# The P Cytotype in *Drosophila melanogaster*: A Maternally Transmitted Regulatory State of the Germ Line Associated With Telomeric *P* Elements

**Michael J. Simmons,[1] John D. Raymond, Jarad B. Niemi, Jeremy R. Stuart and Peter J. Merriman**

*Department of Genetics, Cell Biology and Development, University of Minnesota, Saint Paul, Minnesota 55108-1095*

Manuscript received June 24, 2003
Accepted for publication September 28, 2003

ABSTRACT

The incomplete *P* elements *TP5* and *TP6* are inserted in the TAS repeats near the left telomere of the *Drosophila melanogaster X* chromosome. These telomeric *P* elements repress *P*-induced gonadal dysgenesis and germ-line hypermutability in both sexes. However, their capacity to repress hypermutability is lost when they are transmitted patroclinously in a cross. *TP5* and *TP6* do not repress *P*-element activity in somatic cells, nor do they alter the somatic or germ-line phenotypes of *P*-insertion alleles. In the germ line, these elements suppress the phenotype of a *P*-insertion allele of the *singed* gene that is evoked by other *P* elements, presumably because these other elements encode repressor polypeptides. This suppression is more effective when the telomeric *P* elements are inherited maternally. Regulation by telomeric *P* elements parallels that of the P cytotype, a state that represses *P*-element activity in some strains of Drosophila. This state exists only in the germ line and is maternally transmitted along with the *P* elements themselves. Regulation by known repressor P polypeptides is not restricted to the germ line and does not require maternal transmission of the relevant *P* elements. Regulation by telomeric *P* elements appears to be epistatic to regulation by repressor P polypeptides.

THE transposable *P* elements of *Drosophila melanogaster* were discovered through their involvement in hybrid dysgenesis, a syndrome of abnormalities that occurs in the offspring of crosses between recently established wild-type strains and longstanding laboratory strains (Kidwell *et al.* 1977). These abnormalities are restricted to the germ line and include high frequencies of mutations, chromosome rearrangements, and sterility. They are much more prevalent in the progeny of crosses between wild-type males and laboratory females than in the progeny of the reciprocal cross. This difference indicates that hybrid dysgenesis is regulated by a maternally transmitted condition, which is known as the P cytotype (Engels 1979a). Genetic analyses have demonstrated that this condition depends on the *P* elements themselves (Engels 1979a; Sved 1987).

Flies derived recently from natural populations possess *P* elements in their genomes; they usually also possess the P cytotype, which keeps their *P* elements in check (Bingham *et al.* 1982). Flies from longstanding laboratory strains lack *P* elements; however, they possess a condition called the M cytotype, which permits *P* elements to become active and which can be transmitted maternally through the egg cytoplasm. Hybrid dysgenesis occurs when *P* elements are introduced into the M cytotype by crossing P males to M females. *P* elements are mobilized in the offspring of such crosses by an enzyme, the P transposase, which is encoded by the structurally complete members of the *P*-element family (Engels 1984; Karess and Rubin 1984). Incomplete *P* elements do not encode this enzyme, but they can be mobilized by it if the transposase is provided *in trans* by a complete *P* element present somewhere in the genome (Engels 1984).

In dysgenic crosses, *P*-element activity is restricted to the germ line. The molecular basis of this restriction involves alternate splicing of *P*-element transcripts (Laski *et al.* 1986). In the germ line, all three introns within the complete *P* sequence are removed whereas, in the soma, the last of these introns remains in the transcript. The incompletely spliced somatic RNA is translated into a 66-kD polypeptide instead of the 87-kD transposase. This 66-kD polypeptide does not function catalytically as a transposase; in fact, it acts as a repressor of transposase activity (Robertson and Engels 1989; Misra and Rio 1990).

For many years researchers have hypothesized that the 66-kD polypeptide is responsible for the P cytotype. Transgenes designed to produce this polypeptide repress *P* activity, albeit partially, and models have been constructed to explain its preferential production in the germ lines of P strains (Misra and Rio 1990; O'Hare *et al.* 1992; Gloor *et al.* 1993; Roche *et al.* 1995). Polypeptides encoded by some incomplete *P* elements have also been implicated in the regulation of germ-line *P* activity. One such element, known as *KP*, encodes a small polypeptide that binds to sequences near the *P*-element promoter (Lee *et al.* 1996, 1998). Binding of the KP

[1]*Corresponding author:* Department of Genetics, Cell Biology and Development, 250 BioScience Center, 1445 Gortner Ave., University of Minnesota, St. Paul, MN 55108-1095. E-mail: simmo004@umn.edu

polypeptide may therefore interfere with the expression of the transposase gene in structurally complete *P* elements, or it may prevent the transposase from catalyzing transposition.

Genetic analyses have offered another explanation for the P cytotype. Complete *P* elements inserted at the left end of the *X* chromosome, very near the telomere, repress hybrid dysgenesis almost completely, and repression by these elements has a maternally inherited component (Ronsseray *et al.* 1991). Incomplete *P* elements inserted near the *X* telomere are also strong repressors of hybrid dysgenesis, and repression is seen only when these elements are transmitted maternally (Marin *et al.* 2000; Stuart *et al.* 2002). The regulatory properties of the telomeric *P* elements therefore exactly parallel those of the P cytotype. In contrast, repression by the 66-kD and KP polypeptides occurs even when the elements encoding them are transmitted paternally in crosses (Black *et al.* 1987; Jackson *et al.* 1988; Robertson and Engels 1989; Misra and Rio 1990; Simmons *et al.* 2002a,b).

Different assays have been used to study *P*-element regulation. The most direct approaches have tested for repression of hybrid dysgenesis. For example, one can monitor the frequency of gonadal dysgenesis, a form of sterility due to a developmental failure in the germ line, in flies that do and do not carry putative repressor *P* elements, or one can monitor the frequency of transposase-catalzyed *P*-element excisions in the germ lines of such flies. *P* excisions can also be monitored in somatic cells using a test system in which the flies carry a modified *P* element that lacks the last intron; with such an element, production of the P transposase is not restricted to the germ line (Robertson *et al.* 1988). Putative repressors can also be evaluated for effects on the expression of *P*-insertion mutations or *P* transgenes. For example, the phenotypes of some *P*-insertion mutations are suppressed or enhanced by certain P polypeptides (Williams *et al.* 1988; Robertson and Engels 1989; Gloor *et al.* 1993). The variety of assays available to study *P*-element activity has permitted many questions about *P*-element regulation to be addressed.

In this article we consider questions about telomeric *P* elements and the P cytotype. We focus on two incomplete elements, *TP5* and *TP6*, inserted in the TAS repeats near the left telomere of the *X* chromosome (Stuart *et al.* 2002). *TP5* is 1.8 kb long and *TP6* is 1.9 kb long. Both elements were isolated from wild-type P strains, and neither encodes a catalytically active P transposase. However, both elements are associated with strong repression of gonadal dysgenesis in females and *P*-element excision in males and females. Repression of *P*-element excision by these elements is restricted to the germ line and appears to require maternal transmission of the elements themselves. Can *TP5* and *TP6* repress gonadal dysgenesis in males? Do they repress dysgenesis when they are inherited paternally? Does either of the telomeric *P* elements exert a regulatory effect in the soma? Does either of these elements alter the expression of *P*-insertion alleles in the germ line? Does either element regulate the effects of repressor P polypeptides?

## MATERIALS AND METHODS

**Drosophila stocks and husbandry:** Genetic symbols for the Drosophila stocks are explained in Lindsley and Zimm (1992) or in other references cited in the text. Experimental cultures were maintained on a standard cornmeal-molasses-dried yeast medium at 25° unless stated otherwise. Cultures used to assess the fertility of individual flies contained a sugar-yeast medium (Simmons *et al.* 1980).

