent experiments, with the standard deviation indicated.

alleles, the assay was performed at several temperatures to examine the temperature-dependence of derepression of the reporter transgene. Loss of transcriptional repression of the reporter transgene occurred at both 25° and 29° for all three alleles (Table 3) and at 18° for *E(z)*^{28} and *E(z)*^{32} (data not shown). The *E(z)*^{28} and *E(z)*^{32} alleles displayed the strongest effect on *Lk-P*(1A) P cytotype. These alleles displayed a 12- to 200-fold derepression of the reporter transgene when the female parent contained one chromosomal copy of the 1A *P* elements (*P*(1A) / *FM6*;*E(z)* / *TM3*) and a 20- to 80-fold derepression in the presence of two chromosomal copies (*P*(1A); *E(z)* / *TM3*) (Table 3). The weaker *E(z)*^{61} allele only derepressed the reporter transgene when present in a background with one chromosomal copy of the 1A *P* elements (10- to 68-fold derepression, Table 3).

In a second assay, mutations in *E(z)* were tested for their effects on repression of *P*-element transposition by *Lk-P*(1A). The transposition assay is called a gonadal dysgenesis assay, since a P strain male is crossed to a tester female and the female progeny are examined for gonadal dysgenesis. If the tester female has M cytotype, the *P* elements introduced by the male sperm transpose at high frequencies in the germ line of the offspring, resulting in gonadal dysgenesis and sterility. However, if the tester female has P cytotype, *P*-element transposition is repressed and the offspring are fertile.

Lk-P(1A) P cytotype was abolished in the presence of the *E(z)*^{61}, *E(z)*^{28} and *E(z)*^{32} alleles, as observed by a high percentage of sterile offspring from crosses between P strain males and *Lk-P*(1A) females carrying these *E(z)* alleles (Figure 3). In addition, the *E(z)* alleles behaved in a dominant and maternal manner, with no evidence of a zygotic effect (data not shown). The null allele, *E(z)*^{63}, and the dominant alleles, *E(z)*^{son2} and *E(z)*^{son3}, did not affect repression of gonadal dysgenesis by *Lk-P*(1A), while *E(z)*^{61} only affected this repression when one chromosomal copy of the 1A *P* elements was present (Figure 3 and data not shown). These results parallel those from the *lacZ* activity assay. Taken together, the data from both assays demonstrate that mutations in *E(z)* affect the P cytotype of a strain containing *P*-element insertions within subtelomeric heterochromatin.

The *E(z)*-dependent loss of *Lk-P*(1A) P cytotype does not correlate with increased *P*-element transcription: We previously observed P cytotype-dependent transcrip-

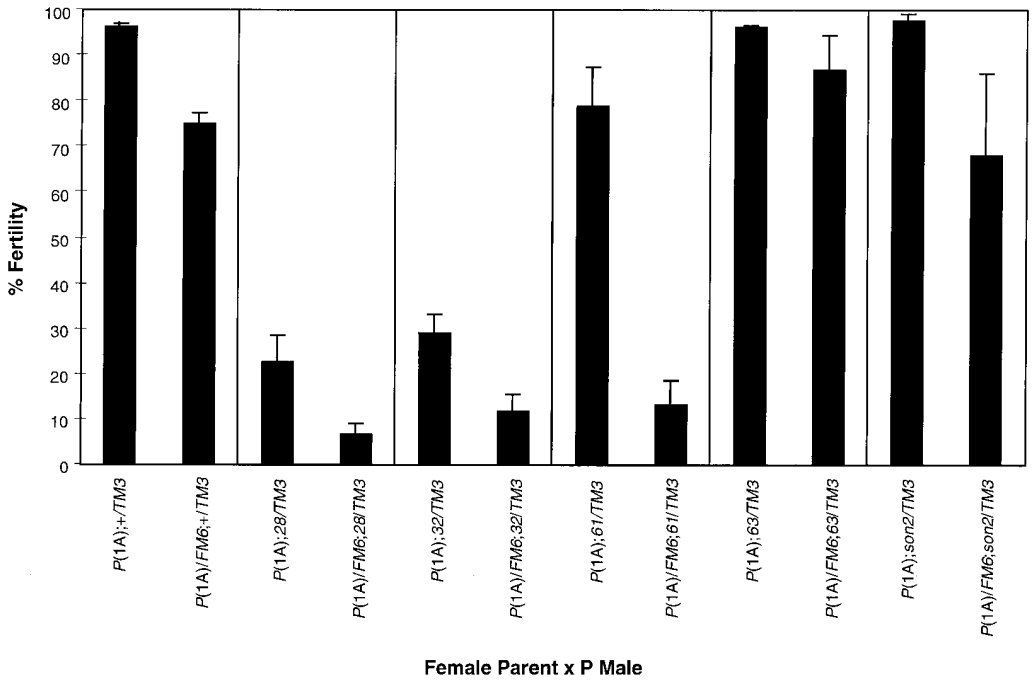

Figure 3.—Mutations in *E(z)* prevent repression of *P*-element transposition. Shown are the results of a gonadal dysgenesis (GD) assay (see materials and methods for details). The genotypes of the females that were crossed to Harwich males for the GD test are given on the horizontal axis. *E(z)* mutations are referred to by allele number. The vertical axis indicates the percent fertility among the female progeny from the crosses. Data were pooled from sibs that inherited the *E(z)* mutation or the *TM3* balancer chromosome, since there was no significant difference in fertility levels between the two classes (data not shown). Complete repression of transposition results in 90–100% fertility. Absence of repression results in 0–5% fertility. The vertical bars represent fertility data from three independent experiments with error bars indicating the standard deviation among experiments.

tional repression of the *hsp83* and *vasa* promoters, when contained within a *P* element (Roche *et al.* 1995), suggesting that P cytotype repression occurs through a chromatin-based transcriptional silencing. We proposed that the 66-kD repressor protein might interact with chromatin-associated proteins to silence the non-*P*-element promoters. The discovery that a gene involved in transcriptional silencing of homeotic genes is a key component of *Lk-P*(1A) P cytotype suggests that E(Z) protein might affect expression of the 1A *P*-element products by maintaining low levels of transcription, which is thought to promote repressor production (O'Hare *et al.* 1992; Lemaitre *et al.* 1993; Roche *et al.* 1995). Loss of wild-type E(Z) protein could result in increased transcription of the 1A *P* elements, reduced 66-kD repressor production, and therefore, increased expression of the reporter transgenes.

To analyze expression of the *Lk-P*(1A) *P* elements, RNase protection experiments were performed using total ovary RNA isolated from *Lk-P*(1A) females, mutant or wild type for *E(z)*, and an antisense RNA probe complementary to sequences surrounding the alternatively spliced *P*-element third intron, IVS3 (Figure 4A) (Misra and Rio 1990). A probe for α-tubulin mRNA was included in the reactions as a standard for the amount of target RNA in each sample (Hedley and Maniatis 1991). In the *P*(1A)/*FM6*;+/*TM3* control strain RNA, a 236 nt RNA fragment was protected by fully-spliced *P*-element mRNA and a 316 nt fragment was protected by incompletely spliced, IVS3-containing, *P*-element mRNA (Figure 4B, lane 1). Both products were detected since IVS3 is incompletely spliced in the germline (Roche *et al.* 1995). Both the spliced and unspliced products were also detected in RNA samples isolated from *Lk-P*(1A) females containing the $E(z)^{61}$ allele (Figure 4B, lane 2), while only the spliced product was detected in *Lk-P*(1A) ovary RNA containing $E(z)^{28}$ or $E(z)^{32}$ alleles (Figure 4B, lanes 3 and 4). Neither product was detected in w^{1118} ovary RNA (Figure 4B, lane 5). Protected RNA products of other sizes were detected but these were not specific to the *P*-element-containing RNAs, as they were also observed in w^{1118} RNA (Figure 4B, lane 5).

