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Impairment of Cytotype Regulation of *P*-Element Activity in *Drosophila melanogaster* by Mutations in the *Su(var)205* Gene

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ABSTRACT

Cytotype regulation of transposable *P* elements in the germ line of *Drosophila melanogaster* is associated with maternal transmission of *P* elements inserted at the left telomere of the *X* chromosome. This regulation is impaired in long-term stocks heterozygous for mutations in *Suppressor of variegation 205* [*Su(var)205*], a gene implicated in the control of telomere length. Regulation by *TP5*, a structurally incomplete *P* element at the *X* telomere, is more profoundly impaired than regulation by *TP6*, a different incomplete *P* element inserted at the same site in a TAS repeat at the *X* telomere. Genetic analysis with the *TP5* element indicates that its regulatory ability is not impaired in flies whose fathers came directly from a stock heterozygous for a *Su(var)205* mutation, even when the flies themselves carry this mutation. However, it is impaired in flies whose grandfathers came from such a stock. Furthermore, this impairment occurs even when the *Su(var)205* mutation is not present in the flies themselves or in their mothers. The impaired regulatory ability of *TP5* persists for at least several generations after *TP5 X* chromosomes extracted from a long-term mutant *Su(var)205* stock are made homozygous in the absence of the *Su(var)205* mutation. Impairment of *TP5*-mediated regulation is therefore not directly dependent on the *Su(var)205* mutation. However, it is characteristic of the six mutant *Su(var)205* stocks that were tested and may be related to the elongated telomeres that develop in these stocks. Impairment of regulation by *TP5* is also seen in a stock derived from Gaiano, a wild-type strain that has elongated telomeres due to a dominant mutation in the *Telomere elongation* (*Tel*) gene. Regulation by *TP6* is not impaired in the Gaiano genetic background. The regulatory abilities of the *TP5* and *TP6* elements are therefore not equally susceptible to the effects of elongated telomeres in the mutant *Su(var)205* and Gaiano stocks.

THE transposable *P* elements of *Drosophila melanogaster* were discovered through their involvement in a syndrome of germ-line abnormalities called hybrid dysgenesis (Kidwell *et al.* 1977; Bingham *et al.* 1982). The traits of this syndrome include sterility due to a failure of the gonads to develop (gonadal dysgenesis, GD), the frequent occurrence of mutations and chromosome rearrangements, recombination in males, and chromosome transmission ratio distortion. These traits are seen when *P* elements are activated in the germ line—an event that occurs in the hybrid offspring of crosses between males with *P* elements in their genomes and females without these elements. Hybrid dysgenesis is usually not seen in the offspring of the reciprocal crosses because *P* elements are repressed by a maternally transmitted condition called the P cytotype, which genetic analyses have shown depends on the *P* elements themselves (Engels 1979a; Sved 1987).

The mechanistic basis of the P cytotype is unknown. For many years it was thought to involve *P*-encoded polypeptides transmitted through the egg cytoplasm (Engels 1989; Rio 1990; Roche *et al.* 1995). However, recent studies have cast doubt on this hypothesis (Simmons *et al.* 2002a,b,c, 2004). The M cytotype, a complementary condition that permits *P*-element movement, is characteristic of Drosophila strains that do not carry *P* elements in their genomes. However, like the P cytotype, it is maternally transmitted. Thus, when P males are crossed to M females, *P* elements are introduced into offspring that inherit the M cytotype and hybrid dysgenesis occurs.

The *P* elements that are found in the genomes of P strains are structurally heterogeneous. Complete *P* elements, 2.9 kilobases (kb) long, encode a transposase that catalyzes *P*-element excision and insertion (Engels 1984; Karess and Rubin 1984). Incomplete *P* elements do not produce the transposase because they lack part of the coding sequence. However, most incomplete *P* elements can be mobilized by the transposase if this enzyme is produced by a complete *P* element somewhere in the genome.