**Synthesis of stocks with "cytotype-dependent" alleles:** Stocks carrying an *X*-linked telomeric *P* element (*TP*, either *TP5* or *TP6*) and the *P*-insertion mutation $vg^{21\text{-}3}$ of the autosomal *vestigial* gene were created in a scheme that began by crossing *TP* males with *C(1)DX, y f;* $vg^{21\text{-}3}$ females (*y, yellow* body; *f, forked* bristles). The *TP;* $vg^{21\text{-}3}/+$ sons were then backcrossed to *C(1)DX, y f;* $vg^{21\text{-}3}$ females to create a stock homozygous for $vg^{21\text{-}3}$ and hemizygous for *TP* in the males. *TP;* $vg^{21\text{-}3}$ males were then crossed to homozygous *TP* females to obtain *TP/TP;* $vg^{21\text{-}3}/+$ daughters, which were backcrossed to *TP;* $vg^{21\text{-}3}$ males. Among the progeny, flies showing the vestigial phenotype were crossed to establish a stock fixed for both the *TP* and the $vg^{21\text{-}3}$ allele.

Stocks carrying an *X*-linked telomeric *P* element and the *P*-insertion mutation $sn^{50e}$ of the *X*-linked *singed* gene were obtained by recombining a *TP w m f* (*w, white* eyes; *m, miniature* wings) *X* chromosome with a *y w* $sn^{50e}$ *X* chromosome in heterozygous females. *TP* $y^+$ *w* $sn^{50e}$ $m^+$ $f^+$ recombinant males were individually crossed to *FM6,* $l(1)^{69a}$*/Df(1)*$^{wrJ1}$ females and to *C(1)DX, y f* females (both from M strains) to obtain, respectively, *TP w* $sn^{50e}$*/FM6,* $l(1)^{69a}$ daughters and *TP w* $sn^{50e}$ sons. These flies were then intercrossed to obtain *TP w* $sn^{50e}$ homozygous females and *TP w* $sn^{50e}$ males, which were used to establish the stocks.

The presence of the telomeric *P* element and the *P*-insertion mutation in all of the stocks was verified by PCR using primers complementary to *P*-element sequences. Synthesis of stocks with the telomeric *P* elements and the $sn^w$ (*weak singed*) allele of the *singed* gene has been described in Stuart *et al.* (2002).

**Synthesis of stocks with chromosomes from an M′ strain:** M′ strains possess *P* elements but do not have the P cytotype. Chromosomes of the M′ strain Sexi.4 (Rasmusson *et al.* 1990) were introduced into stocks carrying the *X*-linked markers *w* and $sn^w$ by a series of backcrosses. In two of these stocks, either the *TP5* or the *TP6* telomeric *P* element, both of which are tightly linked to the *w* locus, was also present. The initial crosses were between *w* $sn^w$ females and Sexi.4 males. Sons from these crosses were mated to Sexi.4 females to produce *w* $sn^w/+$ daughters, which were then mated to Sexi.4 males. The *w* $sn^w$ sons from these last matings were crossed to Sexi.4 females. Thereafter, sib-matings were used to produce homozygous *w* $sn^w$ stocks carrying Sexi.4 chromosomes. The presence of the telomeric *P* elements in the stocks was verified by PCR using primers specific for these elements. See Stuart *et al.* (2002) for the sequences of the *TP5*- and *TP6*-specific primers. Homozygous $sn^w$ females without a telomeric *P* element proved to be sterile when Sexi.4 chromosomes were present. To balance this stock, *w* $sn^w/+$ females with Sexi.4 chromosomes were mated to *FM6* males; the *FM6* balancer chromosome is marked with the recessive *yellow* body (*y*), dominant *Bar* eye (*B*), and recessive *diminutive* (*dm*) muta-

tions. The *w sn$^w$/FM6* daughters from this mating were then crossed to *w sn$^w$* males carrying Sexi.4 chromosomes.

**Gonadal dysgenesis assay for *P*-element activity:** Gonadal dysgenesis (GD) was induced by crossing females from a particular strain to males from the Harwich-w P strain. Thirty replicate cultures of each cross were incubated at 25°, and as many as 20 daughters and 10 sons from each culture were examined for GD. The daughters were examined by squashing them between two glass slides to determine if they carried eggs. Females that did not were judged to have GD. The sons were examined by placing them individually in 13 × 100-mm culture tubes with two *C(1)DX, y f* virgin females at 25° and checking for larvae 6 days later. Males that did not produce any progeny were judged to have GD. The initial cross to induce GD was incubated at 25° instead of the usual 29° to prevent the generalized male sterility that is seen when flies are reared at 29°.

**Assay for female fertility:** Females (not necessarily virgins) to be tested for the sterility associated with certain alleles of the *singed* gene were collected from cultures and incubated *en masse* with males for at least 2 days in vials. Each female was then placed in a separate 13 × 100-mm culture tube, which was incubated at 25° for 9 days. The fertility of each female was assessed by counting the number of pupae on the walls of the tube at the end of this incubation period.

**Mutability assay for *P*-element activity:** Instability of the double *P*-insertion mutation *weak singed* (*sn$^w$*) of the *X*-linked *singed* gene was used to assay for transposase activity. In the presence of the P transposase, one or the other of the *P* elements inserted in the *singed* gene is excised, creating *singed* alleles with different phenotypes: extreme singed (sn$^e$) or pseudo-wild-type (sn$^+$; Roiha *et al.* 1988). The frequency of these changes is a measure of transposase activity. To determine the mutability of *sn$^w$* in the germ line, males carrying this allele and a source of the P transposase were mated individually to three or four *C(1)DX, y f* females; the sons of these matings were then classified for bristle phenotype and counted, and the frequency of the sn$^+$ and sn$^e$ flies among them was used to estimate the *sn$^w$* mutation rate. When *sn$^w$* was destabilized by the *P(ry$^+$, Δ2-3)99B* transgene, which produces the P transposase in the soma as well as in the germ line, the *C(1)DX, y f* females used in the testcrosses came from a P strain; the chromosomes from this strain suppress the bristle mosaicism that would otherwise occur in the offspring (Robertson and Engels 1989). Progeny in the *sn$^w$* mutability assays were counted up to the seventeenth day after the test cultures were started. Typically 30–50 replicate cultures were set up for each test group.

**Molecular analyses:** Initially the *P* element inserted in the *sn$^{50e}$* allele was amplified by PCR using a primer complementary to a segment in the inverted terminal repeats. The product of this amplification was sequenced by a campus facility. Southern blotting experiments with DNA from a homozygous *sn$^{50e}$* stock had indicated that the *P* element in *sn$^{50e}$* was inserted in the 5′ region of the *singed* gene. Primers in this region were used in combination with primers complementary to segments within the *P* element to amplify the DNA around the *P* element's insertion site and these products were sequenced.

## RESULTS

**Telomeric *P* elements repress gonadal dysgenesis in both male and female germ lines:** Previous work has shown that the telomeric *P* elements *TP5* and *TP6* are strong repressors of transposase-catalyzed *P*-element excision in both the male and the female germ lines (Stuart *et al.* 2002). This repression occurs when both the telomeric *P* elements and the targets of the P transposase are transmitted maternally. Maternally transmitted telomeric *P* elements also repress gonadal dysgenesis in the female germ line even though the targets of the transposase are inherited paternally. To extend these results, we tested *TP5* and *TP6* for repression of gonadal dysgenesis in the male germ line.

