Telomeric *P* elements Associated With Cytotype Regulation of the *P* Transposon Family in *Drosophila melanogaster*

Jeremy R. Stuart, Kevin J. Haley, Douglas Swedzinski, Samuel Lockner, Paul E. Kocian, Peter J. Merriman and Michael J. Simmons[1]

Department of Genetics, Cell Biology and Development, University of Minnesota, Twin Cities, St. Paul, Minnesota 55108

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ABSTRACT

P elements inserted at the left end of the Drosophila *X* chromosome were isolated genetically from wild-type P strains. Stocks carrying these elements were tested for repression of P-strain-induced gonadal dysgenesis in females and for repression of transposase-catalyzed *P*-element excision in males and females. Both traits were repressed by stocks carrying either complete or incomplete *P* elements inserted near the telomere of the *X* chromosome in cytological region 1A, but not by stocks carrying only nontelomeric *X*-linked *P* elements. All three of the telomeric *P* elements that were analyzed at the molecular level were inserted in one of the 1.8-kb telomere-associated sequence (TAS) repeats near the end of the *X* chromosome. Stocks with these telomeric *P* elements strongly repressed *P*-element excision induced in the male germline by a P strain or by the transposase-producing transgenes *H(hsp/CP)2*, *H(hsp/CP)3*, a combination of these two transgenes, and *P(ry*$^+$, *Δ2-3)99B*. For *H(hsp/CP)2* and *P(ry*$^+$, *Δ2-3)99B*, the repression was also effective when the flies were subjected to heat-shock treatments. However, these stocks did not repress the somatic transposase activity of *P(ry*$^+$, *Δ2-3)99B*. Repression of transposase activity in the germline required maternal transmission of the telomeric *P* elements themselves. Paternal transmission of these elements, or maternal transmission of the cytoplasm from carriers, both were insufficient to repress transposase activity. Collectively, these findings indicate that the regulatory abilities of telomeric *P* elements are similar to those of the P cytotype.

TRANSPOSABLE elements are ubiquitous components of prokaryotic and eukaryotic genomes. In many species of eukaryotes they account for a significant fraction of the DNA—in humans, for example, nearly 45% (International Human Genome Sequencing Consortium 2001). Many different types of transposable elements have been identified. One large class comprises the elements that have come to be called the "cut-and-paste" transposons. These elements are excised from one position in the genome and inserted into another by an element-encoded enzyme called the transposase. The *P* elements of Drosophila are among the most thoroughly studied cut-and-paste transposons (Engels 1989). These elements are found in natural populations of *Drosophila melanogaster*, but not in long-standing laboratory stocks, probably because they invaded the genome of this species sometime during the twentieth century (Kidwell 1983; Engels 1997).

The excision and insertion of *P* elements is catalyzed by an 87-kD transposase (Rio 1990). The gene for this enzyme comprises four exons numbered 0–3. In the germline, these exons are spliced together in the RNA transcribed from *P* elements to create an mRNA that encodes the transposase. In the somatic cells the last two exons remain separated by an intron to form an mRNA that is translated into a catalytically inactive 66-kD polypeptide instead of the transposase (Laski *et al.* 1986). Tissue-specific expression of the transposase therefore restricts *P*-element activity to the germline. However, this restriction can be bypassed if the last intron is deleted from the transposase gene. *P* elements with this modification produce the transposase in the soma as well as in the germline. Stable transgenes that express the P transposase include *H(hsp/CP)2* and *H(hsp/CP)3* (Simmons *et al.* 2002a), which were inserted in the genome by *hobo* transposable elements, and *P(ry*$^+$, *Δ2-3)99B* (Robertson *et al.* 1988), which was inserted by a *P* element. *P(ry*$^+$, *Δ2-3)99B* produces the transposase in the soma as well as in the germline. However, the peculiar structure of this insertion prevents it from being excised or transposed (Robertson 1996).

Like most cut-and-paste transposons, *P* elements have inverted repeats at their termini. The transposase interacts with these sequences and others nearby to catalyze transposition (Rio 1990; Beall and Rio 1997). The largest *P* elements, called complete *P* elements, are 2907 bp long; incomplete *P* elements are shorter because internal sequences have been deleted (O'Hare and Rubin 1983). Only complete *P* elements encode the transposase. However, most incomplete *P* elements can still be excised and transposed if a transposase-produc-

[1]*Corresponding author:* Department of Genetics, Cell Biology and Development, 140 Gortner Laboratory, 1479 Gortner Ave., University of Minnesota, St. Paul, MN 55108. E-mail: simmo004@tc.umn.edu

ing complete *P* element is present in the genome (Engels 1984).

When transposable elements are mobilized, they cause mutations and chromosome breakage. Selection against these harmful effects provides a basis for the evolution of mechanisms to repress transposable element activity. The mechanisms that regulate the *P* elements of Drosophila have been studied for many years. In the germline *P* element activity is regulated by a maternally transmitted condition called the P cytotype (Engels 1979a). Genetic analyses have shown that this condition depends on the *P* elements themselves and that it is characteristic of most, but not all, strains that have *P* elements in their genomes (Bingham *et al.* 1982). The M cytotype, a complementary condition that permits *P*-element activity, is characteristic of strains that lack *P* elements. When P males are crossed to M females, *P* elements are activated in the germlines of the hybrid progeny because the paternally inherited *P* elements encounter the maternally inherited M cytotype. *P*-element activity in these progeny is detected as increased mutation frequencies, chromosome breakage, and sterility. These germline abnormalities have been consolidated into a syndrome called hybrid dysgenesis (Kidwell *et al.* 1977). The offspring of crosses between P females and M males usually do not exhibit hybrid dysgenesis because they inherit the P cytotype, which represses *P*-element activity, from their mothers.

Although the P cytotype is maternally transmitted, it depends absolutely on the *P* elements themselves. When chromosomes bearing *P* elements are removed from the genome by segregation in females that had P-cytotype mothers, the cytotype switches abruptly from P to M (Sved 1987). Most attempts to explain the P cytotype have assumed that it involves polypeptides encoded by *P* elements. The 66-kD polypeptide produced through alternate splicing of complete *P*-element transcripts has been proposed as a candidate. This polypeptide appears to be made in the germline as well as in the soma, and transgenes designed to express it confer some ability to repress transposase activity (Misra and Rio 1990). In addition, a polypeptide encoded by a geographically widespread incomplete *P* element, called *KP*, has been shown to repress transposase activity (Black *et al.* 1987; Rasmusson *et al.* 1993; Andrews and Gloor 1995). These and possibly other *P*-encoded polypeptides could therefore be responsible for the P cytotype. However, genetic analyses suggest that neither the KP nor the 66-kD polypeptides, separately or together, are able to repress transposase activity as strongly as the P cytotype (Simmons *et al.* 2002a,b). Furthermore, unlike repression by the P cytotype, repression by the KP or 66-kD polypeptides does not require maternal transmission of the elements encoding them.

Other analyses have revealed that *P* elements inserted near the left telomere of the *X* chromosome are powerful regulators of the entire *P* family. The first indication that telomeric *P* elements might be involved in *P* regulation was obtained by Kidwell (1981), who showed that a distal segment from the *X* chromosome of the Mount Carmel P strain was able to repress hybrid dysgenesis. Subsequently, Peterson *et al.* (1986) identified an incomplete *P* element in this chromosomal segment. Ronsseray *et al.* (1991) analyzed telomeric *P* elements derived from the *X* chromosome of the Lerik P strain. Because these strong regulators of the *P* family were complete *P* elements, it was thought that they might preferentially produce the 66-kD repressor polypeptide (Ronsseray *et al.* 1996). Later, an incomplete *P* element at the telomere of the *X* chromosome from the Nasr'Allah P strain was found to regulate the *P* family (Marin *et al.* 2000). In addition, apparently noncoding, telomeric *P* transgenes were observed to enhance the regulatory effects of naturally occurring *P* elements (Ronsseray *et al.* 1998). These intriguing findings have prompted a reassessment of long-standing hypotheses about the P cytotype.