To determine the effects of *E(z)* mutations on *Lk-P*(1A) *P*-element transcript levels, the total amount of protected species in each $E(z)^{-}$ lane was quantitated and compared to that in the $E(z)^{+}$ control lane (Figure 4B and Table 2). A 2-fold (±0.9) increase in *P*-element transcript levels was observed in the presence of $E(z)^{61}$ (Figure 4B, lane 2, and Table 2). Surprisingly, decreased *P*-element transcript levels were detected in the presence of $E(z)^{28}$ and $E(z)^{32}$ alleles. A 3.9-fold (±0.7) reduc-

tion was observed for $E(z)^{28}$ (Figure 4B, lane 3, and Table 2) and a 3.1-fold (±0.8) decrease for $E(z)^{32}$ (Figure 4B, lane 4, and Table 2). When the $E(z)^{61}$ allele was reintroduced into the *Lk-P*(1A) background, increased *P*-element mRNA levels were still observed but P cytotype was not disrupted (data not shown). It was previously observed that the $E(z)^{61}$ allele requires a couple of generations to disrupt *Lk-P*(1A) P cytotype (data not shown). Taken together, these data suggest that there is no correlation between increased *P*-element transcript levels and disruption of P cytotype and that E(Z) protein is not exerting direct transcriptional repression on the *Lk-P*(1A) *P* elements.

In addition to the alterations in *Lk-P*(1A) *P*-element transcript levels, splicing of IVS3 was also altered in the *E(z)* mutant backgrounds. A 2-fold (±0.4) decrease in splicing of IVS3 was observed in the presence of the $E(z)^{61}$ allele and a 1.9- and 2.2-fold (±0.6 and ±0.7) activation of IVS3 splicing in the presence of the $E(z)^{28}$ and $E(z)^{32}$ alleles, respectively (Figure 4B, lanes 2–4, and Table 2). Even though there appears to be no unspliced product present in lanes 3 and 4, this product was detected by phosphorimager quantitation (Table 2). These data are inconsistent with the model for autoregulation of 66-kD repressor production (Lemaitre *et al.* 1993; O'Hare *et al.* 1992; Roche *et al.* 1995). According to the model, low levels of *P*-element transcription in a P cytotype background promote retention of IVS3 and 66-kD repressor production and high levels of transcription in an M cytotype background permit splicing of IVS3 and transposase production. In the presence of the $E(z)^{61}$ allele, steady state *Lk-P*(1A) *P*-element transcript levels increased yet IVS3 splicing decreased. In addition, steady state *P*-element transcript levels decreased and IVS3 splicing increased in the presence of the $E(z)^{28}$ and $E(z)^{32}$ alleles. While these data seem to disagree with the autoregulation model, we do observe effects of steady state *P*-element RNA levels on splicing of IVS3, as seen previously (Roche *et al.* 1995). It is possible that mutations in *E(z)* are somehow affecting aspects other than transcription, such as RNA processing or stability of the *Lk-P*(1A) *P*-element transcripts in the nucleus.

Recombinant *P* elements inserted in subtelomeric *TAS* repeats silence maternal promoters within a *P*-element vector in an *E(z)*-dependent manner: The RNase protection analyses revealed that *Lk-P*(1A);$E(z)^{61}$ females have increased levels of repressor-encoding mRNA and therefore, have the potential to produce more 66-kD repressor protein than normal. However, these females still lose P cytotype suggesting that repression of *P* elements by *Lk-P*(1A) may be independent of the 66-kD repressor protein. Repression of *P* elements by *Lk-P*(1A) may involve pairing interactions between *P* elements. E(Z) protein and the 1A heterochromatin might be involved by directly affecting repression of other *P* elements, such as the reporter transgenes. Pairing interactions between *P* elements at distant loci might stimulate

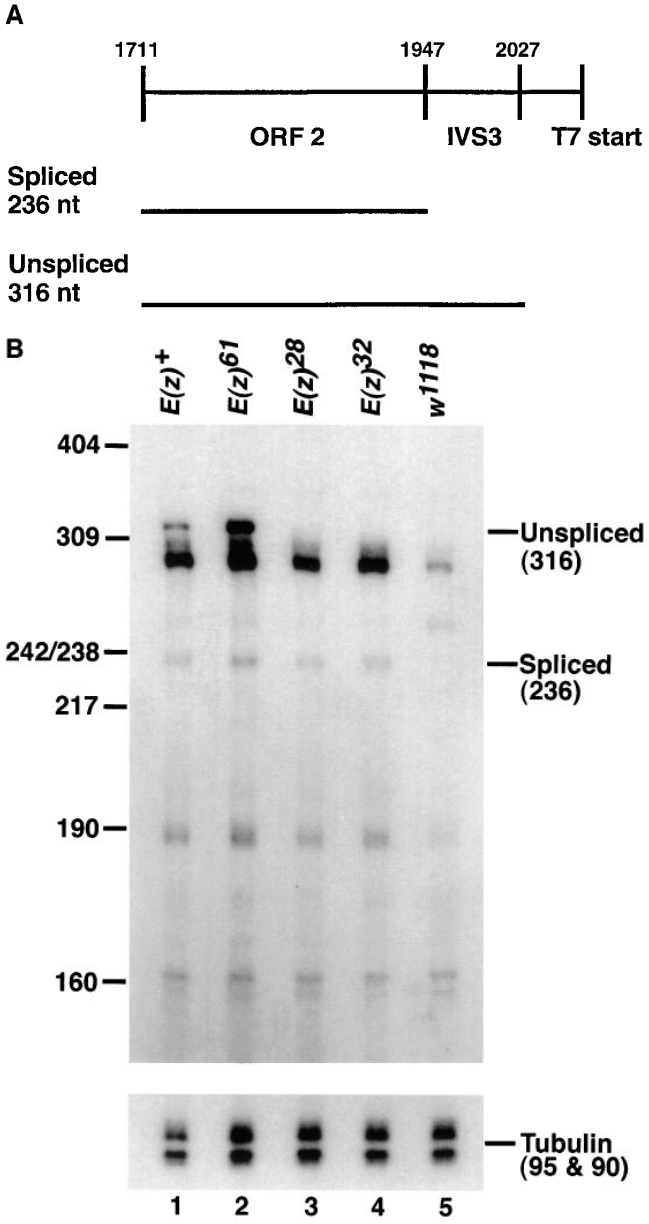

Figure 4.—*E(z)* mutations affect *P*-element transcript levels and splicing of IVS3. (A) Schematic diagram showing the antisense IVS3 probe used for RNase protection (see materials and methods for details) and the probe fragments protected by spliced and unspliced *P*-element RNA, as indicated. The numbers above the diagram refer to the *P*-element nucleotides contained within the antisense probe. (B) RNase protection analysis of total ovary RNA from *P*(1A)/*FM6*;+/*TM3* (lane 1), *P*(1A)/*FM6*;*E(z)*/*TM3* (lanes 2–4) and w^{1118} (lane 5) females. The size and identity of each protected fragment are indicated to the right of each panel. The sizes of DNA molecular weight markers are indicated to the left of the upper panel (in bp). The upper panel shows the probe fragments protected by the *P*-element RNA. Note that the nonspecific protected species appear to be of a weaker intensity in the w^{1118} RNA sample (lane 5) than in the *P*-element-containing samples (lanes 1–4). This difference in intensity was not always apparent. The lower panel shows the probe fragments protected by α-tubulin RNA. Note that the upper panel is a 3-day exposure while the lower panel is a 1-hr exposure of the same gel. Values from phosphorimager quantitation of the products are reported in Table 2.