Homozygous *TP5* or *TP6* females were crossed at 25° to males from the Harwich-w P strain to induce gonadal dysgenesis in the offspring. Daughters from these crosses were examined for the absence of eggs, and sons were crossed individually to *C(1)DX, y f* virgin females to ascertain if they were fertile or sterile. As controls, females from an M (*y w*) or a P (Harwich-w) strain were crossed to Harwich-w males, and the offspring were analyzed for dysgenic sterility. The results of these experiments are summarized in Table 1.

The progeny from the control crosses with the M strain showed high frequencies of gonadal dysgenesis in both females (85.9%) and males (98.6%). The frequency of GD among the females was slightly less than that seen in previous experiments [99.9%, Stuart *et al.* (2002)], where the culturing temperature was higher (29°). The progeny from the control crosses with the P strain showed little or no GD (0% among females and 1.6% among males), indicating strong repression by this strain. Among the offspring of the crosses involving *TP5*, the frequency of GD in the daughters was 32.6%, and that in the sons was 2.3%. *TP5* is therefore a moderate repressor of GD in females and a strong repressor of GD in males. Among the offspring of the crosses involving *TP6*, the frequencies of GD were lower (7.1% in females and 1.6% in males). *TP6* is therefore a strong repressor of GD in both sexes.

In these experiments, the telomeric *P* elements were inherited maternally and both the target *P* elements and the sources of the P transposase were inherited paternally. The results demonstrate that maternally inherited telomeric *P* elements are effective repressors of the GD induced by these paternally inherited factors in both the male and the female germ lines.

**Telomeric *P* elements lose their ability to repress transposase activity when they are inherited paternally:** Maternal inheritance is a key requirement for repression of *P*-element activity by the P cytotype (Engels 1979a,b; Sved 1987). Whether or not telomeric *P* elements are able to repress hybrid dysgenesis when they are paternally inherited cannot be addressed by studying dysgenic sterility because the offspring of P females crossed to males that carry isolated telomeric *P* elements will always be fertile due to maternal transmission of the P cytotype. However, this issue can be addressed by studying the hypermutability of *sn$^w$*, which is an allele due to the insertion of two incomplete *P* elements in the 5′ region of the *singed* gene; the two elements are inserted in opposite orientations (Roiha *et al.* 1988).

**TABLE 1**

**Repression of *P*-induced sterility in females and males by telomeric *P* elements**

| | $F_1$ progeny from cross stock ♀♀ × Harwich-w ♂♂[a] | | | | |
|---|---|---|---|---|---|
| Stock | No. vials | No. females | %GD ± SE[b] | No. males | %GD ± SE[b] |
| M control (*y w*) | 27 | 518 | 85.9 ± 2.2 | 218 | 98.6 ± 0.8 |
| TP5 | 30 | 567 | 32.6 ± 4.2 | 281 | 2.3 ± 0.9 |
| TP6 | 30 | 586 | 7.1 ± 1.2 | 289 | 1.6 ± 0.8 |
| P control (Harwich-w) | 30 | 543 | 0.0 ± 0.0 | 270 | 2.4 ± 1.5 |

[a] $F_1$ flies were reared at 25°; as many as 20 $F_1$ females and 10 $F_1$ males from each vial were tested for sterility.
[b] Unweighted mean percentage gonadal dysgenesis (sterility) ± standard error.

When one or the other of these *P* elements is excised by the action of the P transposase, new, phenotypically distinguishable alleles are created. Transposase activity can be measured by the frequency with which these alleles are induced by defined transposase sources such as *H(hsp/CP)2*, a stable *hobo* transgene that produces the P transposase in the germ line (Simmons *et al.* 2002a), or *P(ry*$^+$*, Δ2-3)99B*, a stable *P* transgene that produces the P transposase in the soma as well as the germ line (Robertson *et al.* 1988). Previous work has indicated that in the germ line telomeric *P* elements repress *sn*$^w$ mutability induced by either of these transposase sources (Stuart *et al.* 2002). However, they do not repress the somatic transposase activity encoded by *P(ry*$^+$*, Δ2-3)99B*. Furthermore, reciprocal crosses between *TP sn*$^w$ (where *TP* is *TP5* or *TP6*) and *H(hsp/CP)2* strains indicate that repression of germ-line *sn*$^w$ mutability occurs only when the telomeric *P* elements are maternally inherited. However, the analysis of these crosses is complicated by maternal transmission of the transposase activity encoded by the *H(hsp/CP)2* transgene (Simmons *et al.* 2002c).

To obtain unequivocal evidence that repression of transposase-induced *sn*$^w$ mutability requires maternal inheritance of the telomeric *P* elements, we carried out a reciprocal-cross analysis using the *P(ry*$^+$*, Δ2-3)99B* transgene, which does not transmit transposase activity maternally (Simmons *et al.* 2002c). *TP sn*$^w$ females were crossed to *P(ry*$^+$*, Δ2-3)99B* males (cross I) and *C(1)DX, y f; P(ry*$^+$*, Δ2-3)99B* females carrying attached-*X* chromosomes were crossed to *TP sn*$^w$ males (cross II). The *TP sn*$^w$*; P(ry*$^+$*, Δ2-3)99B/+* sons from both types of crosses were then mated individually to *C(1)DX, y f* females from a P strain with the $\pi_2$ genetic background to obtain progeny, which were classified and counted for their singed bristle phenotypes. These progeny could be classified unambiguously because their maternally derived P chromosomes repressed the somatic transposase activity encoded by *P(ry*$^+$*, Δ2-3)99B*, which should be present in half of them. Three distinct phenotypes appeared among the progeny: weak singed (sn$^w$), wild type (sn$^+$), and extreme singed (sn$^e$), the latter two types being due to the excision of one of the *P* elements in the *sn*$^w$ allele while it was in the paternal germ line. As controls, we also measured *sn*$^w$ mutability in flies that did not carry a telomeric *P* element.

The results of these experiments are summarized in Table 2. The control flies from the two reciprocal crosses had *sn*$^w$ mutation rates of ~0.80, indicating a high level of transposase activity in the germ line. The near identity of these rates demonstrates that there is no reciprocal-cross effect associated with transposase activity itself. The absence of this effect is expected from previous studies, which showed that the transposase activity encoded by the *P(ry*$^+$*, Δ2-3)99B* transgene is not transmitted through the egg cytoplasm (Simmons *et al.* 2002c). However, the flies that carried the telomeric *P* elements had much lower *sn*$^w$ mutation rates in cross I than in cross II. For *TP5*, the respective rates were 0.085 and 0.852, and for *TP6*, they were 0.435 and 0.839. These results show that when the telomeric *P* elements are transmitted maternally, they repress *sn*$^w$ mutability in the germ line, whereas when they are transmitted paternally, repression ability is lost. Thus, the regulatory abilities of the telomeric *P* elements parallel those of the P cytotype.

It should be noted that all the flies that were tested for *sn*$^w$ mutability in these experiments were somatic mosaics for the singed bristle phenotypes. Thus, as previously demonstrated (Stuart *et al.* 2002), neither of the telomeric *P* elements represses transposase activity in the soma.