To elucidate the role that telomeric *P* elements might play in the P cytotype, we isolated these types of elements from different P strains. Each element was analyzed structurally and genetically and tested for its ability to mimic the P cytotype. The results indicate that both complete and incomplete telomeric *P* elements have a cytotype-like ability to regulate the *P* family.

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. Heat shocks were administered to flies throughout their development and reproductive periods by placing cultures or storage vials in a cabinet incubator at 37° for 40 min each day.

Genetic isolation of the telomeric region at the left end of the *X* chromosome from wild-type P strains: To isolate the telomeric region from different P strains (hereafter referred to as the TP region), P females from different wild-type strains were crossed to M males that were hemizygous for mutations in genes spanning the genetic map of the *X* chromosome: *yellow* (*y*) body, *white* (*w*) eyes, *miniature* (*m*) wings, and *forked* (*f*) bristles. The *TP +/y w m f* F_1 daughters from these crosses were then mated to M males homozygous for the autosomal eye color mutations *brown* (*bw*) eyes and *scarlet* (*st*) eyes, located on chromosomes *2* and *3*, respectively. Among the F_2 progeny, *TP + w m f* males resulting from recombination between the *y* and *w* loci were collected and crossed individually to *C(1)DX, y f; bw; st* females from an M strain. Because these females carried attached-*X* chromosomes, the *TP + w m f X* chromosome was transmitted patroclinously to the offspring. Single *TP + w m f* sons carrying the *bw* and *st* markers were then backcrossed to *C(1)DX, y f; bw; st* females for two generations to fix these markers and thereby clear the genotype of major autosomes derived from the original wild-type strains. No effort was made to control the segregation of chromosome *4* in these crosses. The *TP + w m f X* chromosomes derived from this procedure were then made homozygous or balanced in stocks by using an *FM7 X* chromosome from an M strain.

Each TP stock was subsequently analyzed by Southern blotting and PCR amplification to determine if it contained *P* elements.

Synthesis of *TP sn^w* stocks: *TP y$^+$ w m f* males were crossed to *y sn^w* females carrying the double *P*-element insertion mutation *weak singed* (*sn^w*) bristles (Engels 1979b), and their *TP y$^+$ w m f/y sn^w* daughters were crossed to *y w^{67c23}* males. Recombinant sons with the genotype *TP y$^+$ w sn^w m$^+$ f$^+$* were then crossed individually to *C(1)DX, y f* females to establish stocks in which the distal part of the *X* chromosome was derived from the original TP stock. These chromosomes were made homozygous by using the *FM6, l(1)^{69a}* balancer chromosome. All the *TP sn^w X* chromosomes synthesized in this manner carried the *w* marker but lacked the *y*, *m*, and *f* markers.

Gonadal dysgenesis assay for *P*-element activity: Gonadal dysgenesis (GD) was induced by crossing females from a particular strain to males from a standard P strain. The crosses were initially mass matings at 21°, but after 2 days, the females were placed into individual cultures that were incubated at 29°; these females were allowed to lay eggs for 5–7 days. The F_1 flies that emerged in these cultures before day 14 were transferred to fresh cultures and allowed to mature at 21° for 2 days, after which a sample of the females among them were squashed between two glass slides to determine if they carried eggs. Females that did not were judged to have GD. The percentage of GD was used as a measure of a strain's ability to repress *P*-element activity. Observed differences were evaluated for statistical significance by the Mann-Whitney rank-sum test.

Mutability assay for *P*-element activity: Transposase-catalyzed excision of one or the other of the *P* elements inserted in the double *P* mutation *sn^w* creates *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 male germline, males carrying this allele and a source of the P transposase were mated individually to three to four *C(1)DX, y f* females; the sons of these matings were then classified for bristle phenotype and counted, and the frequency of sn$^+$ and sn^e flies among them was used to estimate the *sn^w* mutation rate. When the *P(ry$^+$, Δ2-3)99B* transgene was used to destabilize *sn^w*, 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). To determine the mutability of *sn^w* in the female germline, females heterozygous for this allele and a source of the P transposase were mated individually to *y sn^3 v car* males; the progeny of these matings were classified as sn^w or sn^e (the sn$^+$ progeny were ignored because of the preexisting *sn$^+$* allele in the mother's genotype), and the proportion that were sn^e was used to estimate the *sn^w* mutation rate. Progeny in the *sn^w* mutability assays were counted up to the seventeenth day after the test cultures were started (see Simmons *et al.* 2002 for further details). *Z*-tests were used to assess the statistical significance of observed differences in mutation rates.

Manipulation and analysis of DNA: Standard procedures were used to extract, clone, and analyze DNA. Southern blotting and PCR procedures are summarized in Simmons *et al.* (2002a,b). A genomic DNA library was constructed by ligating *Xba*I genomic restriction fragments from the TP5 stock into the *Xba*I site of the λGEM11 bacteriophage vector. This library was screened with a ^{32}P-labeled probe derived from the PCR product of a complete *P* element and a single clone containing a 16-kb insert was isolated. The insert was transferred into the plasmid pBS-SK for analysis. DNA sequencing was performed by a campus facility using samples obtained from plasmids or PCR amplifications of genomic DNA. *In situ* hybridizations of a biotinylated *P*-element probe to polytene chromosome squashes from larval salivary glands was carried out according to Spradling *et al.* (1995); the probe was obtained by labeling DNA from a plasmid (pBWC) containing a terminally truncated but otherwise complete *P* element.

DNA primers: The inverted repeat (IR) and *KP*-specific primers were described by Rasmusson *et al.* (1993). The primer used in conjunction with the *KP*-specific primer was 66d (nucleotides 66–85 in the canonical *P* sequence given by O'Hare and Rubin 1983), which primes DNA synthesis toward the 3′ end of the *P* element. The *TP5*-specific (nucleotides 424–437/TG/1524–1528) and *TP6*-specific (nucleotides 818–832/G/1817–1821) primers span the respective deletions in these elements and prime DNA synthesis toward the 3′ end of the *P* element. The primer used in conjunction with the *TP5*- and *TP6*-specific primers was 3′in (nucleotides 2872–2851), which primes DNA synthesis toward the 5′ end of the *P* element. Primers complementary to sequences in the 1.8-kb telomere-associated sequence (TAS) repeat of the *X* chromosome were TAS-A (5′-CACCGGCAAGAACAAAACG-3′, nucleotides 110–128 and 966–984 in the 1.8-kb TAS repeat sequence given by Karpen and Spradling 1992), TAS-B (5′-GCTGCGTGAGG TCCGATC-3′, nucleotides 378–361 and 551–534), and TAS-C (5′-GCCTCTGCCGCAGCGCTC-3′, nucleotides 395–412, 568–585, and 729–746). TAS-A and TAS-C prime DNA synthesis away from the beginning of the 1.8-kb TAS repeat and toward the centromere of the *X* chromosome; TAS-B primes DNA synthesis toward the beginning of the 1.8-kb TAS repeat and toward the telomere of the *X* chromosome. The *P*-element primers that were used in conjunction with the TAS primers were 261s (with TAS-A), 1228d (with TAS-B), and 318d, 780s, and 2575s (with TAS-C). These oligonucleotides prime DNA synthesis toward the 5′ end of the *P* element (primers denoted by "s") or toward the 3′ end of the *P* element (primers denoted by "d"); the position of the primer's 5′ nucleotide in the *P*-element sequence is given by the primer's number.