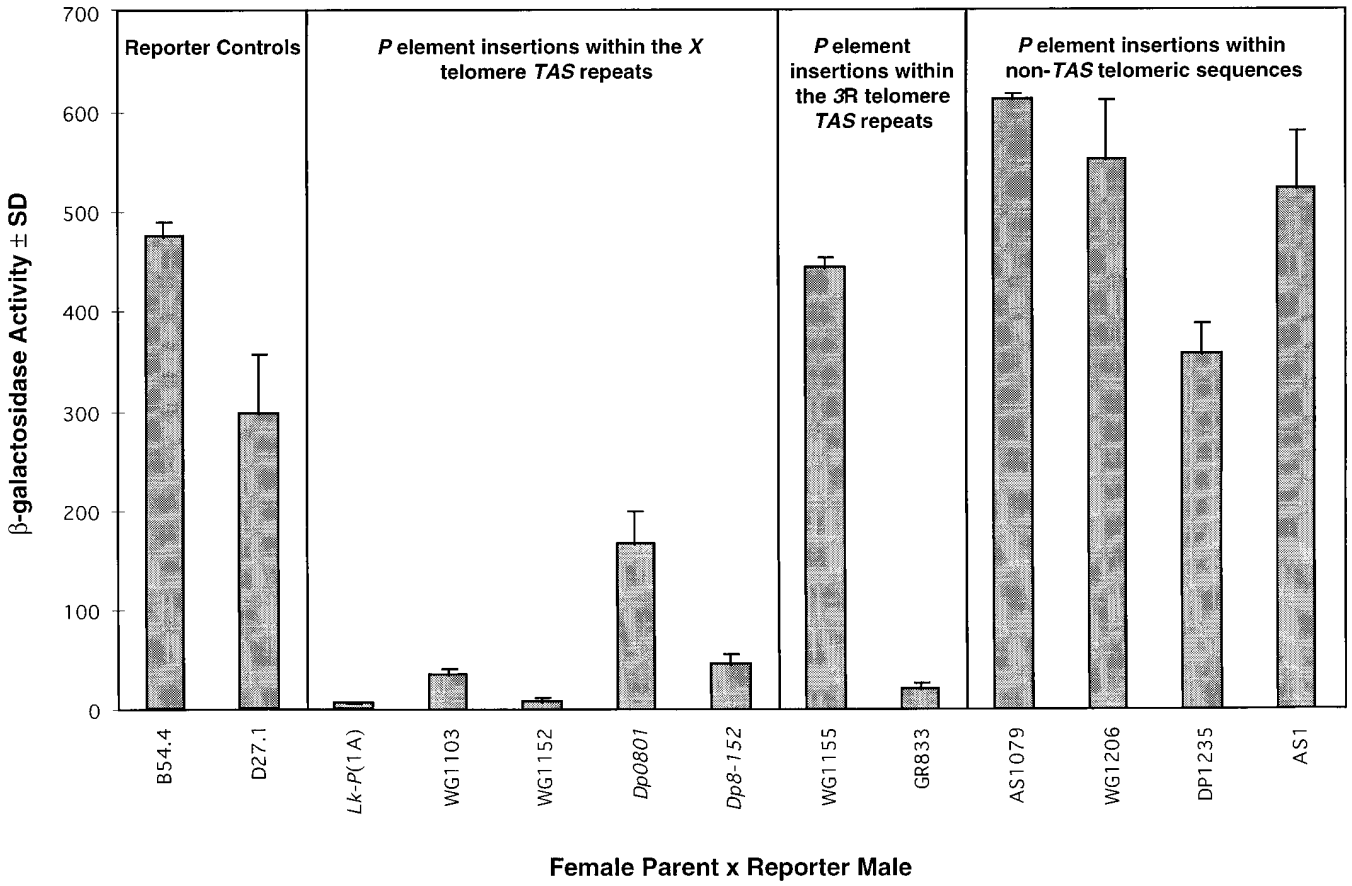

Figure 5.—Telomeric *P-lacZ* elements can repress an *hsp83* or *vasa*-reporter transgene. Shown are the results of a quantitative β-galactosidase assay of ovaries isolated from the progeny of crosses between the females indicated and either *P[hsp83*-IVS3-β-*geo]* (B54.4) or *P[vasa*-IVS3-β-*geo]* (D27.1) males. All lines were crossed to the B54.4 reporter transgene line, except for *Dp0801* and *Dp8-152*, which were crossed to D27.1. The female parents contained autonomous [*Lk-P*(1A), positive control for repression], *P[lArB]* (WG1103, WG1152, WG1155, WG1206 and DP1235), *PZ* (AS1079, *Dp0801* and *Dp8-152*), *P[hsneo]* (AS1) or *P[wA]* (GR833) elements inserted at telomeres. The vertical axis gives the results of the β-galactosidase assay expressed as OD units per minute per gram of total protein assayed. The vertical bars represent average data from three independent samples with error bars indicating the standard deviation between samples.

nucleation of heterochromatin structures at the euchromatic *P* element, resulting in transcriptional silencing. If pairing interactions between *P* elements are independent of the 66-kD repressor protein, then recombinant *P* elements inserted in the *TAS* repeats at 1A would be expected to repress reporter transgene expression. To test this hypothesis, we measured the β-galactosidase activity present in ovaries isolated from the progeny of a cross between females containing *P-lacZ* enhancer trap elements inserted at 1A and males carrying an *hsp83* or *vasa*-IVS3-β-*geo* reporter transgene (lines B54.4 and D27.1, respectively). The *P-lacZ* enhancer trap lines alone did not exhibit β-galactosidase activity in the ovaries (data not shown). *P-lacZ* enhancer trap elements contain 587 nt of 5′- and 232 nt of 3′-*P*-element sequence (Bellen *et al.* 1989; Wilson *et al.* 1989), and therefore, cannot make the 66-kD repressor protein. However, they could encode an N-terminal 144 amino acid *P*-element peptide (see discussion). We discovered that *P-lacZ* enhancer trap elements inserted at 1A could silence the reporter transgenes (Figure 5). The *P-lacZ* elements at 1A repressed euchromatic reporter transgenes inserted on the second and third chromosomes, in addition to the *X* chromosome, indicating that repression was independent of the genomic location of the reporter transgene element (data not shown). Furthermore, the reporter transgene was repressed by *P-lacZ* elements inserted at 1A on a normal *X* chromosome (WG1103 and WG1152) or on the mini-*X* chromosome, *Dp1187* (*Dp0801* and *Dp8-15*2) (Karpen and Spradling 1992; Tower *et al.* 1993).

Lines WG1103 and WG1152 repressed the reporter transgene as strongly as *Lk-P*(1A), even though they only contain a single *P-lacZ* element at 1A. However, with the minichromosome lines, strong repression of the reporter transgene was observed only when two *P-lacZ* enhancer trap elements were present. Line *Dp0801* contains a single *P-lacZ* insertion and only had a small effect on expression of the reporter transgene (Figure 5, compare *Dp0801* to D27.1). Repression was not observed by *lacZ* histochemical staining of ovaries (data not shown). Line *Dp8-152* contains an additional *P-lacZ* insertion,

obtained from a local transposition experiment using *Dp0801* as the starting strain (Tower *et al.* 1993); it strongly repressed activity of the D27.1 reporter transgene (Figure 5, compare *Dp8-152* to D27.1). Of all the repressing elements at 1A, *Dp8-152* had the weakest effects on the reporter transgene expression. Multiple single and double *P-lacZ* mini-chromosome lines were tested. The double insertion lines consistently exhibited strong repression of the reporter transgene while the single insertion lines had little effect (data not shown). Finally, as was observed in the *Lk-P*(1A) repression assays, the reporter transgene was silenced only by maternally inherited *P-lacZ* transgenes (data not shown).

The above data support a model for P cytotype repression through pairing interactions between distantly located *P* elements. An important component of the model is the involvement of a heterochromatic structure that flanks the repressing elements. We therefore determined the sequences flanking the *P-lacZ* elements at 1A. Plasmid rescue experiments mapped the minichromosome *P-lacZ* insertions to the *TAS* repeats at 1A (Karpen and Spradling 1992; Tower *et al.* 1993). By DNA blot hybridization and cycle sequencing of inverse PCR products, we determined that the *P-lacZ* insertions of strains WG1103 and WG1152 are also in *TAS* repeats (data not shown).