**Chromosomes from a P strain partially repress transposase activity when they are inherited paternally:** P strains possess many different *P* elements scattered throughout the genome. The preceding analyses have indicated that *P* elements inserted at the left telomere of the *X* chromosome are associated with a reciprocal-cross effect that is characteristic of germ-line regulation by the P cytotype. Although cytotype is the paramount system of regulating the *P*-element family, other types of regulation that do not exhibit a reciprocal-cross effect are known (Robertson and Engels 1989; Lemaitre *et al.* 1993; Simmons *et al.* 2002a,b). To detect the presence of these types of regulation in a P strain, we tested

**TABLE 2**

**Reciprocal-cross analysis of repression of transposase activity by telomeric *P* elements**

| | Cross I[a] | | | | | | Cross II[a] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Stock | No. vials | $sn^w$ | $sn^+$ | $sn^e$ | Total | Mutation rate[b] | No. vials | $sn^w$ | $sn^+$ | $sn^e$ | Total | Mutation rate[b] |
| $sn^w$ | 35 | 179 | 392 | 331 | 902 | 0.805 ± 0.018 | 38 | 240 | 567 | 448 | 1255 | 0.807 ± 0.016 |
| *TP5* $sn^w$ | 44 | 1174 | 58 | 57 | 1289 | 0.085 ± 0.022 | 38 | 175 | 555 | 476 | 1206 | 0.852 ± 0.015 |
| *TP6* $sn^w$ | 46 | 692 | 326 | 168 | 1186 | 0.435 ± 0.037 | 39 | 207 | 600 | 498 | 1305 | 0.839 ± 0.017 |

[a] See text for details.
[b] Unweighted average mutation rate ±SE.

P chromosomes for repression ability when they were inherited paternally.

The P strain used in these experiments had the *C(1)DX, y f* compound-*X* chromosomes in females and the $sn^w$ mutation in males. It was produced by introgressing chromosomes from $\pi_2$, a standard P strain derived from wild-caught flies, into an M strain that carried the *C(1)DX, y f* compound-*X* chromosomes (ENGELS 1979b). Once established, this synthetic P strain was maintained by crossing *C(1)DX, y f* ($\pi_2$) females with $sn^w$ ($\pi_2$) males each generation.

Few, if any, non-$sn^w$ males are ever observed in this P strain, even in mass cultures. However, if $sn^w$ males from this stock are crossed to *C(1)DX, y f* females from an M strain, and the sons are then crossed to *C(1)DX, y f* females to assess germ-line $sn^w$ mutability, non-$sn^w$ sons are frequently observed (*i.e.*, >20% of all sons). These findings indicate that the synthetic P strain carries transposase-producing *P* elements capable of destabilizing $sn^w$; however, within the strain, the transposase activity encoded by these *P* elements is repressed.

To see if this repression could be due to a noncytotype mechanism, we tested paternally inherited chromosomes from this P strain for repression of $sn^w$ mutability induced by a maternally inherited *P(ry*$^+$*, Δ2-3)99B* transgene. The strategy was to measure *Δ2-3*-induced $sn^w$ mutability in the presence (group I) and absence (group II) of the paternally inherited P chromosomes. Lower $sn^w$ mutability in the presence of the P chromosomes would indicate a noncytotype mechanism of regulation. The flies for these tests were obtained from crosses between *C(1)DX, y f; P(ry*$^+$*, Δ2-3)99B* females and $sn^w$ ($\pi_2$) males (group I) or $sn^w$ (M) males (group II). Sons were tested for $sn^w$ mutability by crossing them individually to *C(1)DX, y f* females from the P strain. As controls, we also measured $sn^w$ mutability induced by the P chromosomes in the absence of the *P(ry*$^+$*, Δ2-3) 99B* transgene (group I′). The flies for these tests were obtained by crossing *C(1)DX, y f* females from an M strain to $sn^w$ ($\pi_2$) males. The results of all of the $sn^w$ mutability experiments are summarized in Table 3.

The paternally inherited P chromosomes repressed somatic transposase activity induced by the *P(ry*$^+$*, Δ2-3) 99B* transgene. Among the three types of tested males, nearly all of those in group II showed somatic transposase, whereas almost none of those in groups I and I′ showed this activity. The presence of transposase activity in group II is due to somatic production of the P transposase by the *P(ry*$^+$*, Δ2-3)99B* transgene, and the absence of this activity in group I′ is due to the lack of this transgene. The absence of somatic transposase activity in group I indicates repression of the *P(ry*$^+$*, Δ2-3)99B* transgene by paternally derived P chromosomes.

The paternally inherited P chromosomes also repressed transposase activity in the germ line. Test groups I and I′ had about the same germ-line $sn^w$ mutability even though the flies in group I carried the *Δ2-3* transposase source. The ability of this transposase source to induce germ-line $sn^w$ mutability is evident in group II, where the mutation rate is more than twice that of group I. Thus, the paternally inherited P chromosomes present in the flies of group I must repress the germ-line $sn^w$ mutability induced by the *P(ry*$^+$*, Δ2-3)99B* transgene.

These findings indicate that the P strain used in these experiments possesses a noncytotype regulatory mechanism. Chromosomes inherited paternally from it repress transposase activity in both the soma and the germ line.

**Telomeric *P* elements do not affect the phenotypes of cytotype-dependent alleles in the soma:** WILLIAMS *et al.* (1988) discovered that some *P*-insertion mutations are phenotypically expressed in M strains, but suppressed in P strains. Other *P*-insertion mutations are expressed in P strains but suppressed in M strains (ROBERTSON and ENGELS 1989). Because of the major regulatory difference between M and P strains, these types of mutations have come to be called cytotype-dependent alleles. However, this convention overlooks the inherent complexity of P strains, which typically carry many different types of *P* elements in their genomes and which may possess different regulatory systems. It is possible that the phenotype of a *P*-insertion allele in the genetic background of a P strain is due to something other than the P cytotype.

To determine if telomeric *P* elements affect so-called cytotype-dependent alleles in the soma, we created stocks homozygous for these elements and the *P*-insertion mutations $vg^{21\text{-}3}$ and $sn^{50e}$.

The $vg^{21\text{-}3}$ mutation is due to the insertion of a 2.6-kb

**TABLE 3**

**Repression of transposase activity by paternally inherited P chromosomes**

| Experimental group[a] | Δ2-3 present[b] | P chromosomes present[c] | Mosaic males[d] | Nonmosaic males[d] | Total males | No. males tested[e] | $sn^w$ | $sn^+$ | $sn^e$ | Total | Mutation rate[f] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| I′ | — | + | 0 | 40 | 40 | 37 | 822 | 144 | 109 | 1075 | 0.236 ± 0.015 |
| I | + | + | 1 | 120 | 121 | 48 | 872 | 136 | 121 | 1129 | 0.229 ± 0.016 |
| II | + | — | 58 | 4 | 62 | 48 | 679 | 538 | 248 | 1465 | 0.539 ± 0.024 |

[a] See text for details. Initial crosses were at 21°; testcrosses were at 25°.
[b] $P(ry^+, \Delta 2\text{-}3)99B$ maternally inherited.
[c] P chromosomes paternally inherited.
[d] Males were classified as mosaic (showing at least two of the three bristle phenotypes) or nonmosaic (showing only $sn^w$ bristles).
[e] Males tested for germ-line mutability of $sn^w$ were crossed to *C(1)DX, y f* ($\pi_2$) females and their sons were scored as $sn^w$, $sn^+$, or $sn^e$.
[f] Unweighted average mutation rate ±SE.

*P* element in the 5′ region of the autosomal *vestigial* gene (Williams *et al.* 1988). In a genetic background devoid of other *P* elements, this mutation is associated with an extreme mutant phenotype. The wings are short and shriveled. In the genetic background of a P strain, flies carrying the $vg^{21\text{-}3}$ allele are wild type in appearance. Moreover, this wild-type phenotype is seen even when the $vg^{21\text{-}3}$ flies inherit their P chromosomes from a male (Williams *et al.* 1988). Suppression of the extreme vestigial phenotype by a P genetic background therefore does not exhibit the reciprocal-cross effect associated with cytotype regulation of *P*-element activity in the germ line. Stocks homozygous for $vg^{21\text{-}3}$ and either *TP5* or *TP6* have extreme mutant phenotypes. Thus, these telomeric *P* elements do not affect the somatic phenotype of the $vg^{21\text{-}3}$ allele.