In some strains, the P cytotype is associated with complete or incomplete *P* elements inserted at the telomeres of chromosomes (Ronsseray *et al.* 1991; Marin *et al.* 2000; Stuart *et al.* 2002). In these strains, a single telomeric *P* element is sufficient to repress the

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entire *P* transposon family. Genetic analyses indicate that this repression is maternally inherited along with the telomeric *P* elements themselves; neither paternal transmission of a telomeric *P* element nor maternal transmission of cytoplasm from a heterozygous carrier without the element itself can repress hybrid dysgenesis (Stuart *et al.* 2002; Niemi *et al.* 2004; Simmons *et al.* 2004). Regulation by the telomeric *P* elements therefore exactly parallels regulation by the P cytotype.

The discovery that telomeric *P* elements have regulatory ability like that of the P cytotype has drawn attention to ways in which telomeric chromatin might affect *P*-element function. The distalmost sequences of Drosophila telomeres consist of retrotransposons from the *HeT-A* and *TART* families (Biessmann *et al.* 1990; Levis *et al.* 1993; Sheen and Levis 1994; Mason and Biessmann 1995). These LINE-type retrotransposons insert specifically at the ends of chromosomes to replenish sequences that are lost because of incomplete DNA replication there. Proximal to the retrotransposon sequences are multiple copies of telomere-associated sequences, known simply as the TAS repeats (Karpen and Spradling 1992). To date, all telomeric *P* elements associated with the P cytotype have been found to be inserted in TAS repeats or at the junction between the *HeT-A* sequences and TAS repeats (Ronsseray *et al.* 1996; Roche and Rio 1998; Marin *et al.* 2000; Stuart *et al.* 2002).

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 at least one protein associated with the organization of heterochromatin is found in the vicinity of Drosophila telomeres (James *et al.* 1989). This protein, called heterochromatin protein 1 (HP1), is the product of the *Suppressor of variegation 205* [*Su(var)205*] gene, also known as *Su(var)2-5* (Eissenberg *et al.* 1990). Mutations in *Su(var)205* act as dominant suppressors of position-effect variegation caused by chromosomal rearrangements that juxtapose euchromatic genes to centric heterochromatin (Sinclair *et al.* 1983). They also appear to impair the regulatory ability of telomeric *P* elements (Ronsseray *et al.* 1996; Marin *et al.* 2000), possibly through a maternal effect (Ronsseray *et al.* 2001). Recent analyses have shown that the telomeres of Drosophila chromosomes are abnormally elongated in stocks heterozygous for *Su(var)205* mutations (Savitsky *et al.* 2002; Perrini *et al.* 2004). This elongation is due to the addition of *HeT-A* and *TART* retrotransposons to the ends of the chromosomes. Telomere elongation is also caused by a mutation in the *Telomere elongation* (*Tel*) gene, which was discovered by analyzing a wild-derived strain called Gaiano (Siriaco *et al.* 2001). It is possible that the elongated telomeres engendered by these types of mutations are directly responsible for the impairment of cytotype regulation that is associated with telomeric *P* elements. If so, cytotype regulation should be impaired in flies that have inherited chromosomes from a strain that carries a *Su(var)205* mutation, even if they have not inherited that mutation itself. In addition, cytotype regulation should be impaired in flies that have inherited chromosomes from the Gaiano strain.

This study presents a genetic analysis of the effects of *Su(var)205* mutations on cytotype regulation mediated by two different incomplete *P* elements inserted near the left telomere of the *X* chromosome. These elements are genetically stable because they do not encode the P transposase. Earlier studies of *P*-element regulation involved complete *P* elements inserted at the *X* telomere (Ronsseray *et al.* 1991,1996). Although seminal, these studies were limited because the *P* elements that were analyzed could produce the P transposase, which catalyzes excision and transposition and therefore causes genetic instability (Roche and Rio 1998; Rio 1999). By studying incomplete *P* elements, we have been able to perform a more comprehensive analysis of the effects of *Su(var)205* mutations on cytotype regulation.