To determine whether the silencing effect of *P-lacZ* elements could occur only when the elements are inserted at the 1A telomere, we tested strains that contain *P*-element transgenes inserted at autosomal telomeres (see materials and methods). Since *TAS*-related sequences have been identified at the telomeres of the autosomes (Karpen and Spradling 1992), we first identified strains that contain insertions within these heterochromatic repeats. Strains GR833 and WG1155 contain *P[wA]* and *P-lacZ* insertions, respectively, within the *TAS*-related sequences at 100F (data not shown). The *white* marker gene of strain GR833 exhibits variegated expression (Hazelrigg *et al.* 1984; Levis *et al.* 1993) as a result of the heterochromatic environment of the *P[wA]* transgene. Surprisingly, when tested for repression of the reporter transgene, only GR833 could prevent reporter transgene expression (Figure 5). GR833 repressed the *hsp83*-IVS3-β-*geo* reporter transgene as strongly as *P-lacZ* insertions that were located within the 1A *TAS* repeats. Strain WG1155 also contains an insertion in the *TAS*-related sequences at 100F, yet had no effect on the expression of the β-*geo* reporter transgene (Figure 5).

We also tested whether strains that contain *P-lacZ* or *P[hsneo]* elements inserted in non-*TAS*-related telomeric sequences (strains AS1079, WG1206, DP1235, and AS1, data not shown) could prevent expression of the reporter transgene. We did not observe repression of the reporter transgene by any of these strains (Figure 5). *P-lacZ* insertions within euchromatin and euchromatic, variegating *P-lacZ* transgene arrays (Dorer and Henikoff 1994) also exhibited no repression of the reporter transgene (data not shown). These data reveal a strong correlation between the insertion of *P* elements within the *TAS* repeats or *TAS*-related sequences and reporter transgene silencing. However, the results with strain WG1155 indicate that insertion within these heterochromatic repeats is not sufficient for repression of the reporter transgene.

Because the role of *E(z)* in *Lk-P*(1A) P cytotype does not appear to be through controlling expression of the *Lk-P*(1A) *P* elements, we decided to test its role(s) in the silencing effects of recombinant *P* elements inserted in the *TAS* repeats at 1A or the *TAS*-related sequences at 100F. The $E(z)^{32}$ allele was crossed into the *P-lacZ* or *P[wA]* backgrounds (see materials and methods for details) and females containing both the recombinant *P* element and *E(z)* mutant allele were crossed to males containing the reporter transgene. Repression of the reporter transgene was assayed in the ovaries of the resulting offspring (Figure 6). The presence of the $E(z)^{32}$ allele in strain WG1103 resulted in an 8- to 12-fold increase in the β-galactosidase activity of the reporter transgene (Figure 6, compare WG1103;+ / *TM3* to WG-1103;*E(z)* / *TM3*). Mutations in *E(z)* had a weaker effect on the reporter transgene repression by WG1152 (2- to 3.5-fold derepression) and *Dp8-152* (3- to 4.5-fold derepression; Figure 6). It is unclear why the *E(z)* mutations have such different effects on the three strains. However, strain WG1152 exhibited stronger repression of the reporter transgene than WG1103 (Figure 5), so its repression may be more difficult to disrupt. The minichromosome line, *Dp8-152*, did not exhibit stronger repression capability than WG1103. The *Dp1187* minichromosome contains a 1-Mb block of centromeric heterochromatin near to 1A (Karpen and Spradling 1990), which may substitute for a potential loss of telomeric heterochromatin in the *Dp8-152;*$E(z)^{32}$ females. When $E(z)^{32}$ was crossed into strain GR833, we observed a threefold derepression of the reporter transgene (Figure 6, compare GR833 / *TM3* to GR833 / *E(z)*). Again, strain GR833 strongly repressed the reporter transgene, which may explain the observed weak derepression. The $E(z)^{32}$ allele had no effect on the variegated expression of the *P[wA]* transgene (data not shown). $E(z)^{32}$ exhibited greater disruption of repression by the *Lk-P*(1A) *P* elements (10- to 68-fold derepression, Table 3, repression by *P*(1A);$E(z)^{32}$ / *TM3* females at 25°) than by the recombinant *P* elements at 1A or 100F (2- to 12-fold derepression; Figure 6). Overall, these data suggest that *E(z)* and the *TAS* repeats are not affecting *Lk-P*(1A) P cytotype by controlling expression of the two *P* elements at 1A. Instead, we propose that *P* elements in telomeric *TAS* repeats interact in *trans* with other *P*-element sequences in the genome to cause gene silencing.

DISCUSSION

We have shown that the *Lk-P*(1A) *P* elements are inserted into subtelomeric heterochromatin and that

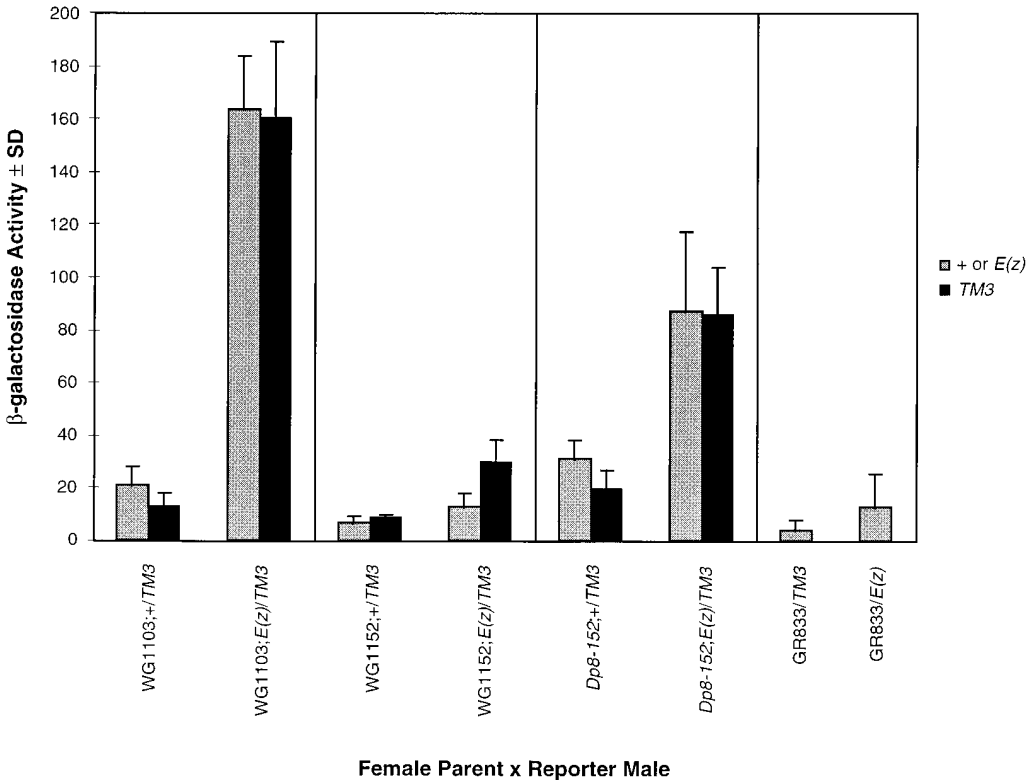

Figure 6.—Repression by telomeric *P*-element transgenes is affected by mutations in *E(z).* Shown are the β-galactosidase activities of ovaries dissected from the progeny of crosses between females containing telomeric *P-lacZ* or *P[wA]* insertions, that were either wild type or mutant for *E(z),* and β-*geo* reporter transgene males. The *P-lacZ* lines were crossed to the reporter line, B54.4, and the *P[wA]* line was crossed to a strain containing an insertion of the same reporter on the second chromosome. See the legend to Figure 5 for a description of the graph. For all strains tested, except GR833, the progeny of the crosses were separated according to whether they inherited the *E(z)* (or wild-type chromosome) or the *TM3* balancer chromosome. Grey vertical bars represent the activities of ovaries from females that inherited the wild-type or *E(z)* chromosome and not the balancer chromosome. Black vertical bars represent the data from progeny that inherited the balancer chromosome. For line GR833, only progeny that inherited the *P[wA]* element and not the *E(z)* or *TM3* chromosomes were assayed. See materials and methods for a description of these crosses.

the P cytotype of this strain is sensitive to mutations in *E(z)*, a Pc-G gene involved in gene silencing. The maternal effect of *E(z)* mutations on *Lk-P*(1A) P cytotype suggests that a complex required for P cytotype repression is established early in development and is stably maintained throughout the life cycle of the fly. Our data suggest that repression of transcription by the *Lk-P*(1A) *P* elements may involve pairing interactions between *P* elements, since recombinant *P*-element transgenes incapable of encoding the 66-kD repressor protein can silence a β-*geo* reporter transgene on a homologous or nonhomologous chromosome. Furthermore, silencing of the reporter transgene may be heterochromatin-based, since it requires insertion of the repressing *P* elements within the *TAS* repeats.