RESULTS

Genetic isolation and molecular characterization of telomeric *P* elements: *P* elements inserted at or near the left telomere of the *X* chromosome were isolated genetically by selecting *y$^+$ w m f* recombinants among the progeny of *+/y w m f* females derived from crosses between wild-type P females and *y w m f* M males. Because the *y* and *w* loci are close to the left telomere of the *X* chromosome, only the distalmost segment of the recombinant *X* chromosome was expected to come from the P strain. Eight inbred, wild-type P strains representing five different natural populations in the midwestern and eastern United States were used to obtain the recombinant *X* chromosomes (Table 1). Two of these populations (MC and ν_6) had previously been shown to carry *P* elements inserted in the region of interest (Simmons *et al.* 1984; Peterson *et al.* 1986). Nothing was known about the genomic distribution of the *P* elements in the other populations. Each of the recombinant *X* chromosomes was made homozygous or balanced in an M genetic background, and the resulting stocks were designated as telomeric P (TP) stocks. Nine such stocks were obtained. TP1–TP6 were homozygous viable and fertile; TP7–TP9 were maintained with the *FM7* balancer chromosome because of poor homozygous fertility.

TABLE 1

TP stocks in which the left telomeric region of the *X* chromosome was derived from a wild-type P strain

P strain[a]	Location	Year collected	Reference	Derived TP stock
Edina	Edina, MN	1994	This article[b]	TP1
P-4	Pittsburgh, PA	1978	Kocur *et al.* (1986)	TP2
P-5	Pittsburgh, PA	1978	Kocur *et al.* (1986)	TP3
Mtk-1	Minnetonka, MN	1994	This article[c]	TP4
ν_6-E	Madison, WI	1975/1989[d]	Engels and Preston (1981)	TP5
MC	Mt. Carmel, IL	1970	Kidwell (1981)	TP6
ν_6-A	Madison, WI	1975/1989[d]	Engels and Preston (1981)	TP7, TP8[e]
ν_6-D	Madison, WI	1975/1989[d]	Engels and Preston (1981)	TP9[e]

The wild-derived segment was distal to the *w* gene located in cytological band 3C2 on the polytene *X* chromosome.

[a] Nine other strains representing seven other populations were unsuccessfully screened for wild-derived segments of the *X* chromosome distal to *w*.

[b] Collected by Dieter Roggy.

[c] Collected by Craig Grimes.

[d] Sublines of the ν_6 strain were established in 1989.

[e] Maintained with the *FM7* balancer because females homozygous for the TP *X* chromosome have reduced fertility.

Each of the TP stocks was analyzed by Southern blotting to determine if *P* elements were present. Genomic DNA was digested with *Bam*HI, which does not cleave within the *P*-element sequence, fractionated by electrophoresis, and hybridized with a radiolabeled *P*-element probe. The resulting autoradiogram (Figure 1A) revealed that *P* elements were present in all nine of the TP stocks. Under the assumption that each hybridizing band represents a restriction fragment with a single *P* element, five of the stocks (TP1, TP2, TP5, TP6, and TP9) contained just one element, two stocks (TP3 and TP4) contained two elements, and two stocks (TP7 and TP8) contained three elements.

To determine the sizes of the *P* elements isolated in the TP stocks, genomic DNA templates from each stock were amplified by PCR using a primer (IR) complementary to a sequence in the terminal inverted repeats of *P* elements. Eight of the nine stocks yielded PCR products (Figure 1B); 2.9-kb-long products, indicating the presence of complete *P* elements, were obtained from two stocks (TP1 and TP4), and smaller products, indicating the presence of incomplete *P* elements, were obtained from six stocks (TP3, TP5, TP6, TP7, TP8, and TP9). Because no PCR product was obtained from TP2 in this type of amplification, the *P* element present in TP2 must have at least one abnormal terminal inverted repeat. Attempts to amplify this element with other *P*-element primers were also unsuccessful. Analysis of the TP2 stock was therefore discontinued.

An ~1.1-kb-long IR PCR product was obtained from three of the stocks (TP3, TP7, and TP8). The size of this product suggested that these stocks might carry a geographically widespread incomplete *P* element called *KP* (Black *et al.* 1987; Jackson *et al.* 1988), which is known to be involved in regulation of the *P*-element family. To test this possibility, template DNA from each of these three stocks was PCR amplified with a primer that spans the internal deletion characteristic of *KP* elements; a second primer in the 5′ region of the *P* sequence was used as a partner with this *KP*-specific primer. In all three cases, as well as in a control using template DNA from a known *KP*-containing strain, the expected PCR product was obtained (data not shown). By contrast, this product was not obtained when DNA from any of the other TP stocks was amplified using the *KP*-specific primer and its upstream partner. Thus *KP* elements were present in TP3, TP7, and TP8 but not in the other TP stocks.

By combining the results of the Southern and PCR analyses, and by noting from which populations the TP stocks had been derived, it was possible to infer the basic features of the *P* elements that had been isolated in these stocks. TP1 contains a single, apparently complete *P* element that could encode the P transposase. TP3 contains two *P* elements, both apparently *KP* elements. TP4 contains two *P* elements, both apparently complete and capable of encoding the P transposase. TP5 contains a single incomplete *P* element ~1.8 kb long. TP6 contains a single incomplete *P* element ~1.9 kb long. TP7 and TP8 both contain the same three incomplete *P* elements, at least one of which is a *KP* element. Finally, TP9 appears to contain the same *P* element as TP5.

The IR PCR products from three of the incomplete *P* elements in the TP stocks were sequenced. As expected, the 1.1-kb-long element in TP3 was a *KP* element with a deletion from bp 810 to 2562. The 1.8-kb-long element in TP5 proved to have a deletion from bp 438

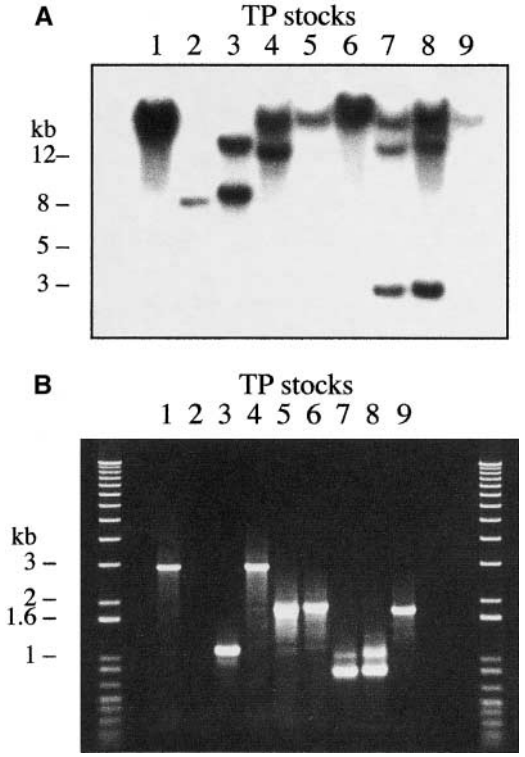

FIGURE 1.—(A) Southern blot of *Bam*HI-digested genomic DNA from nine TP strains. The blot was hybridized with a ^{32}P-labeled probe made by randomly priming DNA synthesis from a clone containing a terminally truncated but otherwise complete *P* element. (B) PCR products of the *P* elements in nine TP strains. A primer complementary to sequences in the inverted terminal repeats was used to amplify genomic DNA from each of the strains. The products were analyzed by agarose gel electrophoresis.

to 1523 with a TG dinucleotide inserted between the deletion breakpoints, and the 1.9-kb-long element in TP6 proved to have a deletion from bp 833 to 1816 with a single G inserted between the deletion breakpoints. From this information it was possible to construct element-specific primers spanning the deletions in the *TP5* and *TP6* elements. These primers were then used with an appropriate partner primer to amplify template DNA from other Drosophila strains (data not shown). The *TP5*-specific primer was able to amplify DNA from TP5 and TP9, but not from any of the other TP stocks. Thus, as suspected, TP9 appears to contain the same *P* element as TP5. The *TP5*-specific primer was also able to amplify DNA from the two v_6 stocks from which the TP5 and TP9 stocks were derived. The *TP6*-specific primer amplified DNA from TP6 and the MC stock from which it was derived. It did not amplify DNA from any of the other TP stocks. The *TP5*- and *TP6*-specific primers were also used to screen 91 wild-type stocks derived from natural populations from all over the world for the *TP5* and *TP6* elements. None of the tested stocks contained either of these elements.