MATERIALS AND METHODS

Telomeric *P* elements: *TP5* and *TP6* are incomplete *P* elements inserted at the same nucleotide position in one of the 1.8-kb repeats within the telomere-associated sequences (TAS) at the left end 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 because they are structurally incomplete, neither encodes a catalytically active P transposase. However, both elements are associated with strong abilities to regulate the entire *P* family; furthermore, these abilities are maintained stably over time (Niemi *et al.* 2004). All stocks containing these elements are marked with a recessive mutation in the *white* (*w*) eyes gene and a wild-type allele of the *yellow* (*y*) body gene. These two genes are tightly linked to the left telomere of the *X* chromosome.

Drosophila stocks and husbandry: Genetic symbols for the Drosophila stocks are explained in the FlyBase website, in Lindsley and Zimm (1992), or in other references cited in the text. The *Su(var)205* gene, which encodes the 206 amino-acid-polypeptide HP1, is located on chromosome *2* in cytological region 29A, map position 30.8. The *Su(var)205* alleles *1*, *2*, *03*, *4*, and *5* were obtained from Joel Eissenberg and Barbara Wakimoto. Because these alleles are homozygous lethal, they were maintained in stocks with a *Cy Roi* [= $In(2L)Cy^{L}t^{R}$ + *In(2R)Cy, Cy Roi cn sp bw*] balancer chromosome. Each mutant *Su(var)205* allele was introduced into stocks that were homozygous for the *TP5* or *TP6* telomeric *P* elements. After balancing these alleles with the *Cy Roi* chromosome, the resulting stocks were checked for the presence of the telomeric *P* element by PCR with appropriate primers (either the IR or *TP5*- and *TP6*-specific primers described in Stuart *et. al.* 2002). They were also checked for the *Su(var)205* mutation by testing for suppression of the variegating eye phenotype of the *white-mottled* (w^{m4}) *X* chromosome (provided by Joel Eissenberg). For these tests, males from the *TP*; *Su(var)205/Cy Roi* stocks were crossed to w^{m4} females, and their non-Curly sons were examined for suppression of the w^{m4} phenotype. Stocks that had both the telomeric *P* element and the balanced *Su(var)205* mutation were maintained by mass matings for many generations before any experimental analysis was attempted. The deficiencies *Df(2L)Trf-C6R31* and *Df(2L)TE29Aa-11* were

obtained from the Bloomington, Indiana, Drosophila Stock Center as part of a "deficiency kit" for constructing segmental aneuploids. All experimental cultures were maintained on a standard cornmeal-molasses-dried yeast medium at 25°; stock cultures were maintained on the same medium at 21°.

Assays for *P*-element activity: *P*-element activity was monitored quantitatively using the GD and *singed-weak* (sn^w) mutability assays, which are described in detail in Stuart *et al.* (2002). In the GD assay, females from the stock to be tested were crossed to P males and their daughters were examined for gonadal dysgenesis, a condition caused by transposase-induced *P*-element excision and transposition in the germ line. The frequency of GD was therefore used as an index of *P*-element activity in the female germ line. Statistical differences between sets of GD data were evaluated by the Mann-Whitney rank sum test. In the sn^w mutability assay, females carrying sn^w, a double *P*-element-insertion mutation of the *X*-linked *singed* gene that is destablized by the P transposase (Engels 1979b,1984; Roiha *et al.* 1988), were crossed to males carrying either *H(hsp/CP)2* or *H(hsp/CP)3*, which are genetically stable *hobo* transgenes that encode the P transposase (Simmons *et al.* 2002a). Their sn^w; *H(hsp/CP)2* or *3/+* sons were then crossed individually to *C(1)DX, y f* females, which have attached-*X* chromosomes, to produce males that inherit the sn^w allele or a transposase-induced derivative of it from their fathers. Two types of derivatives can be detected in this assay: *extreme singed* (sn^e) and pseudowild-type [$sn^{(+)}$], each due to the excision of one or the other of the *P* elements inserted in the sn^w allele. The progeny that emerged from these crosses were scored on days 14 and 17 after the crosses were set up. The combined frequency of the sn^e and $sn^{(+)}$ flies among all those scored was used as a measure of transposase activity in the male germ line. Statistical differences in sn^w mutability were evaluated by *z*-tests.