Genomic position effects, subtelomeric heterochromatin, and P cytotype: P cytotype is highly sensitive to the genomic position of the *P* elements, since not all strains containing *P* elements can repress transposition (Engels 1981; Sved 1987; Preston and Engels 1989; Misra *et al.* 1993). The 1A telomere, which was identified as a hotspot for *P*-element insertion (Ajioka and Eanes 1989; Biemont *et al.* 1990; Karpen and Spradling 1992), is a region of the genome that strongly promotes P cytotype (Biemont *et al.* 1990; Ronsseray *et al.* 1991). *Lk-P*(1A) contains only two *P* elements, both at 1A, but it represses transposition as effectively as a P strain containing 15 or more complete *P* elements (Ronsseray *et al.* 1991). Molecular characterization of the 1A genomic region revealed that a series of noncoding, repetitive sequences (*TAS* repeats), are found approximately 40 kb from the telomere (Karpen and Spradling 1992). In a screen for *P*-element insertions into the mini-*X* chromosome, *Dp1187*, 39 of the 45 insertions recovered were actually in the *TAS* repeats, indicating that these repeats represent a *P*-element hotspot.

A heterochromatic structure for the *TAS* repeats was suggested by their repetitive nature, the variegated expression of the *P-lacZ* enhancer trap elements inserted within them, their lack of essential genes and their underrepresentation in polytene chromosomes (Karpen and Spradling 1990; Karpen and Spradling 1992). *In situ* hybridizations to polytene chromosomes localized the *TAS* repeats to centromeric heterochromatin and

to the telomeres (Karpen and Spradling 1992). The genes required for the variegated expression of genes at telomeres in Drosophila have not yet been identified. Variegation of a telomeric *P[w; hsp26]* transgene is not suppressed by known modifiers of position effect variegation (PEV), such as increased *Y* chromosome dosage and mutations in the *Su(var)* genes (Wallrath and Elgin 1995). However, mutations in the Pc-G gene, *Su(z)2*, abolish variegation (Elgin 1996). Similarly, a human homolog of another Pc-G gene, *E(z)*, can complement telomeric silencing defects in yeast (Laible *et al.* 1997). We have shown that *E(z)* is required for the P cytotype repression of strains containing *P*-element insertions within subtelomeric heterochromatin. These data raise the interesting possibility that the Pc-G genes might play a role in telomeric silencing in Drosophila.

A role for the Polycomb group gene, *E(z)*, in *Lk-P*(1A) P cytotype: The Pc-G genes are responsible for maintaining the homeotic genes in a transcriptionally inactive state (Paro 1993; Pirrotta 1997). The Pc-G gene products may exist as a large multimeric complex in cells (Franke *et al.* 1992) and bind to more than 100 loci on polytene chromosomes (Rastelli *et al.* 1993; Carrington and Jones 1996), at sites known as polycomb response elements (PREs) (Chan *et al.* 1994). It is unclear how the Pc-G gene products transcriptionally silence genes. The observation that genes inserted within PRE-containing transposons exhibit variegated expression (Fauvarque and Dura 1993; Chan *et al.* 1994; Kassis 1994) and are less accessible to the *E. coli dam* methyltransferase (A. Boivin and J. M. Dura, personal communication), suggests that alterations of chromatin structure may be involved. Furthermore, members of the Pc-G contain structural motifs also found in suppressors of PEV, such as the chromodomain and SET domain (a domain of homology between *Su(var)3-9*, *E(z)*, and trithorax; Paro and Hogness 1991; Jones and Gelbart 1993; Tschiersch *et al.* 1994) and the Pc-G gene, *E(z)*, exhibits haplo-suppressor/triplo-enhancer dosage effects on variegation of the heterochromatin-associated white gene, w^{m4h} (Laible *et al.* 1997). Mutations in *Enhancer of Polycomb* (E(Pc)) also suppress w^{m4h} variegation (Sinclair *et al.* 1998). Most Pc-G genes, however, do not affect PEV (Grigliatti 1991; Sinclair *et al.* 1998).

We have shown here that the Pc-G gene, *E(z)*, is involved in P cytotype repression by the P strain *Lk-P*(1A). Mutant alleles of *E(z)* exhibit defects in oogenesis, maternal-effect lethality, and zygotic lethality (Shearn *et al.* 1978; Jones and Gelbart 1990; Phillips and Shearn 1990). Consistent with these maternal effects, the *E(z)* transcript is most abundant in 0–2 hr embryos (Jones and Gelbart 1993). The loss of *Lk-P*(1A) P cytotype in the presence of *E(z)* mutations also exhibited a maternal effect, suggesting that an *E(z)*-dependent state is set up during oogenesis, or early in development of the embryo, that is important for P cytotype repression by *Lk-P*(1A). The establishment of an *E(z)*-dependent state might contribute to the maternal inheritance of P cytotype. Once established, the *E(z)*-dependent state may no longer require *E(z)*, since introduction of the mutations zygotically through the male germ line, had no effect on repression by *Lk-P*(1A) (data not shown). Similarly, silencing of a Gal4 DNA-binding domain-*lacZ* reporter transgene is maintained after the Gal4-Polycomb fusion protein is no longer expressed (Muller 1995). The demonstration of a role for a Pc-G member in P cytotype repression helps explain, at least in the case of *Lk-P*(1A), how repression of a natural or engineered *P* element is maintained throughout the entire life cycle of the fly.

The observation that mutations in *E(z)* affected *Lk-P*(1A) P cytotype in a dominant fashion, that was not due to a haplo-insufficiency, was unexpected. The *E(z)* alleles were previously characterized as recessive, loss-of-function mutations (Shearn *et al.* 1978; Jones and Gelbart 1990; Phillips and Shearn 1990), and therefore, should have behaved in the same way as the null allele of *E(z)*. Flies homozygous for the *E(z)* alleles that affected P cytotype still produce E(Z) protein at the restrictive temperature (Carrington and Jones 1996). However, binding of the mutant E(Z) proteins to polytene chromosomes is reduced or eliminated. Since the Pc-G gene products may form multimeric complexes in cells (Franke *et al.* 1992), it is possible that the *E(z)* alleles affect P cytotype by producing proteins that poison complexes involved in P cytotype repression but not those responsible for homeotic gene repression or repression of *white* by z^1.

Also unexpected was the lack of a clear temperature-sensitive effect on P cytotype in the $E(z)^{61}$, $E(z)^{28}$ and $E(z)^{32}$ mutant backgrounds. However, the $E(z)^{32}$ mutation is not strictly temperature-sensitive. Flies homozygous for the $E(z)^{32}$ allele exhibit reduced viability and ectopic sex combs at nonrestrictive temperatures (Phillips and Shearn 1990). Also, the $E(z)^{61}$ allele partially suppresses the z^1-*w* interaction at 25° (Jones and Gelbart 1990). It is possible that the mutant E(Z) proteins might be resistant to temperature effects when part of a complex involved in P cytotype repression but not when in a complex required for homeotic gene repression or repression of *white* by z^1.