Cytological localization of the *P* elements in the TP stocks: The isolated *P* elements in six of the TP stocks (TP1, TP3–TP7) were analyzed by *in situ* hybridization of a *P*-element probe to polytene chromosomes from larval salivary glands. TP8 and TP9 were omitted from this analysis because they were apparently identical to TP7 and TP5, respectively. The stocks used in these labeling experiments carried the *X*-linked *P*-insertion mutation sn^w, which served as a positive control for the hybridization signal. This mutation had been introduced into the *TP X* chromosomes by recombination. During the creation of these recombinants, it became clear that sn^w was unstable in both the TP1 and the TP4 stocks, indicating that the complete *P* elements in these stocks could produce the P transposase. Although a largely stable stock of *TP1* sn^w was eventually obtained, it was not possible to establish a stable *TP4* sn^w stock. Consequently, the TP4 stock that was analyzed by *in situ* hybridization did not carry sn^w. Table 2 summarizes the results of the *in situ* hybridization experiments.

All the stocks showed hybridization at sites near the left end of the *X* chromosome. The TP3 *X* chromosome showed hybridization signal at only one such site (2C1-2). However, two *P*-hybridizing bands were seen in the initial Southern analysis of TP3; thus, this site must

TABLE 2

Cytological localization of *P* elements in the TP stocks by *in situ* hybridization of a *P*-element probe to polytene chromosome squashes

Stock[a]	Cytologically labeled loci[b]	*P* elements present
TP1	1A1-4; 1B9-10; 60F5	Complete *P*
TP3	2C1-2	*KP* element
TP4	Two sites in 1A	Complete *P*
TP5	1A1-4	1.8-kb element in which nucleotides 438–1523 are replaced by TG
TP6	1A1-4	1.9-kb element in which nucleotides 833–1816 are replaced by G
TP7	1B1-2; 1E5-F1; 2B1-2; 2B17-18	*KP* element and other incomplete elements

[a] Except for TP4, all stocks carried the sn^w mutation.
[b] Excluding 7D, which contains the *singed* locus.

TABLE 3

Effect of the TP stocks on gonadal dysgenesis induced by moderate and strong P strains

	Still2 inducer			Harwich-w inducer		
TP stock	No. of vials	No. of flies	% GD ± SE[a]	No. of vials	No. of flies	% GD ± SE[a]
y w M control	45	891	67.0 ± 2.5	80	1528	99.9 ± 0.1
TP1	27	416	12.7 ± 3.6*	32	252	64.0 ± 6.6*
TP3	24	242	66.9 ± 5.4	30	479	99.3 ± 0.5
TP4	16	287	25.9 ± 6.2*	33	315	49.9 ± 6.5*
TP5	37	577	19.2 ± 3.2*	44	435	84.6 ± 2.8*
TP6	24	312	4.8 ± 1.5*	35	350	28.7 ± 4.2*
TP7/*FM7*[b]	22	269	79.1 ± 3.0	30	360	100.0 ± 0.0
TP8/*FM7*[b]	23	244	61.1 ± 5.5	29	380	99.7 ± 0.2
TP9/*FM7*[b]	20	210	58.7 ± 6.0	30	405	99.4 ± 0.4
Harwich-w P control	23	440	1.1 ± 0.9*	20	302	0.9 ± 0.6*

*Significantly less than M control value by the Mann-Whitney rank-sum test.
[a] Unweighted mean percentage of GD ± standard error.
[b] Only non-*FM7* daughters scored for GD.

contain two *P* elements, both apparently *KP*s. The other *KP*-containing stock, TP7, showed four hybridizing sites near the left end of the *X* chromosome. However, only three *P*-hybridizing bands were seen in the initial Southern analysis of TP7; thus, one of these bands must have contained two *P* elements. None of the cytologically localized *P* elements in TP3 or TP7 was inserted at or near the telomere.

Four TP stocks (TP1, TP4, TP5, and TP6) had *P* elements in the distalmost cytological region (1A) of the *X* chromosome, which includes the telomere. TP4 had two labeled sites in this region; TP1, TP5, and TP6 each had one. The TP1 stock had two additional hybridization sites: 1B9-10, which is a nontelomeric locus on the *X* chromosome, and 60F5, which is near the telomere of chromosome *2R*. The initial Southern analysis of TP1 revealed only one *P*-hybridizing band. Thus, these additional *P* elements may have been generated by transposition in the *TP1 sn*^{w} stock.

Molecular positioning of the *P* elements in cytological region 1A: The molecular positions of the *X*-linked *P* elements in the TP1, TP5, and TP6 stocks were determined by sequencing cloned or PCR-amplified genomic DNA. The *TP5* element was cloned in a 16-kb *Xba*I fragment isolated from a genomic DNA library constructed from the TP5 strain. Sequencing established that the cloned *P* element was identical to the element found by sequencing PCR-amplified DNA from TP5 and that it was situated within one of the 1.8-kb TAS repeats of the *X* chromosome (see, for example, bp 2372–4243 in the subtelomeric DNA sequence given by Karpen and Spradling 1992). Analysis of the clone indicated that the *TP5* element was inserted in the second of three 161- to 173-bp-long subrepeats within the 1.8-kb TAS repeat. Counting from the start of the 1.8-kb repeat, the *TP5* element was positioned between bp 479 and 486 (the 8-bp target site duplication) with the 5′ end of the element oriented toward the telomere.

With the expectation that the telomeric *P* elements in the TP1 and TP6 stocks might also be inserted in TAS repeats, oligonucleotide primers complementrary to these repeats were used in conjunction with primers complementary to the *TP1* and *TP6* elements to amplify genomic DNA by PCR. Products were obtained from the TP6 stock using the TAS-C and 318d primers and the TAS-A and 261s primers. DNA sequencing of these products indicated that the *TP6* element was inserted at the same position and in the same orientation within a 1.8-kb TAS repeat as the *TP5* element was. PCR products were obtained from the *TP1 sn*^{w} stock using the TAS-B and 1228d primers and the TAS-C and 780s primers. DNA sequencing of these products indicated that the *TP1* element was inserted between nucleotides 255 and 262 (the 8-bp target site duplication) near the beginning of the first 173-bp-long subrepeat (nucleotides 252–425) within a 1.8-kb TAS repeat with the element's 5′ end oriented toward the telomere.

Tests for regulation of *P* elements by the TP stocks: Each of the eight TP stocks was tested for repression of GD induced by moderate (Still2, listed as S2 in Kocur *et al.* 1986) and strong (Harwich-w, see Kidwell *et al.* 1977) P strains (Table 3). These strains induced 67.0 and 99.9% GD, respectively, in tests with an M strain control (*y w*). By contrast, they induced virtually no GD in tests with a P strain control (Harwich-w). Four of the TP stocks (TP1, TP4, TP5, and TP6) repressed the GD induced by each of these P strains. The other four TP stocks did not. TP6 was the strongest repressor in both sets of tests. TP9, which apparently contains the same *P* element as TP5, did not repress GD in either set of tests, possibly because the *TP9 X* chromosome carries fertility-reducing factors that prevent it from being maintained in homozygous condition.

TABLE 4

Effect of the TP stocks on *sn*^{w} mutability induced in the male germline by different transposase sources

	Harwich-w inducer						*H(hsp/CP)2* inducer					
TP stock[a]	No. of vials	*sn*^{w}	sn^{+}	sn^{e}	Total	Mutation rate[b]	No. of vials	*sn*^{w}	sn^{+}	sn^{e}	Total	Mutation rate[b]
M control	49	905	211	215	1331	0.323 ± 0.012	50	537	332	324	1193	0.549 ± 0.019
TP1	49	1370	0	0	1370	0.000 ± 0.000	49	805	0	2	807	0.002 ± 0.001
TP3	49	683	262	232	1177	0.429 ± 0.019	49	342	380	292	1014	0.677 ± 0.018
TP5	49	1716	4	3	1723	0.004 ± 0.002	50	1666	0	1	1667	0.001 ± 0.001
TP6	49	1485	9	10	1504	0.012 ± 0.007	50	1425	23	27	1475	0.034 ± 0.009
TP7	50	678	166	151	995	0.325 ± 0.019	50	550	336	303	1189	0.523 ± 0.026
TP8	50	739	206	177	1122	0.343 ± 0.021	43	494	232	225	951	0.488 ± 0.032
TP9	50	1667	1	2	1670	0.002 ± 0.001	50	1534	7	1	1542	0.005 ± 0.004

[a] All stocks carried *w sn*^{w}.
[b] Unweighted mean proportion (sn^{+} + sn^{e}) among total ± standard error.