Synthesis of *TP5* and *TP6* stocks with the Gaiano genetic background: Females from the basic *TP5* and *TP6* stocks (both marked with the *w* mutation) were crossed to males from the Gaiano wild-type strain, and their white-eyed sons were crossed to Gaiano females. Heterozygous *w/+* females from these crosses were then mated to Gaiano males. After two more cycles of matings in this pattern, homozygous *w/w* females and hemizygous *w* males were intercrossed to produce *TP5* Gaiano and *TP6* Gaiano stocks, in which the presence of the telomeric *P* element was confirmed by PCR. The sn^w allele was introduced into these stocks by crossing females from the basic *TP5* sn^w and *TP6* sn^w stocks to *TP5* Gaiano and *TP6* Gaiano males. The *TP* sn^w*/+* daughters were backcrossed to *TP* Gaiano males to obtain *TP* sn^w sons, which were then crossed to *TP* Gaiano females. After one more cycle of matings in this pattern, *TP* sn^w*/+* females were crossed to *TP* Gaiano males, and their *TP* sn^w sons were backcrossed to a reserved group of *TP* sn^w*/+* females from the previous generation to obtain lines fixed for the sn^w allele. The stocks created from these lines are denoted *TP5* sn^w Gaiano and *TP6* sn^w Gaiano. A similar procedure was used to create a sn^w Gaiano stock without either telomeric *P* element.

RESULTS

***TP5* and *TP6* stocks with the *Su(var)205*^{4} allele show impaired repression of gonadal dysgenesis:** Previous work has indicated that repression of GD by structurally complete *P* elements inserted in the TAS repeats of the left telomere of the *X* chromosome is impaired in flies carrying the *Su(var)205*^{4} mutation (Ronsseray *et al.* 1996). We investigated whether stocks with this mutation might also compromise repression by two incomplete *P* elements located in these repeats. The elements, denoted *TP5* and *TP6*, have been implicated in the repression of GD and have also been shown to repress transposase-catalyzed *P*-element excision (Stuart *et al.* 2002). To determine if repression by *TP5* or *TP6* is impaired in stocks carrying the *Su(var)205*^{4} mutation, we crossed *Su(var)205*^{4}*/Cy Roi* females from replicate stocks that were homozygous for *TP5* or *TP6* to P males from two different strains, Harwich-w (Kidwell *et al.* 1977) and Nem12 (N-12 in Kocur *et al.* 1986). We then observed the frequency of GD among their non-Curly [*i.e.*, *Su(var)205*^{4}*/+*] daughters. As positive controls, we crossed females from *TP5* and *TP6* strains that did not have the *Su(var)205*^{4}*/Cy Roi* genotype to these two types of P males, and as negative controls, we crossed females from two M strains [*w m f* and w^{m4}; *Su(var)205*^{4}*/Cy Roi*] to the two types of P males. The results of all these tests are summarized in Table 1.

In the crosses with *w m f* M females, Harwich-w and Nem12 induced 100 and 54.3% GD, respectively. Similar frequencies of GD were observed in the crosses involving the w^{m4}; *Su(var)205*^{4}*/Cy Roi* females. Thus, by itself, the *Su(var)205*^{4}*/Cy Roi* genotype does not seem to influence the frequency of GD induced by either strong (Harwich-w) or moderate (Nem12) P males. The crosses with the basic *TP5* and *TP6* strains show that the telomeric *P* elements significantly reduced the frequency of GD. With Harwich-w as the inducer, the frequency was 73% for *TP5* and 19.6% for *TP6*, and with Nem12 as the inducer, it was 18% for *TP5* and 0% for *TP6*. Thus, both *TP5* and *TP6* repressed the GD induced by either strong or moderate P males, and *TP6* did so more effectively. These results are consistent with previous analyses (Stuart *et al.* 2002).