The *E(z)* alleles that affected P cytotype contain point mutations that map to different domains of the protein and therefore did not identify a single domain of the E(Z) protein as being important for P cytotype repression (Jones and Gelbart 1993; Carrington and Jones 1996). However, the C-terminal SET domain does not seem to be required since an allele containing a mutation in this domain did not affect *LK-P*(1A) P cytotype ($E(z)^{son3}$).

Finally, $E(z)^{61}$ exhibited an unusual effect in our assays for P cytotype repression. It only affected *Lk-P*(1A) P cytotype when in the background with one chromosomal copy of the *P* elements at 1A, *i.e.*, *P*(1A)/*FM6*. In

the *P*(1A)/*P*(1A);*E(z)*^{61}/*TM3* background, interactions between the homologous *X* chromosomes might have stabilized an E(Z)-containing complex formed in the vicinity of the two *P* elements, making it resistant to disruption by the mutant protein encoded by *E(z)*^{61}. The lack of interchromosomal stabilizing effects in the *P*(1A)/*FM6*;*E(z)*^{61}/*TM3* background might have enabled the *E(z)*^{61} product to disrupt complex formation. Such interchromosomal stabilizing effects are observed in Pc-G silencing. For instance, the PRE-induced variegation of a *white* reporter transgene is stronger in flies homozygous or *trans*-heterozygous for PRE-*white* transgenes, when compared to hemizygous flies (Fauvarque and Dura 1993; Kassis 1994; Sigrist and Pirrotta 1997), suggesting that the silencing activity of a PRE may depend on interactions with many PREs in the genome.

As mentioned, mutations in the gene that encodes HP1 also affect the P cytotype of *Lk-P*(1A) (Ronsseray *et al.* 1996). However, the *Su(var)205*-induced loss of P cytotype is zygotic and not maternal and is a result of a reduced dosage of the wild-type gene product. The differences in the behaviors of *Su(var)205* and *E(z)* alleles in assays for P cytotype repression suggest that HP1 and E(Z) may play separate, but related, roles in *Lk-P*(1A) P cytotype.

***Trans*-silencing of *P* elements:** Analysis of the *Lk-P*(1A) *P*-element transcripts in the presence of mutations in *E(z)* revealed a lack of correlation between levels of 66-kD repressor mRNA and P cytotype repression. This suggests that *Lk-P*(1A) P cytotype repression may not require the 66-kD repressor protein but instead may involve interactions between *P* elements in different genomic locations. We have shown that recombinant *P*-element transgenes inserted into subtelomeric heterochromatin are able to silence a β-*geo* reporter transgene driven by a heterologous promoter. We refer to this repression as *trans*-silencing. The repression mediated by the recombinant *P* elements strongly resembled that mediated by *Lk-P*(1A). Reporter transgene repression exhibited a maternal effect, was only observed with elements that are inserted in the subtelomeric *TAS* repeats, and was dependent on *E(z)*. Repression by both natural and recombinant *P* elements was independent of the genomic location of the reporter transgene. Euchromatic *P-lacZ* elements were also silenced by the recombinant *P* elements in the *TAS* repeats (S. Ronsseray and L. Marin, personal communication). Therefore, repression by the telomeric recombinant *P* elements may be used as an assay to study the components of *Lk-P*(1A) P cytotype. Ronsseray *et al.* (1998, accompanying article) have shown that although the same recombinant *P* elements in the *TAS* repeats cannot repress *P*-element transposition, they can promote the repression of transposition by paternally inherited autonomous *P* elements. This phenomenon is sensitive to mutations in HP1 and may be a result of altered expression of the autonomous *P* elements in the presence of the telomeric *P* elements.

The *trans*-silencing experiments with the recombinant *P* elements revealed that their insertion within the heterochromatic *TAS* repeats was necessary but not sufficient for reporter transgene repression. In the dominant *trans*-inactivation of repeated transgenes in plants, there is also a correlation between *trans*-inactivation capabilities and insertion of the *trans*-inactivating transgene in a transcriptionally inactive region of the chromosome (Matzke and Matzke 1995). Elements inserted at different locations within the *TAS* repeats exhibited variable repression capabilities and were not equally affected by mutations in *E(z)*. Position effects within the *TAS* repeats have previously been observed. Different *P-lacZ* insertions within the *TAS* repeats of *Dp1187* exhibit variable levels of variegation (Karpen and Spradling 1992). Also, when the *P* elements of *Lk-P*(1A) are separated from each other, the two isolated elements do not behave identically in genetic assays for P cytotype repression (Ronsseray *et al.* 1996).

These experiments also revealed a new aspect of P cytotype repression. *P*-element repression may involve interactions between *P* elements inserted at different genomic positions to allow for the spread of inactive chromatin structures. Pairing interactions between *P* elements may be mediated solely by sequence homology. The conversion of sequences from one *P* element to an element on a homologous or nonhomologous chromosome, or to the site of a *P*-element-induced break, suggests that homology searching mechanisms do exist in Drosophila (Gloor *et al.* 1991; Engels *et al.* 1994). Homology searching might permit sequence-dependent interactions between *P* elements in different genomic locations. Indeed, the variegated expression of *P*-element transgene arrays might be a result of pairing interactions between the repeated *P*-element transgenes (Dorer and Henikoff 1994).

Alternatively, maternally-inherited proteins, such as the 66-kD repressor protein, might be involved in pairing interactions between *P* elements. The transposase-mediated *trans*-cleavage of the *P*-element ends, when contained on separate plasmids, indicates that interactions between *P*-element-encoded products bound to different transposons can occur (Beall and Rio 1997). Although the *P-lacZ* or *P[wA]* elements cannot encode the 66-kD protein, they are capable of encoding an N-terminal 144 amino acid *P*-element peptide. This putative peptide would contain the DNA-binding and leucine zipper dimerization domains of the KP and 66-kD repressor proteins (Andrews and Gloor 1995; Lee *et al.* 1996; C. C. Lee and D. C. Rio, personal communication) and could mediate interactions between the different *P* elements by dimerization of bound monomers. However, the 144 amino acid *P*-element peptide would be fused to β-galactosidase in the case of the *P-lacZ* ele-

ments at 1A and none of the *P-lacZ* strains exhibit β-galactosidase activity in the ovary.

E(Z), like the 66-kD repressor protein, is maternally inherited and could also be mediating interactions between the different *P* elements. The Pc-G genes have been implicated in *trans*-silencing interactions, such as the z^1-*w* interaction (Wu and Goldberg 1989) and the cosuppression of repeated *w-Adh* transgenes (Pal-Bhadra *et al.* 1997). Whatever the mechanism, interactions between the β-*geo* reporter transgene and the 1A (or 100F) *P* elements, could allow a chromatin complex to spread to the site of the reporter transgene. It is possible that E(Z) and HP1 are components of this complex. The repressive chromatin complex could stably repress expression of the reporter transgene throughout the life cycle of the fly. The heterochromatic *TAS* repeats might promote formation of the repressive protein complex that spreads, which may explain why only *P* elements inserted within the *TAS* repeats silence the reporter transgene.

It is also possible that pairing interactions between *P* elements may result in silencing by altering the subnuclear localization of regions of the genome. Altered nuclear localization of the *brown* gene accompanies its silencing by a dominant, variegating allele of *brown* on the homologous chromosome (Csink and Henikoff 1996; Dernburg *et al.* 1996). Association of the telomeric *P* elements with the reporter transgene may relocate this region of the genome to the nuclear periphery, where telomeres in Drosophila tend to cluster (Hill and Whytock 1993).

It is becoming clear that P cytotype repression of *P* elements will be a good model system for studying gene regulation at a global chromosome level. By analyzing the silencing interactions of *P* elements, we may be able to learn more about how chromosome structure and nuclear architecture influence gene expression not only in the fruitfly, *Drosophila melanogaster*, but also in other organisms that silence repeated sequences.