All the TP stocks except TP4 were also tested for repression of transposase-catalyzed excisions of the *P* elements in the *sn*^{w} allele. This double *P*-element insertion mutation of the *singed* gene is a sensitive target for transposase activity. The *sn*^{w} allele was recombined into the *TP X* chromosomes to produce homozygous *TP sn*^{w} stocks, which were all viable and fertile. The fertility-reducing factors that were present in the original TP7, TP8, and TP9 stocks were evidently lost during the synthesis of these recombinant stocks. Three sets of experiments were initially carried out to test for TP-mediated repression of *sn*^{w} mutability (Table 4). In the first set, mutability was induced in the germlines of F_1 males from crosses between *TP sn*^{w} females and Harwich-w males (Table 4). The mutation rate of males from the control crosses (*w sn*^{w} females × Harwich-w males) was 0.323, which is consistent with previous estimates (Simmons *et al.* 2002a). Males from the crosses involving TP3, TP7, and TP8 showed mutation rates as great or greater than those of the control rate, indicating that the isolated *P* elements in these three stocks were not able to repress *sn*^{w} mutability in the male germline. Males from the crosses involving TP1, TP5, TP6, and TP9 showed mutation rates close to or equal to zero. Thus, the isolated *P* elements in these four stocks were powerful repressors of Harwich-w-induced transposase activity in the male germline.

In the second set of experiments, *sn*^{w} mutability was induced in the male germline by the *H(hsp/CP)2* transgene (Table 4), a stable source of the P transposase located within a *hobo* transposon inserted on chromosome *2* (Simmons *et al.* 2002a). Although the transposase gene in this insertion is fused to a heat-shock gene promoter, no heat shock is needed to stimulate its expression. In control males, this transgene induced a high level of *sn*^{w} mutability, 0.549, which is consistent with previous estimates (Simmons *et al.* 2002a). In males from the TP1, TP5, TP6, and TP9 stocks, it induced almost no *sn*^{w} mutability. The highest rate was 0.034 for TP6; the other rates were close to zero. Thus, the isolated *P* elements in these four stocks were strong repressors of *H(hsp/CP)2*-induced transposase activity in the male germline. The other TP stocks, TP3, TP7, and TP8, did not repress *H(hsp/CP)2*-induced *sn*^{w} mutability in the male germline.

In the third set of experiments, *sn*^{w} mutability was induced by the *H(hsp/CP)2* transgene in the female germline (Table 5). The *sn*^{w} mutation rate in control females carrying the transgene was 0.146. (Note that this rate is not directly comparable to the mutation rate in males because the pseudo-wild derivatives of *sn*^{w} cannot be enumerated in experiments to measure the female mutation rate.) For the TP1, TP5, TP6, and TP9 stocks, the mutation rate in females was close to zero. Thus the same four stocks that repressed transposase activity in the male germline also did so in the female germline. The TP7 and TP8 stocks, which carried duplicate isolates of a TP region from one of the original strains, showed a tendency to repress *H(hsp/CP)2*-induced *sn*^{w} mutability in the female germline (mutation rate is 0.105); however, even when the results from these two stocks were pooled, the observed mutation rate was not significantly less than the control rate. Finally, the TP3 stock showed no ability to repress *sn*^{w} mutability in the female germline.

From these results it is clear that both complete and incomplete *P* elements can be effective repressors of transposase activity, no matter if the activity is induced by naturally occurring complete *P* elements or by a transposase-encoding transgene. Furthermore, this repression is seen in both male and female germlines. Two different types of incomplete *P* elements—*TP5* (also apparently present in the TP9 stock) and *TP6*—can repress transposase activity. Finally, all the TP stocks that repressed transposase activity had *P* elements inserted at or near the telomere of the *X* chromosome. A telomeric location of the *P* element therefore appears to be important for repression ability.

TABLE 5

Effect of the TP stocks on *sn*^{w} mutability induced in the female germline by the *H(hsp/CP)2* transposase source

TP stock[a]	No. of vials	*sn*^{w}	sn^{e}	Total	Mutation rate[b]
M control	49	1532	259	1791	0.146 ± 0.012
TP1	50	1963	2	1965	0.001 ± 0.001
TP3	45	1336	271	1607	0.165 ± 0.010
TP5	48	2059	7	2066	0.004 ± 0.002
TP6	47	1840	14	1854	0.007 ± 0.002
TP7	45	1460	165	1625	0.105 ± 0.015[c]
TP8	32	983	112	1095	0.105 ± 0.021[c]
TP9	44	2038	4	2042	0.002 ± 0.001

[a] All stocks carried *w sn*^{w}.

[b] Unweighted mean proportion sn^{e} among total ± standard error.

[c] When pooled, the mutation rate is 0.105 ± 0.012.

Further tests of TP-mediated repression of *sn*^{w} mutability: The TP1, TP5, and TP6 stocks were tested for repression of *sn*^{w} mutability induced by different transposase sources under different experimental conditions. These stocks were selected because they each carried a different telomeric *P* element. For comparative purposes, the nonrepressing TP3 stock, which carries two isolated *P* elements, both *KP*s, was also included in the analyses. The transposase sources included the *H(hsp/CP)2* transgene on chromosome *2*; another insertion of this transgene, *H(hsp/CP)3*, on chromosome *3*; and the *P(ry*$^{+}$, Δ*2-3)99B* transgene on chromosome *3*. This last transgene lacks the intron between exons 2 and 3 in the transposase coding region and therefore produces the transposase in the somatic cells as well as in the germline (Robertson *et al.* 1988; Robertson 1996). Each stock was also tested for repression of *sn*^{w} mutability induced by the *H(hsp/CP)2* or *P(ry*$^{+}$, Δ*2-3)99B* transgenes under heat-shock conditions. The purposes of these experiments were to determine if the telomeric *P* elements could repress (1) *sn*^{w} mutability induced by transposase transgenes located on different chromosomes, (2) *sn*^{w} mutability induced by the combined forces of two transposase transgenes, (3) *sn*^{w} mutability induced by the partially preprocessed Δ*2-3* transposase transgene in the soma as well as in the germline, and (4) *sn*^{w} mutability induced by a transposase transgene stimulated to higher levels of expression by heat-shock treatments. The results of all these experiments are summarized in Table 6.

Not surprisingly, the TP1, TP5, and TP6 stocks effectively repressed *sn*^{w} mutability induced in the male germline by the *H(hsp/CP)3* transgene. The mutation rate for the controls in this set of experiments was 0.513 and for the nonrepressing TP3 flies it was 0.597; for the TP1 and TP5 flies it was essentially zero, and for the TP6 flies it was 0.057. Thus, the chromosomal location of the transposase transgene does not compromise the ability of the telomeric *P* elements to repress *sn*^{w} mutability.

The TP1, TP5, and TP6 stocks were also effective repressors of germline *sn*^{w} mutability when this mutability was induced by a combination of the *H(hsp/CP)2* and *H(hsp/CP)3* transgenes or when it was induced by the highly mutagenic *P(ry*$^{+}$, Δ*2-3)99B* transgene. The controls in these experiments showed mutation rates of 0.639 (for the double transgene combination) and 0.796 (for the Δ*2-3* transgene); the TP3 flies showed even higher mutation rates. By contrast, the mutation rates for the TP1 and TP5 flies were <0.022, and those for the TP6 flies were 0.077 (for the double transgene combination) and 0.172 (for the Δ*2-3* transgene). Thus, the telomeric *P* elements were able to counter the high level of germline transposase activity induced by the combination of two *H(hsp/CP)* transgenes or by the *P(ry*$^{+}$, Δ*2-3)99B* transgene. However, TP6 was clearly less capable of coping with the higher level of germline transposase activity than either TP1 or TP5.