The $sn^{50e}$ allele is due to the insertion of a 0.6-kb *P* element in the 5′ region of the *X*-linked *singed* gene. The inserted *P* element contains a segment of non-*P* DNA 125 bp long in place of base pairs 162–2540 in the canonical *P* sequence (O'Hare and Rubin 1983); the non-*P* DNA is 100% AT. Using the coordinates in the Berkeley *Drosophila* Genome Project annotation RELEASE3.1 (Adams *et al.* 2000), the $sn^{50e}$ *P* element is inserted at base 5697 in the *singed* gene [base −660 in the coordinates of Roiha *et al.* (1988)], with bases 5690–5697 duplicated on both sides of the element; it is inserted within the first (noncoding) exon of one of the *singed* transcription units (defined by cDNA CG32858-RB), but in the opposite orientation.

In a genetic background devoid of other *P* elements, $sn^{50e}$ is associated with an extreme mutant phenotype. The bristles are very short and twisted. In the genetic background of a P strain, flies with the $sn^{50e}$ allele have moderately mutant bristles—longer and less twisted than those seen in the extreme mutant phenotype (Robertson and Engels 1989). We have determined that this suppression occurs when the P chromosomes are inherited from either parent (Table 4). Thus, like $vg^{21\text{-}3}$, $sn^{50e}$ is suppressed by P chromosomes *per se* rather than by the maternally inherited P cytotype. In contrast, stocks homozygous for $sn^{50e}$ and either *TP5* or *TP6* have extreme mutant phenotypes. These telomeric *P* elements therefore do not suppress the somatic phenotype of $sn^{50e}$. Furthermore, even when these telomeric *P* elements are maternally derived, they do not prevent the suppression of $sn^{50e}$ by P chromosomes (Table 4).

To follow up these experiments, we tested a complete *P* element for its ability to suppress the extreme phenotype of $sn^{50e}$ in the presence or absence of telomeric *P* elements. This complete *P* element is contained within a *hobo* transgene, *H(hsp/CP)2*, inserted on chromosome *2*. Previous analyses have shown that this transgene encodes the P transposase as well as a repressor of transposase activity, presumably the 66-kD polypeptide, in the germ line (Simmons *et al.* 2002a). In the soma, *H(hsp/CP)2* does not encode the transposase but it does en-

**TABLE 4**

**Reciprocal-cross analysis of the $sn^{50e}$ bristle phenotype in males**

| Male[a] | Female[b] | Singed phenotype of sons |
|---|---|---|
| $sn^{50e}$ | M | Extreme |
| *TP5* $sn^{50e}$ | M | Extreme |
| *TP6* $sn^{50e}$ | M | Extreme |
| $sn^{50e}$ | P | Moderate |
| *TP5* $sn^{50e}$ | P | Moderate |
| *TP6* $sn^{50e}$ | P | Moderate |
| M | $sn^{50e}$ | Extreme |
| M | *TP5* $sn^{50e}$ | Extreme |
| M | *TP6* $sn^{50e}$ | Extreme |
| P | $sn^{50e}$ | Moderate |
| P | *TP5* $sn^{50e}$ | Moderate |
| P | *TP6* $sn^{50e}$ | Moderate |

Crosses were incubated at 21°; only sons were scored for their bristle phenotype.

[a] M males were from a *w* $sn^{w}$ strain; P males were from a $sn^{w}(\pi_2)$ strain.

[b] Both M and P females carried the *C(1)DX, y f* compound *X* chromosome. P females had the genetic background of the $\pi_2$ P strain.

code the repressor polypeptide. Females homozygous for $sn^{50e}$ were crossed to males homozygous for *H(hsp/CP)2* and their $sn^{50e}$*; H(hsp/CP)2/+* sons were examined for bristle phenotype. All of the sons had the moderate mutant bristles observed when P chromosomes are combined with the $sn^{50e}$ mutation. Thus, the *H(hsp/CP)2* transgene suppresses the extreme bristle phenotype of $sn^{50e}$. When homozygous *TP5* $sn^{50e}$ or *TP6* $sn^{50e}$ females were crossed to *H(hsp/CP)2* males, the moderate mutant phenotype was also observed in their sons. Thus, neither of these telomeric *P* elements affects suppression of the $sn^{50e}$ phenotype by the *H(hsp/CP)2* transgene.

**Telomeric *P* elements affect a cytotype-dependent allele in the germ line:** The $sn^{w}$ allele is due to the insertion of two incomplete *P* elements in the first noncoding exon of one of the transcription units of the *singed* gene. In males this mutation is associated with a weak malformation of the bristles. In females, the bristles are close to wild type, but in some cases, slight malformations can be seen. In males, the bristle phenotype of $sn^{w}$ is the same in M and P genetic backgrounds, as well as in the presence of the telomeric *P* elements *TP5* and *TP6*.

The $sn^{w}$ allele also causes homozygous females to be sterile. This "singed sterility" occurs because of faulty vitellogenesis in the nurse cells, which are part of the female germ line (Robertson and Engels 1989). However, it is seen only when *P* elements that have been implicated in the production of polypeptide repressors of P transposase activity are present in the genome (Robertson and Engels 1989).

We discovered that *P* elements in the genome of Sexi.4, an M′ strain devoid of complete *P* elements (Rasmusson *et al.* 1990; Simmons *et al.* 1990), cause homozygous $sn^{w}$ females to be sterile. A stock carrying the $sn^{w}$ mutation and *P* elements from the Sexi.4 strain was constructed by backcrossing. However, because the females in this stock were sterile, it was necessary to balance the $sn^{w}$ allele over the *FM6* chromosome, which contains *diminutive* (*dm*), an unrelated recessive, female-sterilizing mutation. Stocks carrying the telomeric *P* elements *TP5* or *TP6*, $sn^{w}$, and *P* elements from the Sexi.4 strain were also constructed. In these stocks, homozygous $sn^{w}$ females were fertile; consequently it was not necessary to introduce the *FM6* balancer chromosome into their genotypes.

Quantitative data on the fertility of individual homozygous $sn^{w}$ females from the original stocks and their Sexi.4 derivatives were obtained by counting progeny at the pupal stage in fertility assay cultures (Table 5). Most of the females from the original stocks produced many progeny; very few were completely sterile. By contrast, none of the homozygous $sn^{w}$ females from the Sexi.4 stocks produced any progeny unless a telomeric *P* element was present. With either *TP5* or *TP6*, most of the tested females produced some progeny, although not as many as the females from the corresponding original stocks. Thus, the sterility caused by the Sexi.4 background is partially suppressed by the telomeric *P* elements.

To determine if this suppression follows the pattern of the P cytotype, we performed reciprocal crosses between the $sn^{w}$; Sexi.4 and *TP* $sn^{w}$; Sexi.4 strains and tested their daughters for fertility. In series A, $sn^{w}$*/FM6*; Sexi.4 females were crossed to *TP* $sn^{w}$; Sexi.4 males and in series B, *TP* $sn^{w}$; Sexi.4 females were crossed to $sn^{w}$; Sexi.4 males. We analyzed the *TP* $sn^{w}/sn^{w}$; Sexi.4 daughters from both series. These daughters are expected to be genetically identical. As controls, we also analyzed the *TP* $sn^{w}$*/FM6*; Sexi.4 daughters from series A. To distribute the effort in these experiments, the tested females were divided into two groups, one assayed within a few days of eclosion (the young group) and the other about a week later (the old group).