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LITERATURE CITED

Adams, M. D., R. S. Tarng and D. C. Rio, 1997 The alternative splicing factor PSI regulates P-element third intron splicing in vivo. Genes Dev. **11:** 129–138.

Adler, P. N., J. Charlton and B. Brunk, 1989 Genetic interactions of the suppressor 2 of zeste region genes. Dev. Genet. **10:** 249–260.

Ajioka, J. W., and W. F. Eanes, 1989 The accumulation of P-elements on the tip of the X chromosome in populations of Drosophila melanogaster. Genet. Res. **53:** 1–6.

Andrews, J. D., and G. B. Gloor, 1995 A role for the KP leucine zipper in regulating P element transposition in Drosophila melanogaster. Genetics **141:** 587–594.

Ashburner, M., 1989 *Drosophila: A Laboratory Manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman *et al.*, editors, 1987 *Current Protocols in Molecular Biology.* Greene/John Wiley, New York.

Beall, E. L., and D. C. Rio, 1996 Drosophila IRBP/Ku p70 corresponds to the mutagen-sensitive *mus309* gene and is involved in *P*-element excision *in vivo.* Genes Dev. **10:** 921–933.

Beall, E. L., and D. C. Rio, 1997 Drosophila *P*-element transposase is a novel site-specific endonuclease. Genes Dev. **11:** 2137–2151.

Bellen, H. J., C. J. O'Kane, C. Wilson, U. Grossniklaus, R. K. Pearson *et al.*, 1989 *P*-element-mediated enhancer detection: a versatile method to study development in Drosophila. Genes Dev. **3:** 1288–1300.

Biemont, C., S. Ronsseray, D. Anxolabehere, H. Izaabel and C. Gautier, 1990 Localization of *P* elements, copy number regulation, and cytotype determination in *Drosophila melanogaster*. Genet. Res. Camb. **56:** 3–14.

Black, D. M., M. S. Jackson, M. G. Kidwell and G. A. Dover, 1987 KP elements repress *P*-induced hybrid dysgenesis in *Drosophila melanogaster.* EMBO J. **6:** 4125–4135.

Breen, T. R., and I. M. Duncan, 1986 Maternal expression of genes that regulate the bithorax complex of *Drosophila melanogaster.* Dev. Biol. **118:** 442–456.

Carrington, E. A., and R. S. Jones, 1996 The Drosophila *Enhancer of zeste* gene encodes a chromosomal protein: examination of wild-type and mutant protein distribution. Development **122:** 4073–4083.

Chan, C. S., L. Rastelli and V. Pirrotta, 1994 A Polycomb response element in the *Ubx* gene that determines an epigenetically inherited state of repression. EMBO J. **13:** 2553–2564.

Coen, D., 1990 *P* element regulatory products enhance *zeste* repression of a *P*[*white* duplicated] transgene in *Drosophila melanogaster*. Genetics **126:** 949–960.

Csink, A. K., and S. Henikoff, 1996 Genetic modification of heterochromatic association and nuclear organization in Drosophila. Nature **381:** 529–531.

Dernburg, A. F., K. W. Broman, J. C. Fung, W. F. Marshall, J. Philips *et al.*, 1996 Perturbation of nuclear architecture by long-distance chromosome interactions. Cell **85:** 745–759.

Dorer, D. R., and S. Henikoff, 1994 Expansions of transgene repeats cause heterochromatin formation and gene silencing in Drosophila. Cell **77:** 993–1002.

Eissenberg, J. C., G. D. Morris, G. Reuter and T. Hartnett, 1992 The heterochromatin-associated protein HP-1 is an essential protein in Drosophila with dosage-dependent effects on position-effect variegation. Genetics **131:** 345–352.

Elgin, S. C., 1996 Heterochromatin and gene regulation in Drosophila. Curr. Opin. Genet. Dev. **6:** 193–202.

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

Engels, W. R., 1983 The *P* family of transposable elements in Drosophila. Annu. Rev. Genet. **17:** 315–344.

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 for Microbiology, Washington, DC.

Engels, W. R., 1996 P elements in Drosophila. Curr. Top. Microbiol. Immunol. **204:** 103–123.

Engels, W. R., C. R. Preston and D. M. Johnson-Schlitz, 1994 Long-range cis preference in DNA homology search over the length of a Drosophila chromosome. Science **263:** 1623–1625.

Fauvarque, M. O., and J. M. Dura, 1993 *polyhomeotic* regulatory sequences induce developmental regulator-dependent variegation and targeted *P*-element insertions in Drosophila. Genes Dev. **7:** 1508–1520.

Franke, A., M. DeCamillis, D. Zink, N. Cheng, H. W. Brock *et al.*, 1992 *Polycomb* and *polyhomeotic* are constituents of a multimeric protein complex in chromatin of *Drosophila melanogaster.* EMBO J. **11:** 2941–2950.

Gloor, G. B., N. A. Nassif, D. M. Johnson-Schlitz, C. R. Preston and W. R. Engels, 1991 Targeted gene replacement in Dro-

sophila via *P* element-induced gap repair. Science **253:** 1110–1117.

Grigliatti, T., 1991 Position-effect variegation—an assay for nonhistone chromosomal proteins and chromatin assembly and modifying factors. Methods Cell Biol. **35:** 587–627.

Hazelrigg, T., R. W. Levis and G. R. Rubin, 1984 Transformation of *white* locus DNA in Drosophila: dosage compensation, *zeste* interaction, and position effect. Cell **36:** 469–481.

Hedley, M. L., and T. Maniatis, 1991 Sex-specific splicing and polyadenylation of dsx pre-mRNA requires a sequence that binds specifically to tra-2 protein in vitro. Cell **65:** 579–586.

Hill, R. J., and S. Whytock, 1993 Cytological structure of the native polytene salivary gland nucleus of *Drosophila melanogaster*: a microsurgical analysis. Chromosoma **102:** 446–456.

James, T. C., and S. C. Elgin, 1986 Identification of a nonhistone chromosomal protein associated with heterochromatin in *Drosophila melanogaster* and its gene. Mol. Cell Biol. **6:** 3862–3872.

Jones, R. S., and W. M. Gelbart, 1990 Genetic analysis of the *Enhancer of zeste* locus and its role in gene regulation in *Drosophila melanogaster*. Genetics **126:** 185–199.

Jones, R. S., and W. M. Gelbart, 1993 The Drosophila Polycomb-group gene *Enhancer of zeste* contains a region with sequence similarity to *trithorax*. Mol. Cell Biol. **13:** 6357–6366.

Jurgens, G., 1985 A group of genes controlling spatial expression of the bithorax complex in *Drosophila*. Nature **316:** 153–155.

Kalisch, W.-E., and B. Rasmuson, 1974 Changes in the *zeste* phenotype induced by autosomal mutations in *Drosophila melanogaster*. Hereditas **78:** 97–104.

Karpen, G. H., and A. C. Spradling, 1990 Reduced DNA polytenization of a minichromosome region undergoing position-effect variegation in Drosophila. Cell **63:** 97–107.

Karpen, G. H., and A. C. Spradling, 1992 Analysis of subtelomeric heterochromatin in the Drosophila minichromosome *Dp1187* by single *P* element insertional mutagenesis. Genetics **132:** 737–753.

Kassis, J. A., 1994 Unusual properties of regulatory DNA from the Drosophila *engrailed* gene: three "pairing-sensitive" sites within a 1.6 kb region. Genetics **136:** 1025–1038.

Kaufman, P. D., and D. C. Rio, 1991 Drosophila *P*-element transposase is a transcriptional repressor in vitro. Proc. Natl. Acad. Sci. USA **88:** 2613–2617.

Kaufman, P. D., R. F. Doll and D. C. Rio, 1989 Drosophila *P* element transposase recognizes internal *P* element DNA sequences. Cell **59:** 359–371.

Kennison, J. A., and J. W. Tamkun, 1988 Dosage-dependent modifiers of polycomb and antennapedia mutations in Drosophila. Proc. Natl. Acad. Sci. USA **85:** 8136–8140.