The experiments with the *P(ry*$^{+}$, Δ*2-3)99B* transgene provided an opportunity to evaluate the TP stocks for repression of transposase activity in somatic cells. All the flies obtained for these experiments were somatic mosaics of sn^{w}, sn^{+}, and sn^{e} bristles, regardless of which TP stock was involved. No effort was made to quantify the somatic transposase activity engendered by *P(ry*$^{+}$, Δ*2-3)99B* in these flies. However, previous experiments have shown that one copy of the *H(hsp/CP)2* transgene is able to repress this activity in an appreciable fraction of flies that carry *sn*^{w} and *P(ry*$^{+}$, Δ*2-3)99B* (Simmons *et al.* 2002), presumably because the *H(hsp/CP)2* transgene produces the 66-kD repressor polypeptide. Thus, compared to the *H(hsp/CP)2* standard, none of the telomeric *P* elements could repress the somatic transposase activity encoded by the Δ*2-3* transgene.

The TP stocks were also tested for repression of *H(hsp/CP)2*-encoded transposase activity in the male and female germlines when daily heat shocks were administered to the tested flies—a condition expected to increase the expression of the *H(hsp/CP)2* transgene. The TP1, TP5, and TP6 stocks all repressed *sn*^{w} mutability in these experiments, but the repression was not nearly as complete as it was in the experiments without heat shock (*cf.* Tables 4 and 5). Either the heat shock stimulates the production of transposase by a mechanism that is not effectively repressed by the telomeric *P* elements, or it interferes with the ways in which these elements normally repress transposase activity.

The magnitude of the heat-shock-inducible component of transposase activity can be estimated by comparing the *sn*^{w} mutation rates of the control flies with and without heat shocks. For males, the control rates were 0.549 (no heat shock) and 0.583 (heat shock), and for females, they were 0.146 (no heat shock) and 0.197 (heat shock); all these values are consistent with previous estimates (Simmons *et al.* 2002a). Similar differences

TABLE 6

Effect of selected TP stocks on *sn*^{w} mutability induced in the male germline by different transposase sources

TP stock[a]	No. of vials	*sn*^{w}	sn^{+}	sn^{e}	Total	Mutation rate[b]
		H(hsp/CP)3 inducer				
M control	50	965	463	582	1956	0.513 ± 0.017
TP1	49	1477	1	1	1479	0.001 ± 0.001
TP3	50	690	508	497	1695	0.597 ± 0.020
TP5	43	1298	0	0	1298	0.000 ± 0.000
TP6	50	1395	51	36	1482	0.057 ± 0.016
		H(hsp/CP)2; *H(hsp/CP)3* inducer				
M control	50	551	476	476	1503	0.639 ± 0.013
TP1	48	1220	0	0	1220	0.000 ± 0.000
TP3	50	205	467	420	1092	0.795 ± 0.022
TP5	42	1204	5	5	1214	0.007 ± 0.004
TP6	48	1086	59	39	1184	0.077 ± 0.013
		P(ry^{+}*, Δ2-3)99B* inducer				
M control	46	399	988	575	1962	0.796 ± 0.015
TP1	46	1086	11	17	1114	0.022 ± 0.007
TP3	36	110	406	366	882	0.880 ± 0.026
TP5	44	1352	2	0	1354	0.002 ± 0.001
TP6	45	1058	124	84	1266	0.172 ± 0.025
		H(hsp/CP)2 inducer with heat shock (male germline)				
M control	99	976	684	678	2338	0.583 ± 0.013
TP1	45	1212	8	7	1227	0.012 ± 0.005
TP3	49	172	400	373	945	0.821 ± 0.020
TP5	49	1459	44	39	1542	0.052 ± 0.017
TP6	49	1452	70	44	1566	0.074 ± 0.013
		H(hsp/CP)2 inducer with heat shock (female germline)				
M control	85	3479	—	847	4326	0.197 ± 0.009
TP1	46	1079	—	11	1090	0.015 ± 0.008
TP3	41	1461	—	457	1918	0.239 ± 0.019
TP5	49	2790	—	47	2837	0.017 ± 0.006
TP6	44	2150	—	97	2247	0.044 ± 0.009
		P(ry^{+}*, Δ2-3)99B* inducer with heat shock				
M control	94	536	1183	1062	2781	0.807 ± 0.013
TP1	34	881	3	2	886	0.006 ± 0.002
TP3	41	219	302	301	882	0.761 ± 0.039
TP5	46	1191	29	16	1236	0.034 ± 0.013
TP6	48	1112	212	101	1425	0.213 ± 0.025

[a] All stocks carried *w sn*^{w}.

[b] For male germline, unweighted mean proportion (sn^{+} + sn^{e}) among total ± standard error; for female germline, unweighted mean proportion sn^{e} among total ± standard error.

were observed in the data from the TP3 flies. Thus, the heat-shock-inducible component of germline transposase activity is 10–20% of the non-heat-shock level in males and 30–50% of this level in females.

To investigate whether the slightly impaired repression of transposase activity seen with heat shock was due to an inability to cope with the heat-shock-inducible component of transgene expression or to a failing in the normal repression mechanism, the TP stocks were tested for repression of *sn*^{w} mutability induced in the male germline by the *P(ry*$^{+}$*, Δ2-3)99B* transgene under heat-shock conditions. As the control data show, this transgene was not associated with a heat-shock-inducible component of transposase activity (mutation rate 0.796 without heat shock and 0.807 with heat shock). Nevertheless, two stocks, TP5 and TP6, appeared to be less effective repressors of the *Δ2-3* transgene when heat shocks were given to the tested flies, although the effects of the heat shocks were small and in the case of TP6, not statistically significant. The diminished repression ability of these two telomeric *P* elements under heat-shock conditions may, therefore, be at least partly due

TABLE 7

Effect of paternal transmission from the TP stocks on *H(hsp/CP)2*-induced sn^w mutability in the male germline

TP stock[a]	No. of vials	sn^w	sn^+	sn^e	Total	Mutation rate[b]
M control	49	555	428	358	1341	0.576 ± 0.021
TP1	49	535	461	442	1438	0.628 ± 0.014
TP3	50	297	444	362	1103	0.730 ± 0.018
TP5	48	499	581	529	1609	0.685 ± 0.021
TP6	50	437	545	417	1399	0.687 ± 0.017

[a] All stocks carried *w* sn^w.
[b] Unweighted mean proportion (sn^+ + sn^e) among total ± standard error.

to an impairment of the normal repression mechanisms. However, for TP6 an inability to cope with increased expression of a heat-shocked *H(hsp/CP)2* transgene cannot be ruled out because this telomeric *P* element was clearly not as effective at repressing sn^w mutability induced by high transposase levels.

Maternal and zygotic components of TP-mediated repression: All the previous tests for repression of transposase activity involved maternal transmission of the telomeric *P* elements to the test offspring. Can these elements repress transposase activity when they are paternally transmitted in a cross? To answer this question, *TP* sn^w males were crossed to *C(1)DX, y w f* females homozygous for the *H(hsp/CP)2* transgene. Patroclinous transmission of the *TP* sn^w *X* chromosome produced *TP* sn^w*; H(hsp/CP)2/+* sons, which were individually mated to *C(1)DX, y f* females to measure transposase activity in the germline. The experimental results (Table 7) show that none of the telomeric *P* elements tested was able to repress sn^w mutability under these conditions. Maternal transmission of the telomeric *P* elements therefore appears to be necessary for their ability to repress transposase activity.