Table 6 summarizes the data from these experiments. The control females, which were $sn^{w}/sn^{+}$ heterozygotes, produced many progeny; very few of these females were completely sterile and among those that were fertile, the median number of progeny ranged from 38 to 43. The females that were homozygous for $sn^{w}$ produced significantly fewer progeny. Among those from cross A, many were completely sterile: 77 and 96% of the *TP5* females and 36 and 81% of the *TP6* females, from the young and old groups, respectively. Among the females from cross B, the corresponding sterility frequencies were lower: 14 and 60% of the *TP5* females and 7 and 7% of the *TP6* females. By *z*-tests based on binomial variances, these frequencies are significantly lower than those for the females from cross A ($P < 0.05$ in all four comparisons). Thus, this form of sterility exhibits

**TABLE 5**

**Fertility of homozygous $sn^w$ females from stocks with and without the Sexi.4 genetic background**

| Stock | No. females tested[a] | No. sterile | Proportion sterile | No. progeny per fertile female | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Low | Median | High | Mean[b] | SD[c] |
| $sn^w$ | 69 | 2 | 0.03 | 4 | 31 | 53 | 29.7 | 10.7 |
| *TP5* $sn^w$ | 43 | 4 | 0.09 | 4 | 36 | 56 | 34.0 | 13.9 |
| *TP6* $sn^w$ | 38 | 0 | 0.00 | 15 | 41 | 53 | 41.1 | 8.0 |
| $sn^w$; Sexi.4 | 28 | 28 | 1.00 | — | — | — | — | — |
| *TP5* $sn^w$; Sexi.4 | 52 | 4 | 0.08 | 2 | 20.5 | 43 | 21.4 | 10.6 |
| *TP6* $sn^w$; Sexi.4 | 44 | 9 | 0.21 | 1 | 16 | 35 | 16.4 | 11.4 |

[a] Females were collected from stock cultures, incubated with males for 2–3 days, and then assayed for fertility.
[b] Unweighted mean.
[c] Standard deviation.

a reciprocal-cross effect: less sterility in the daughters of cross B, where a telomeric *P* element had been transmitted maternally, than in the daughters of cross A. The number of progeny produced by the fertile females from these two crosses also showed a reciprocal-cross effect. Among the $sn^w$ homozygotes from cross A, the median numbers of progeny were 2 and 1.5 for the *TP5* females and 4 and 1 for the *TP6* females, from the young and old groups, respectively. Among the $sn^w$ homozygotes from cross B, the corresponding numbers were 4 and 2 and 12 and 13. By the Mann-Whitney rank-sum test, the cross B females had significantly more progeny than did the cross A females in the three comparisons that were made (*TP5* young, *TP6* young, and *TP6* old females; $P < 0.05$ in each case). Thus, maternal transmission of a telomeric *P* element appears to be associated with suppression of sterility and reduced fertility in $sn^w$ homozygotes—a phenomenon that parallels the maternally transmitted repression of germ-line transposase activity by the telomeric *P* elements. Furthermore, the data suggest that the suppression of $sn^w$ sterility is more obvious among young females than among old ones and that it is more pronounced with *TP6* than with *TP5*.

To show that the sterility caused by the Sexi.4 genetic background specifically involves a malfunction of the *singed* gene, we analyzed females heterozygous for $sn^w$ and $sn^{x2}$. The latter mutation is an X-ray-induced null allele that has an extreme singed bristle phenotype and that causes female sterility. If $sn^w/sn^{x2}$ females with the Sexi.4 background are sterile but $sn^w/+$ females with the same background are not, then the observed sterility must involve a malfunction of $sn^w$ evoked by the Sexi.4 background. Males from the original $sn^w$ stocks and their Sexi.4 derivatives were crossed to *FM7,* $y^{31d}$ $sn^{x2}$ *B*/+ fe-

**TABLE 6**

**Reciprocal-cross analysis of female fertility in the Sexi.4 genetic background**

| Cross[a] | Origin of *TP* | Genotype (all Sexi.4) | Experimental group[b] | No. females tested | No. sterile | Proportion sterile | No. progeny per fertile female | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Low | Median | High | Mean[c] | SD[d] |
| A | Father | *TP5* $sn^w/FM6$ | Young | 68 | 1 | 0.02 | 22 | 41 | 53 | 40.6 | 7.2 |
| | | | Old | 84 | 1 | 0.01 | 9 | 43 | 67 | 41.7 | 12.2 |
| A | Father | *TP5* $sn^w/sn^w$ | Young | 65 | 50 | 0.77 | 1 | 2 | 8 | 3.5 | 2.2 |
| | | | Old | 106 | 102 | 0.96 | 1 | 1.5 | 3 | 1.8 | 1.0 |
| B | Mother | *TP5* $sn^w/sn^w$ | Young | 65 | 9 | 0.14 | 1 | 4 | 25 | 6.1 | 5.0 |
| | | | Old | 134 | 80 | 0.60 | 1 | 2 | 7 | 2.3 | 1.3 |
| A | Father | *TP6* $sn^w/FM6$ | Young | 58 | 0 | 0.00 | 23 | 40.5 | 58 | 40.3 | 7.4 |
| | | | Old | 101 | 1 | 0.01 | 11 | 38 | 72 | 40.0 | 12.1 |
| A | Father | *TP6* $sn^w/sn^w$ | Young | 53 | 19 | 0.36 | 1 | 4 | 25 | 6.1 | 5.8 |
| | | | Old | 93 | 75 | 0.81 | 1 | 1 | 7 | 2.0 | 1.9 |
| B | Mother | *TP6* $sn^w/sn^w$ | Young | 61 | 4 | 0.07 | 1 | 12 | 40 | 13.7 | 9.4 |
| | | | Old | 117 | 8 | 0.07 | 1 | 13 | 48 | 15.3 | 11.5 |

[a] See text for details.
[b] Females were collected from cultures on day 13. One group of females (young) was incubated with males 2–3 days and then assayed for fertility. Another group (old) was incubated with males 9–11 days and then assayed for fertility.
[c] Unweighted mean.
[d] Standard deviation.