Laible, G., A. Wolf, R. Dorn, G. Reuter, C. Nislow *et al.*, 1997 Mammalian homologues of the Polycomb-group gene *Enhancer of zeste* mediate gene silencing in Drosophila heterochromatin and at *S. cerevisiae* telomeres. EMBO J. **16:** 3219–3232.

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.

Lee, C. C., Y. M. Mul and D. C. Rio, 1996 The Drosophila *P*-element KP repressor protein dimerizes and interacts with multiple sites on P-element DNA. Mol. Cell. Biol. **16:** 5616–5622.

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.

Lemaitre, B., S. Ronsseray and D. Coen, 1993 Maternal repression of the *P* element promoter in the germline of *Drosophila melanogaster*: a model for the *P* cytotype. Genetics **135:** 149–160.

Levis, R., R. Ganesan, K. Houtchens, L. A. Tolar and F. M. Sheen, 1993 Transposons in place of telomeric repeats at a Drosophila telomere. Cell **75:** 1083–1093.

Lindsley, D. L., and G. G. Zimm, 1992 *The Genome of Drosophila Melanogaster*. Academic Press, San Diego.

Maniatis, T., E. F. Fritsch and J. Sambrook, 1982 *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Matzke, M. A., and A. J. Matzke, 1995 Homology-dependent gene silencing in transgenic plants: what does it really tell us? Trends Genet. **11:** 1–3.

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

Misra, S., R. M. Buratowski, T. Ohkawa and D. C. Rio, 1993 Cytotype control of *Drosophila melanogaster P* element transposition: genomic position determines maternal repression. Genetics **135:** 785–800.

Muller, J., 1995 Transcriptional silencing by the *Polycomb* protein in Drosophila embryos. EMBO J. **14:** 1209–1220.

Nusslein-Volhard, C., E. Weischaus and H. Kluding, 1984 Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster*. I. Zygotic loci on the second chromosome. Wilhelm Roux's Arch. **193:** 267–282.

O'Hare, K., and G. M. Rubin, 1983 Structures of *P* transposable 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 cloned *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 elements in Drosophila. Proc. Natl. Acad. Sci. USA **84:** 9123–9127.

Pal-Bhadra, M., U. Bhadra and J. A. Birchler, 1997 Cosuppression in Drosophila: gene silencing of *Alcohol dehydrogenase* by *white-Adh* transgenes is *Polycomb* dependent. Cell **90:** 479–490.

Paro, R., 1993 Mechanisms of heritable gene repression during development of Drosophila. Curr. Opin. Cell. Biol. **5:** 999–1005.

Paro, R., and D. S. Hogness, 1991 The *Polycomb* protein shares a homologous domain with a heterochromatin-associated protein of Drosophila. Proc. Natl. Acad. Sci. USA **88:** 263–267.

Pelegri, F., and R. Lehmann, 1994 A role of polycomb group genes in the regulation of gap gene expression in Drosophila. Genetics **136:** 1341–1353.

Phillips, M. D., and A. Shearn, 1990 Mutations in *polycombeotic*, a Drosophila polycomb-group gene, cause a wide range of maternal and zygotic phenotypes. Genetics **125:** 91–101.

Pirrotta, V., 1997 Pc-G complexes and chromatin silencing. Curr. Opin. Genet. Dev. **7:** 249–258.

Preston, C. R., and W. R. Engels, 1989 Spread of *P* transposable elements in inbred lines of *Drosophila melanogaster*. Prog. Nucleic Acids Res. Mol. Biol. **36:** 71–85.

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.

Rastelli, L., C. S. Chan and V. Pirrotta, 1993 Related chromosome binding sites for *zeste, suppressors of zeste* and Polycomb group proteins in Drosophila and their dependence on *Enhancer of zeste* function. EMBO J. **12:** 1513–1522.

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

Rio, D. C., 1991 Regulation of Drosophila *P* element transposition. Trends Genet. **7:** 282–287.

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.

Roche, S. E., M. Schiff and D. C. Rio, 1995 *P*-element repressor autoregulation involves germ-line transcriptional repression and reduction of third intron splicing. Genes Dev. **9:** 1278–1288.

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., M. Lehmann and D. Anxolabehere, 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:** 510–512.

Ronsseray, S., B. Lemaitre and D. Coen, 1993 Maternal inheritance of *P* cytotype in *Drosophila melanogaster*: a "pre-*P* cytotype" is strictly extra-chromosomally transmitted. Mol. Gen. Genet. **241:** 115–123.

Ronsseray, S., M. Lehmann, D. Nouaud and D. Anxolabehere, 1996 The regulatory properties of autonomous subtelomeric *P* elements are sensitive to a *Suppressor of variegation* in *Drosophila melanogaster*. Genetics **143:** 1663–1674.

Ronsseray, S., L. Marin, M. Lehmann and D. Anxolabehere, 1998 Repression of hybrid dysgenesis in *Drosophila melanogaster* by com-

binations of telomeric *P*-element reporters and naturally occurring *P* elements. Genetics **149:** 1857–1866.

Sambrook, J., E. F. Fritsch and T. Maniatis, 1989 *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Shearn, A., G. Hersperger and E. Hersperger, 1978 Genetic analysis of two allelic temperature-sensitive mutants of *Drosophila melanogaster* both of which are zygotic and maternal-effect lethals. Genetics **89:** 341–353.

Sigrist, C. J. A., and V. Pirrotta, 1997 Chromatin insulator elements block the silencing of a target gene by the Drosophila *Polycomb* response element (PRE) but allow trans interaction between PREs on different chromosomes. Genetics **147:** 209–221.

Simmons, M. J., J. D. Raymond, C. D. Grimes, C. Belinco, B. C. Haake *et al.*, 1996 Repression of hybrid dysgenesis in *Drosophila melanogaster* by heat-shock-inducible sense and antisense constructs. Genetics **144:** 1529–1544.

Sinclair, D. A. R., N. J. Clegg, J. Antonchuk, T. A. Milne, K. Stankunas *et al.*, 1998 *Enhancer of Polycomb* is a suppressor of position-effect variegation in *Drosophila melanogaster*. Genetics **148:** 211–220.

Struhl, G., 1981 A gene product required for correct initiation of segmental determination in Drosophila. Nature **293:** 36–41.

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

Tower, J., G. H. Karpen, N. Craig and A. C. Spradling, 1993 Preferential transposition of Drosophila *P* elements to nearby chromosomal sites. Genetics **133:** 347–359.

Tschiersch, B., A. Hofmann, V. Krauss, R. Dorn, G. Korge *et al.*, 1994 The protein encoded by the Drosophila position-effect variegation suppressor gene *Su(var)3-9* combines domains of antagonistic regulators of homeotic gene complexes. EMBO J. **13:** 3822–3831.

Wallrath, L. L., and S. C. Elgin, 1995 Position effect variegation in Drosophila is associated with an altered chromatin structure. Genes Dev. **9:** 1263–1277.

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

Wilson, C., R. K. Pearson, H. J. Bellen, C. J. O'Kane, U. Grossniklaus *et al.*, 1989 *P*-element-mediated enhancer detection: an efficient method for isolating and characterizing developmentally regulated genes in Drosophila. Genes Dev. **3:** 1301–1313.

Wu, C. T., and M. L. Goldberg, 1989 The Drosophila *zeste* gene and transvection. Trends Genet. **5:** 189–194.

Wu, C. T., and M. Howe, 1995 A genetic analysis of the Suppressor 2 of zeste complex of Drosophila melanogaster. Genetics **140:** 139–181.

Wu, C. T., R. S. Jones, P. F. Lasko and W. M. Gelbart, 1989 Homeosis and the interaction of *zeste* and *white* in Drosophila. Mol. Gen. Genet. **218:** 559–564.

Yisraeli, J. K., and D. A. Melton, 1989 Synthesis of long, capped transcripts in vitro by SP6 and T7 RNA polymerases. Methods Enzymol. **180:** 42–50.

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