Another possibility, however, is that repression is due to a factor that depends in some way on the telomeric *P* elements, but that can be transmitted independently of them through the egg cytoplasm. To investigate this possibility, reciprocal crosses were performed between each *TP* sn^w stock and a *y* sn^w stock. Because the *TP* sn^w stocks used in these crosses were all marked with the y^+ allele, which is tightly linked to the *X* telomere, it was possible to follow the transmission of the telomeric *P* elements in subsequent generations. In cross I, the *y* sn^w stock was used as the female parent and in cross II, it was used as the male parent. For each *TP* sn^w stock, the F_1 *TP* y^+ sn^w*/y* sn^w daughters from these crosses were mass-mated to *y w; H(hsp/CP)2* males and their *TP* y^+ sn^w*; H(hsp/CP)2/+* (class A) and *y* sn^w*; H(hsp/CP)2/+* (class B) sons were then individually tested for germline sn^w mutability. In the males derived from cross I, the telomeric *P* elements would be able to repress sn^w mutability through a combination of maternal and zygotic effects in class A, but only through strictly maternal (*i.e.*, cytoplasmic) effects in class B. In the males derived from cross II, a grandmaternal effect was superimposed on the maternal and zygotic (class A) or strictly maternal (class B) components of repression. The results of these experiments are given in Table 8.

For the control flies, the four sn^w mutation rates ranged from 0.515 to 0.585. For the TP3 flies, the mutation rates were higher, ranging from 0.661 to 0.682; these higher rates are consistent with previous results (*cf.* Tables 4 and 6). For TP1, the data indicated strong repression by the combination of grandmaternal, maternal, and zygotic effects (0.008), moderate repression by the combination of maternal and zygotic effects (0.249), and weak (although statistically significant) repression by the combination of grandmaternal and maternal effects (0.448); a maternal effect alone did not repress sn^w mutability (0.584). For TP5, strong repression was observed with a combination of grandmaternal, maternal, and zygotic effects (0.054), and moderate repression was observed with a combination of maternal and zygotic effects (0.288); however, no repression was seen with a maternal effect alone (0.617) or with a combination of grandmaternal and maternal effects (0.518). For TP6, the data revealed fairly strong repression by a combination of grandmaternal, maternal, and zygotic effects (0.139) and weak repression by a combination of maternal and zygotic effects (0.400); however, as with TP5, no repression was observed either with a maternal effect alone (0.673) or with a combination of grandmaternal and maternal effects (0.710). The combined data of Tables 7 and 8 therefore indicate (1) that TP-mediated repression of sn^w mutability requires maternal transmission, regardless of the TP involved; (2) that this repression is significantly enhanced by a grandmaternal effect; (3) that except for TP1, TP-mediated repression of sn^w mutability requires a zygotic effect; and (4) that, for TP1, this repression is significantly enhanced by a zygotic effect.

DISCUSSION

The results of this study indicate that different types of *P* elements inserted near the telomere of the *X* chromosome are able to repress *P*-element activity. By contrast, *P* elements inserted at nontelomeric locations in the distal part of the *X* chromosome do not have this ability. It is tempting to conclude that the inability of the nontelomeric *P* elements to repress *P*-element activity is due to their chromosomal position; however, some other feature of these elements, such as size, sequence, or coding capacity, could account for their lack of repression ability. All the nontelomeric *P* elements included in this study were smaller than the telomeric *P* elements. Perhaps a *P* element in the distal region of the *X* chromosome must be a minimum size for it to repress *P* activity. Another possibility is that such an

TABLE 8

Partitioning TP-mediated repression of *H(hsp/CP)2*-induced *sn*^{w} mutability in the male germline into grandmaternal, maternal, and zygotic effects

	Effects[b]								
Cross[a]	Gmtl	Mtl	Zyg	No. of vials	*sn*^{w}	sn$^+$	sn^e	Total	Mutation rate[c]
M control									
IA	−	+	+	50	950	484	516	1950	0.515 ± 0.013
IB	−	+	−	50	883	557	612	2052	0.569 ± 0.014
IIA	+	+	+	50	919	530	574	2023	0.548 ± 0.013
IIB	+	+	−	50	936	667	659	2272	0.585 ± 0.015
TP1									
IA	−	+	+	50	989	188	141	1318	0.249 ± 0.018
IB	−	+	−	48	597	379	445	1421	0.584 ± 0.014
IIA	+	+	+	50	1473	5	6	1484	0.008 ± 0.003
IIB	+	+	−	48	737	280	312	1329	0.448 ± 0.014
TP3									
IA	−	+	+	50	553	564	523	1640	0.661 ± 0.024
IB	−	+	−	50	658	625	675	1958	0.666 ± 0.019
IIA	+	+	+	49	517	588	539	1644	0.687 ± 0.017
IIB	+	+	−	47	637	725	628	1990	0.682 ± 0.018
TP5									
IA	−	+	+	50	1650	291	385	2326	0.288 ± 0.022
IB	−	+	−	48	816	648	655	2119	0.617 ± 0.016
IIA	+	+	+	50	2069	53	62	2184	0.054 ± 0.011
IIB	+	+	−	50	962	494	579	2055	0.518 ± 0.016
TP6									
IA	−	+	+	48	858	318	252	1427	0.400 ± 0.022
IB	−	+	−	46	511	505	546	1562	0.673 ± 0.022
IIA	+	+	+	49	1271	119	100	1490	0.139 ± 0.019
IIB	+	+	−	49	402	545	485	1432	0.710 ± 0.020

[a] Cross I or II, cross A or B (see text).
[b] Gmtl, grandmaternal; Mtl, maternal; Zyg, zygotic.
[c] Unweighted proportion (sn$^+$ + sn^e) among total ± standard error.

element must contain certain sequences or have a certain coding capacity to function as a repressor. Curiously, some of the nontelomeric *P* elements were *KP* elements, which are known to repress *P* activity by producing a polypeptide that apparently binds to *P*-element DNA (Lee *et al.* 1996, 1998). However, none of the *KP* elements in this study showed any significant repression ability.

Telomeric *P* elements repress gonadal dysgenesis induced by different P strains, and they repress *sn*^{w} mutability induced by different transposase sources, including naturally occurring P strains, *H(hsp/CP)* transgenes inserted on either of the major autosomes (separately or together and with or without heat-shock induction), and the *P(ry*$^+$, *Δ2-3)99B* transgene, which lacks the last intron in the transposase gene. Repression of *P*-element activity by the telomeric *P* elements occurs in both the male and the female germlines. However, it does not occur in the somatic tissues of flies carrying the *P(ry*$^+$, *Δ2-3)99B* transgene.

Genetic analyses indicate that repression by the telomeric *P* elements requires that they be transmitted maternally and that the cytoplasm of females carrying these elements is, by itself, unable to cause much, if any, repression. With the TP5 and TP6 strains, no independent cytoplasmic component of repression was detected, and, with the TP1 strain, only a weak cytoplasmic component was observed; however, the results with TP1 are complicated by the fact that this strain carries complete *P* elements capable of transposing and by the fact that it has both telomeric and nontelomeric *X*-linked *P* elements and a telomeric *P* element on chromosome *2R*. The weak cytoplasmic component of repression seen with TP1 therefore may have been due to the zygotic effect of a *P* element not linked to the *X* telomere. For TP5 and TP6, however, the data are unambiguous; repression occurs only when these elements themselves are transmitted to the offspring from the mother. Thus, they mimic exactly the effects of the P cytotype.

Although both TP5 and TP6 were powerful repressors of *sn*^{w} mutability, TP5 was consistently the stronger repressor. The difference between these strains was most apparent under conditions where high levels of transposase activity were produced in control experiments, for

example, with *P(ry*$^+$*, Δ2-3)99B* or both *H(hsp/CP)2* and *H(hsp/CP)3* as the transposase source. Under these conditions, TP6 was clearly less effective than TP5 as a repressor of *sn*^{w} mutability (see Table 6). The inferiority of TP6 was also seen when repression ability was partitioned into grandmaternal, maternal, and zygotic effects. With maternal and zygotic effects, but no grandmaternal effect, TP6 was a much weaker repressor than TP5 (or TP1), and even with all three effects present, TP6 was still a weaker repressor than TP5 (or TP1; see Table 8). Paradoxically, however, TP6 was the strongest repressor of gonadal dysgenesis. It is not clear why the gonadal dysgenesis and *sn*^{w} mutability assays should yield a different ranking of repression ability.