The remaining data in Table 1 show that this *TP*-mediated repression of GD was impaired in flies from stocks with the *Su(var)205*^{4}*/Cy Roi* genotype. With Harwich-w as the inducer, the frequency of GD among the non-Curly daughters of the *TP*; *Su(var)205*^{4}*/Cy Roi* females ranged from 64.9 to 99.7% for *TP5* and from 31.6 to 61.5% for *TP6*. With Nem12 as the inducer, it ranged from 29.7 to 51.5% for *TP5* and from 7.6 to 29.8% for *TP6*. Thus, repression of GD by both telomeric *P* elements was compromised in a *Su(var)205*^{4} genetic background.

A *TP5* stock with the *Su(var)205*^{4} allele shows impaired repression of *P*-element excision: Flies from stocks with the *Su(var)205*^{4}*/Cy Roi* genotype were also tested for impaired repression of transposase-catalyzed *P*-element excisions. Females homozygous for the *X*-linked, double *P*-element insertion mutation *singed-weak* (sn^w), which is a sensitive target for P transposase activity (Engels 1984), were crossed to males homozygous for *H(hsp/CP)2*, a *hobo* transgene on chromosome *2* that encodes the P transposase. The sn^w sons from these crosses were then individually tested for transposase-induced

TABLE 1

Gonadal dysgenesis in females from stocks with the *Su(var)205⁴* mutation

Parental stock[a]	Harwich-w inducer			Nem12 inducer		
	No. vials	No flies	%GD[b]	No. vials	No. flies	%GD[b]
M stocks						
w m f	30	416	100.0 ± 0.0	33	454	54.3 ± 3.5
w^m4; Su(var)205⁴/Cy Roi	25	259	100.0 ± 0.0	30	180	55.9 ± 5.5
TP5 stocks						
TP5	27	428	73.0 ± 4.6	19	196	18.0 ± 4.7
TP5; Su(var)205⁴/Cy Roi line 1	30	253	99.7 ± 0.6	30	309	51.5 ± 4.1
TP5; Su(var)205⁴/Cy Roi line 2	31	219	64.9 ± 6.0	24	236	29.7 ± 5.2
TP5; Su(var)205⁴/Cy Roi line 3	32	235	86.5 ± 3.0	30	283	50.0 ± 4.3
TP6 stocks						
TP6	26	429	19.6 ± 4.1	21	151	0.0 ± 0.0
TP6; Su(var)205⁴/Cy Roi line 1	30	165	31.6 ± 5.4	28	223	7.6 ± 3.0
TP6; Su(var)205⁴/Cy Roi line 2	30	207	61.5 ± 5.0	22	165	18.5 ± 5.1
TP6; Su(var)205⁴/Cy Roi line 3	30	206	56.7 ± 5.9	28	220	29.8 ± 4.0

[a] Females from these stocks were crossed to Harwich-w or Nem12 males. Only their non-Curly daughters were examined for GD.

[b] Unweighted average ± standard error.

excisions of either of the *sn^w P* elements occurring in their germ lines. The tests involving the basic stocks provided data on the extent to which *sn^w* is destabilized by the *H(hsp/CP)2* transposase source; they also provided data on the repression of this instability by the telomeric *P* elements *TP5* and *TP6*. The tests with the *Su(var)205⁴/Cy Roi* derivatives of the basic stocks provided data on the effect of the *Su(var)205⁴/Cy Roi* genotype; in these tests, both the non-Curly [*Su(var)205⁴/H(hsp/CP)2*] and Curly [*Cy Roi/H(hsp/CP)2*] sons were assayed for *sn^w* mutability.

The results of these experiments (Table 2) show that both *TP5* and *TP6* were strong repressors of transposase-induced excisions. In the absence of any *TP* element, the *sn^w* mutation rate was 0.508, whereas in the presence of *TP5*, it was 0, and in the presence of *TP6* it was 0.069. These results are in agreement with previous analyses showing that although both of these telomeric *P* elements repress *sn^w* mutability, *TP5* is consistently the stronger repressor (Stuart *et al.* 2002; Simmons *et al.* 2004).