**TABLE 7**

**Effect of the Sexi.4 genetic background on female fertility assayed in $sn^w/sn^{x2}$ heterozygotes**

| Cross[a] | Origin of $sn^w$ | Genotype | No. females tested | No. sterile | Proportion sterile | No. progeny per fertile female | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Low | Median | High | Mean[b] | SD[c] |
| | | | | Without Sexi.4 chromosomes | | | | | | |
| A | Father | $sn^w/+$ | 75 | 2 | 0.03 | 13 | 46 | 76 | 45.6 | 12.2 |
| A | Father | $sn^w/sn^{x2}$ | 69 | 1 | 0.01 | 3 | 28 | 46 | 28.3 | 8.9 |
| B | Mother | $sn^w/sn^{x2}$ | 115 | 6 | 0.05 | 1 | 17 | 35 | 16.5 | 8.4 |
| A | Father | *TP5* $sn^w/+$ | 69 | 3 | 0.04 | 2 | 48 | 70 | 45.8 | 12.7 |
| A | Father | *TP5* $sn^w/sn^{x2}$ | 62 | 1 | 0.02 | 3 | 30 | 48 | 28.5 | 11.7 |
| B | Mother | *TP5* $sn^w/sn^{x2}$ | 70 | 2 | 0.03 | 1 | 20 | 35 | 18.0 | 8.7 |
| A | Father | *TP6* $sn^w/+$ | 76 | 1 | 0.01 | 1 | 45 | 82 | 43.5 | 14.6 |
| A | Father | *TP6* $sn^w/sn^{x2}$ | 90 | 5 | 0.06 | 5 | 25 | 52 | 25.2 | 11.4 |
| B | Mother | *TP6* $sn^w/sn^{x2}$ | 134 | 4 | 0.03 | 1 | 15.5 | 42 | 15.2 | 9.2 |
| | | | | With Sexi.4 chromosomes | | | | | | |
| A | Father | $sn^w/+$ | 130 | 10 | 0.08 | 5 | 38 | 64 | 38.7 | 12.7 |
| A | Father | $sn^w/sn^{x2}$ | 113 | 113 | 1.00 | — | — | — | — | — |
| B | Mother | $sn^w/sn^{x2}$ | 86 | 86 | 1.00 | — | — | — | — | — |
| A | Father | *TP5* $sn^w/+$ | 84 | 3 | 0.04 | 4 | 46 | 66 | 45.4 | 10.4 |
| A | Father | *TP5* $sn^w/sn^{x2}$ | 91 | 86 | 0.95 | 1 | 1 | 2 | 1.2 | 0.4 |
| B | Mother | *TP5* $sn^w/sn^{x2}$ | 86 | 72 | 0.84 | 1 | 2 | 6 | 2.4 | 1.6 |
| A | Father | *TP6* $sn^w/+$ | 69 | 3 | 0.04 | 7 | 45 | 74 | 44.5 | 13.3 |
| A | Father | *TP6* $sn^w/sn^{x2}$ | 71 | 62 | 0.87 | 1 | 2 | 4 | 1.9 | 1.1 |
| B | Mother | *TP6* $sn^w/sn^{x2}$ | 56 | 36 | 0.64 | 1 | 3.5 | 17 | 4.7 | 4.4 |

[a] See text for details. Females were collected from cultures on day 13, incubated with males 2–9 days, and then assayed for fertility. The data from females of different ages were similar and have been pooled.
[b] Unweighted mean.
[c] Standard deviation.

males (cross A) and the two types of daughters, $sn^w/sn^{x2}$ and $sn^w/+$, were assayed for fertility. We also assayed $sn^w/sn^{x2}$ daughters from the reciprocal cross, *FM7,* $y^{31d}$ $sn^{x2}$ *B* males × $sn^w$ (or $sn^w$*/FM6*) females (cross B). The results of these experiments are summarized in Table 7.

As expected, the $sn^w/+$ heterozygotes produced many progeny, even when they carried Sexi.4 chromosomes. A vast majority of these females were fertile and, among those that were, the median number of progeny ranged from 38 to 48. These results are similar to those from the controls in Table 6. Different results were obtained from the $sn^w/sn^{x2}$ females, which were phenotypically weak singed. From cross A, most of the females with the Sexi.4 background were sterile, even when a telomeric *P* element was present (100% sterile without a *TP*, 95% sterile with *TP5*, and 87% sterile with *TP6*). By contrast, very few (<6%) of the $sn^w/sn^{x2}$ females without the Sexi.4 background were completely sterile. Although these females did produce fewer progeny (median numbers 24, 28, and 30) than the $sn^w/+$ controls, they produced many more progeny than the few fertile $sn^w/sn^{x2}$ females that had the Sexi.4 background (median numbers 1 and 2 for the *TP5* and *TP6* females, respectively). Thus, the sterility and reduced fertility of the $sn^w/sn^{x2}$ females appears to be caused by an adverse effect of the Sexi.4 background on the function of the $sn^w$ allele.

The $sn^w/sn^{x2}$ females from cross B provided an opportunity to observe suppression of singed sterility by maternally inherited telomeric *P* elements. Of course, without the Sexi.4 background, little sterility was seen. With the Sexi.4 background, the majority of the $sn^w/sn^{x2}$ females from cross B were sterile, even when a telomeric *P* element was present. However, for both *TP5* and *TP6* the sterility frequencies were slightly less than those seen with the corresponding females from cross A (by *z*-tests, $P < 0.05$ for *TP6*, but not for *TP5*). There were not enough fertile females to test for differences in the numbers of progeny produced by the A and B females. Thus, in these experiments, there is some evidence that maternal transmission of a telomeric *P* element suppresses singed sterility.

## DISCUSSION

The telomeric *P* elements *TP5* and *TP6* are strong repressors of *P*-element activity in the germ line. Both elements repress *P*-induced gonadal dysgenesis and transposase-catalyzed *P*-element excision. However, the repression abilities of these telomeric *P* elements are manifested only when they are transmitted maternally to the offspring of a dysgenic cross. Paternal transmission abolishes repression ability completely. This reciprocal-

cross effect is not seen in tests with *KP* elements or transgenes containing *KP* elements or with transgenes that produce other repressor polypeptides. Such elements and transgenes repress germ-line *P* activity when they are transmitted from either parent (Robertson and Engels 1989; Misra and Rio 1990; Simmons *et al.* 2002b). The reciprocal-cross effect seen with *TP5* and *TP6* therefore distinguishes their mode of repression from other types of *P*-element regulation. A similar reciprocal-cross effect is characteristic of cytotype. Thus, telomeric *P* elements appear to be associated with cytotype regulation of the *P*-element family.

Passage through the female germ line is a *sine qua non* for repression by telomeric *P* elements. However, the repression itself is not limited to the female germ line. Males that have inherited a telomeric *P* element from their mothers effectively repress gonadal dysgenesis and $sn^{w}$ mutability in their germ lines. The regulatory state associated with telomeric *P* elements is therefore transmitted maternally to offspring of both sexes. However, a telomeric *P* element that has passed from a female to a male will lose its repression ability if that element is transmitted to a male in the next generation. The regulatory state associated with telomeric *P* elements is therefore established and maintained in the female germ line.

Although maternally transmitted telomeric *P* elements are strong repressors of *P* activity in the germ line, they appear to be without effect in the soma. Some P polypeptides repress the somatic transposase activity encoded by the *P(ry*$^{+}$, *Δ2-3)99B* transgene (Robertson and Engels 1989; Misra and Rio 1990), and they also alter the somatic expression of *P*-insertion mutations such as $vg^{21\text{-}3}$ and $sn^{50e}$ (Williams *et al.* 1988; Robertson and Engels 1989). Furthermore, these effects are observed no matter which parent contributes the repressor-encoding *P* element in a cross. Chromosomes from P strains also show these somatic regulatory effects regardless of parental origin. By contrast, neither *TP5* nor *TP6* represses somatic transposase activity, nor does either of them alter the somatic phenotypes of *P*-insertion mutations. Ronsseray *et al.* (1991) also observed that telomeric *P* elements do not affect the somatic phenotype of a *P*-insertion mutation ($vg^{21\text{-}3}$). However, they found that structurally complete telomeric *P* elements partially repressed somatic transposase activity, even when the elements were paternally derived. This latter finding could be due to the 66-kD repressor polypeptide, which might have been produced in the soma by these *P* elements. Subsequent tests with these and other telomeric *P* elements yielded no evidence for repression of somatic transposase activity (Ronsseray *et al.* 1996; Marin *et al.* 2000). The absence of regulatory effects in the soma is one more feature that distinguishes telomeric *P* elements from other forms of *P*-element regulation.