How do the telomeric *P* elements repress transposase activity? The simplest hypothesis is that they titrate the transposase away from other target *P* elements (Simmons and Bucholz 1985). However, this hypothesis is ruled out by the complete lack of repression when the telomeric *P* elements are paternally transmitted. Another hypothesis is that RNA transcribed from these elements titrates the splicing apparatus responsible for removing the last intron from the transcripts of complete *P* elements. However, this hypothesis is excluded by the fact that TP1, TP5, and TP6 all repress the transposase activity encoded by *P(ry*$^+$*, Δ2-3)99B*, which lacks this intron by construction.

A third hypothesis is that these elements produce polypeptide repressors of transposase activity in the germline. TP1 and TP4 contain complete *P* elements inserted at or near the telomere of the *X* chromosome; through alternate splicing, these elements might produce the 66-kD polypeptide, which is known to repress *P*-element activity. Ronsseray *et al.* (1996) have speculated that complete *P* elements situated near the *X* telomere preferentially produce this polypeptide because their transcripts are exported to the cytoplasm before splicing can be completed. The rapid export of transcripts is hypothesized to result from the juxtaposition of the *X* telomere to pores in the nuclear membrane. Current data cannot exclude this hypothesis. However, the fact that TP1 does not repress somatic *sn*^{w} mutability argues that its telomeric *P* element does not produce the 66-kD polypeptide in somatic cells. Thus, production of the 66-kD repressor polypeptide by this telomeric *P* element would have to be germline specific.

The structurally incomplete *P* elements in the TP5 and TP6 strains cannot produce the 66-kD polypeptide. They might, however, encode other proteins. *TP5* could encode a polypeptide of 113 amino acids, the first 95 of which would be identical to those of the P transposase. The putative TP5 polypeptide would contain a domain implicated in binding to *P*-element DNA (Lee *et al.* 1998). *TP6*, which may be the same as the telomeric *P* element isolated from the Mount Carmel strain by Peterson *et al.* (1986), could encode a polypeptide of 441 amino acids, which is identical to the first 208 and the last 232 amino acids of the transposase with an extra glycine present at the junction of these two regions. The amino portion of this putative polypeptide would therefore contain the KP polypeptide, which has been implicated in moderate repression of *P*-element activity (Andrews and Gloor 1995; Lee *et al.* 1996, 1998; Simmons *et al.* 2002b). However, PCR-based screens of 91 P strains from around the world have failed to show that either of these elements is present anywhere except in the strains from which they were isolated. Thus, unlike the geographically widespread *KP* element, the *TP5* and *TP6* elements have not been spread by natural selection throughout the worldwide population of Drosophila. The implication is that these two elements do not produce repressor polypeptides. Marin *et al.* (2000) have identified another incomplete *P* element with regulatory ability inserted at the *X* telomere. Because this element is truncated at its 5′ end by a deletion that removes the promoter, it too may not produce a repressor polypeptide. In another investigation, Ronsseray *et al.* (1998) have shown that unexpressed *P* transgenes inserted at telomeric sites can enhance the regulatory abilities of other *P* elements, and Roche and Rio (1998) have shown that these transgenes inhibit the expression of transgenes inserted at nontelomeric sites. All these findings suggest that regulation by telomeric *P* elements may not necessarily involve *P*-encoded polypeptides. However, if *P*-encoded polypeptides are involved, the evidence from TP5 and TP6 indicates that they (or the RNAs encoding them) are not transmitted independently of the *P* elements themselves through the egg cytoplasm. Furthermore, these polypeptides are evidently not produced in somatic cells.

Another hypothesis is that *P*-element regulation involves antisense *P* RNAs. However, transgenes designed to produce antisense *P* RNAs do not repress transposase activity nearly as well as telomeric *P* elements (Simmons *et al.* 1996). Thus, it is necessary to consider the possibility that repression by telomeric *P* elements is not mediated by any type of *P*-element product, either an RNA or a polypeptide, but rather by a telomeric factor that acts in conjunction with the *P* elements themselves.

Drosophila telomeres exhibit some of the features of heterochromatin. Transgenes inserted into telomeric regions show reduced expression compared to insertions in euchromatin (Wallrath and Elgin 1995; Cryderman *et al.* 1999), and HP1, a protein involved in the organization of heterochromatin, is found at telomeres (James *et al.* 1989; Fanti *et al.* 1998). Apropos of this latter observation, Ronsseray *et al.* (1996) have reported that the regulatory ability of telomeric *P* elements is impaired by mutations in *Su(var)2-5* [also known as *Su(var)205*], the gene that encodes HP1. These observations suggest that regulation of the *P* family by telomeric *P* elements may involve some aspect of telomeric chromatin. Perhaps telomeric *P* elements pair with nontelomeric *P* elements and a component of the

telomeric chromatin is transferred to the nontelomeric elements. This component is assumed to be associated with gene silencing—a natural condition near telomeres—and, once transferred to a nontelomeric site, would presumably act as a silencer there as well. Thus, a transposase-encoding element at a nontelomeric site could be silenced through the "contaminating" effect of telomeric chromatin. A contaminated element might then contaminate other elements, thereby spreading the repressed state throughout the entire *P* family. This epigenetic mechanism of ectopic pairing and chromatin contamination might be responsible for the P cytotype.

Two observations suggest that this mechanism is feasible. First, studies of DNA gap repair have shown that ectopic pairing occurs in the germline (Gloor *et al.* 1991; Nassif and Engels 1993; Engels *et al.* 1994). Damage at one site in the genome can be repaired by copying DNA sequences into it from another, partially homologous site, which can be located far from the damaged site, even on a different chromosome. Second, repression of transcription from nontelomeric *P-lacZ* transgenes by telomeric *P* elements is homology dependent (Roche and Rio 1998; Marin *et al.* 2000). A telomeric *P* element that was truncated at its 5′ end was unable to repress a *P-lacZ* transgene that did not share *P* sequences with it. However, the same truncated telomeric *P* element was able to repress *P-lacZ* transgenes that had *P* sequences in common.

The P cytotype and the regulatory properties of strains such as TP5 and TP6 are transmitted maternally along with the *P* elements themselves. Paternal transmission and maternal transmission without the elements abolishes repression ability completely. These observations indicate that the repressive state associated with telomeric *P* elements must be established, maintained, and transmitted through the female germline. Thus, in terms of the epigenetic model of *P* regulation, the hypothesized telomeric factor must be produced in the female germline and transmitted through the egg, whereupon it acts in conjunction with a telomeric *P* element to repress *P* activity in the germline of the offspring, regardless of its sex. Furthermore, the inability of telomeric *P* elements to repress *P*-element activity in the soma suggests that this factor is germline specific.

Although it is necessary for the telomeric *P* element to be transmitted maternally if transposase activity is to be repressed, it is not necessary for either the source of the P transposase or the *P*-element targets of the transposase to be transmitted along with the telomeric *P* element. They can be transmitted paternally and repression of *P*-element activity will still take place in the germline of the offspring—an effect that is seen, for example, when gonadal dysgenesis is repressed in the offspring of crosses between females from strains such as TP5 or TP6 and males from a P strain. A minimal, maternally transmitted repression system consisting of a single telomeric *P* element and some epigenetic factor is therefore able to control a multi-element, paternally transmitted system set to induce *P*-element activity.

Although an epigenetic mechanism may be responsible for the P cytotype, it is still possible that other mechanisms contribute to *P* regulation in nature. This regulation may involve a complex blend of mechanisms—minor regulation by transposase titration, *P*-encoded polypeptides such as the 66-kD and KP repressors, and antisense *P* RNA and major regulation by the P cytotype.

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