In the course of this study, we found that the *H(hsp/CP)2* transgene, which is capable of producing the 66-kD repressor polypeptide in the soma, partially suppresses the phenotype of $sn^{50e}$, a *P*-insertion allele of the *singed* gene. Chromosomes from a P strain also had this effect, presumably because they carry *P* elements that encode the 66-kD or other repressor polypeptides. The mechanism of this suppression is unknown. However, it might involve the formation of a secondary structure in the DNA of the inserted *P* element. Hodgetts and O'Keefe (2001) have hypothesized that the terminal inverted repeats and internal inverted repeats in *P* elements can form a stem-loop (or "racket frame") structure that influences transcription through the *P* element. Further, they hypothesize that this structure is stabilized when repressor polypeptides bind to it. The *P* element in $sn^{50e}$ is inserted in the first exon of one of the *singed* transcription units, but in an orientation opposite to that of the *singed* gene. Transcription initiated at the *P*-element promoter would therefore be expected to interfere with the progress of transcription initiated at the *singed* promoter. In the presence of polypeptides such as the 66-kD repressor, transcription from the *P* promoter might be impaired by the formation of a racket frame in the *P* DNA, thereby allowing transcription to progress more easily from one of the *singed* promoters—most likely, the one that is far upstream and that is active at the time of bristle formation in somatic cells. Repressor polypeptide binding would therefore lead to better expression of the *singed* gene—that is, to a partial suppression of the mutant bristle phenotype.

Repressor P polypeptides also alter the expression of some *P*-insertion mutations in the germ line—for example, $sn^{w}$. This double *P* insertion of the *singed* gene is associated with reduced female fertility, but only when repressor P polypeptides are present (Robertson and Engels 1989). We found that the *P* elements in the M′ strain Sexi.4 evoke this "singed sterility" in $sn^{w}$ homozygotes and in $sn^{w}/sn^{x2}$ heterozygotes. Robertson and Engels (1989) also observed singed sterility in flies with chromosomes from M′ strains, including the strain from which Sexi.4 was derived. We cannot say what type of *P* element was responsible for this sterility, but one possibility is the *KP* element, which is present in the Sexi.4 genome (Simmons *et al.* 1990); complete *P* elements capable of producing the 66-kD repressor are not present in this genome. The KP (or some other) repressor polypeptide might facilitate the formation of stem-loop structures within either or both of the *P* elements inserted in $sn^{w}$, or it might facilitate the formation of stem-loops between these elements. Such structures might interfere with transcription initiated at the germ-line *singed* promoter immediately upstream of the *P*-insertion site. In the female germ line, this impaired transcription could be responsible for the sterility phenotype.

Our most significant finding, however, is that the singed sterility evoked by the Sexi.4 *P* elements is partially suppressed by telomeric *P* elements—more so in

$sn^w$ homozygotes than in $sn^w/sn^{x2}$ heterozygotes. Furthermore, this suppression is more effective when the telomeric *P* elements are transmitted from females in a cross. This reciprocal-cross effect, reminiscent of cytotype regulation, indicates that maternally transmitted telomeric *P* elements can control the synthesis or behavior of repressor P polypeptides either by silencing the expression of the *P* elements that encode these polypeptides or by altering the chromatin around the *P* elements with which these polypeptides interact. However, this control of repressor P polypeptides is not absolute. Heterozygous $sn^w/sn^{x2}$ females show high levels of singed sterility even though they have inherited a telomeric *P* element maternally (Table 7). Robertson and Engels (1989) made a similar observation with $sn^w/sn^{x2}$ females that were derived from P cytotype mothers. However, singed sterility is less frequent in $sn^w$ homozygotes that have inherited a telomeric *P* element maternally (Table 6). Thus, it appears that repressor P polypeptides have more difficulty inactivating two $sn^w$ alleles—as opposed to one—in the presence of a maternally inherited telomeric *P* element.

Singed sterility is also partially suppressed in homozygous $sn^w$ females that have inherited a telomeric *P* element paternally (Table 6). This result may seem at odds with the loss of repression ability when telomeric *P* elements are paternally transmitted. However, this loss is observed in father-to-son, *i.e.*, patroclinous, transmission. When transmission is from father to daughter, the telomeric *P* element is returned to a female germ line, where it might be able to reestablish some of its repression ability. The partial suppression of singed sterility seen in Table 6 may therefore be evidence for a revival of the regulatory ability of a telomeric *P* element that has passed through the male germ line.

Given what we have learned about *P*-insertion mutations such as $vg^{21\text{-}3}$, $sn^{50e}$, and $sn^w$, it seems appropriate to revise the terminology that has been used to describe them. Heretofore, these kinds of *P*-insertion mutations have been called "cytotype-dependent" alleles because their expression seemed to be conditioned on the cytotype of the fly. We believe there are several reasons for replacing this term with "repressor sensitive." First, cytotype regulation is limited to the germ line; *P*-insertion alleles with somatic phenotypes therefore cannot be cytotype dependent. Second, telomeric *P* elements, which are associated with the P cytotype, do not alter the germ-line phenotype of $sn^w$; thus, cytotype itself does not regulate the expression of this allele. Third, the expression of $vg^{21\text{-}3}$, $sn^{50e}$, and $sn^w$ is altered by *P* elements that encode repressor polypeptides; moreover, these alterations occur no matter which parent contributes the repressor *P* element in a cross—that is, they are cytotype independent. Fourth, the germ-line phenotype of $sn^w$ is altered by chromosomes from a strain with the M cytotype, presumably because these chromosomes carry *P* elements that encode some type of repressor polypeptide; thus, it is the repressor-encoding *P* elements, not the cytotype, that alter the $sn^w$ phenotype. Fifth, this alteration of phenotype is suppressed by maternally inherited telomeric *P* elements that are associated with the P cytotype; the P cytotype therefore actually reverses the phenotype of the repressor-sensitive allele.

At its inception, the analysis of hybrid dysgenesis defined two broad classes of strains, M and P, on the basis of their ability to induce and repress gonadal dysgenesis in pairwise crosses (Kidwell *et al.* 1977). A third category, Q, was also defined. Later, when the molecular basis of hybrid dysgenesis was discovered, one more category, M′, was added to the list (Bingham *et al.* 1982). True M strains, which are devoid of *P* elements, have the M cytotype. P and Q strains, which carry *P* elements in their genomes, have the P cytotype. In crosses to M females, P males are strong inducers of hybrid dysgenesis, whereas Q males are very weak inducers. Both P and Q strains are strong repressors of hybrid dysgenesis when they contribute maternally in a cross. M′ strains possess *P* elements in their genomes but instead of the P cytotype, they have a variable ability to repress hybrid dysgenesis.

The regulation of germ-line *P*-element activity in P, Q, and M′ strains involves a complex blend of mechanisms. In some strains, *P* elements may be quiescent due to the absence of complete *P* elements that can produce the transposase. In other strains, repressor P polypeptides may regulate transposase activity. Studies suggest that this form of regulation does not depend on the parental origin of the repressor *P* elements and that it has rather modest effects. In P and Q strains, *P* activity is repressed by the maternally inherited P cytotype. Although the mechanistic basis of this condition is still unclear, it appears to be associated with *P* elements inserted near the left telomere of the *X* chromosome. It seems unlikely that the strong regulatory abilities of these elements are mediated by repressor polypeptides (Stuart *et al.* 2002). It is not known if *P* elements inserted at other genomic locations, including other telomeres, can bring about the P cytotype. However, *P* transgenes inserted near autosomal telomeres and a repeated array of *P* transgenes inserted at a nontelomeric autosomal location have some ability to repress *P* activity or to enhance repression of this activity by *X*-linked telomeric *P* elements (Ronsseray *et al.* 1998, 2001). Thus, *P* elements at different chromosomal locations may be able to contribute to the P cytotype.

Mark Liszewski provided technical help. Funding was provided by National Institutes of Health grant GM-40263, the University of Minnesota Graduate School, and the Minnesota Medical Foundation.

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Communicating editor: R. S. Hawley