When the males that were tested for *sn^w* mutability came from mothers with the *Su(var)205⁴/Cy Roi* genotype, the mutation rates were elevated in both the non-Curly and Curly classes. In the absence of a telomeric *P* element, the mutation rates for the non-Curly and Curly classes of males were 0.607 and 0.663, respectively. By *z*-statistics, these rates are significantly greater than the control rate of 0.508 seen in the tests with the basic *sn^w* stock. Thus, the *Su(var)205⁴/Cy Roi* genotype in the mother appears to enhance the mutability of *sn^w* in the germ lines of her sons.

In the presence of a telomeric *P* element, the mutation rates of the non-Curly and Curly males were also elevated more than those of the basic stocks. With *TP5* the mutation rates of the non-Curly and Curly males were both ~0.35, and with *TP6* they were 0.145 and 0.108, respectively. The increased mutability observed with *TP6* might be due to a generalized effect of the *Su(var)205⁴/Cy Roi* genotype on *sn^w* mutability *per se*. However, the increased mutability seen with *TP5* suggests a *bona fide* impairment of *TP5*-mediated repression. Moreover, because an increase occurred in both the non-Curly and Curly classes of males, this impairment must involve more than a simple zygotic effect of the *Su(var)205⁴* mutation (*i.e.*, an effect due to the presence of the mutation itself).

***TP5*-mediated repression is not impaired in the hybrid sons of crosses between *TP5 sn^w* and *Su(var)205⁴/Cy Roi* stocks:** The previous analysis shows that *TP5*-mediated repression of *sn^w* mutability is impaired when the *TP5* element comes from a *Su(var)205⁴/Cy Roi* stock. Can chromosomes from a *Su(var)205⁴/Cy Roi* stock compromise repression by a *TP5* element inherited maternally from the basic *TP5 sn^w* stock? To answer this question, we performed three types of crosses to produce males for a series of *sn^w* mutability tests. In cross I, *TP5 sn^w* females were mated to males homozygous for *H(hsp/CP)3*, a *hobo* transgene inserted on chromosome *3* that produces the P transposase; the resulting *TP5 sn^w*; *H(hsp/CP)3/+* sons were then tested individually for *sn^w* mutability. In cross II, *TP5 sn^w*; *Su(var)205⁴/Cy Roi* females were mated to homozygous *H(hsp/CP)3* males and their *TP5 sn^w*; *Su(var)205⁴/+*; *H(hsp/CP)3/+* (phenotypically non-Curly) and *TP5 sn^w*; *Cy Roi/+*; *H(hsp/CP)3/+* (phenotypically Curly) sons were individually tested for *sn^w* mutability. In cross III, *TP5 sn^w* females

TABLE 2

Germ-line mutability of *sn*^{w} in sons derived from *(TP) sn*^{w} and *(TP) sn*^{w}*; Su(var)205*^{4}*/Cy Roi* mothers

	Non-Curly sons			Curly sons		
Genotype of mother[a]	No. vials	No. flies	Mutation rate[b]	No. vials	No. flies	Mutation rate[b]
Basic stocks						
sn^{w}	49	1342	0.508 ± 0.020			
TP5 sn^{w}	48	1404	0.000 ± 0.000			
TP6 sn^{w}	49	1528	0.069 ± 0.021			
Su(var)205^{4}*/Cy Roi* derivatives						
sn^{w}*; Su(var)205*^{4}*/Cy Roi*	48	1295	0.607 ± 0.014	42	924	0.663 ± 0.023
TP5 sn^{w}*; Su(var)205*^{4}*/Cy Roi*	49	1439	0.357 ± 0.025	48	1539	0.340 ± 0.020
TP6 sn^{w}*; Su(var)205*^{4}*/Cy Roi*	49	1470	0.145 ± 0.024	48	1086	0.108 ± 0.016

[a] Mothers were crossed to homozygous *H(hsp/CP)2* males to obtain sons for the *sn*^{w} mutability test.
[b] Unweighted average ± standard error.

were mated to *Su(var)205*^{4}*/Cy Roi*; *H(hsp/CP)3* males and their *TP5 sn*^{w}; *Su(var)205*^{4}*/+*; *H(hsp/CP)3/+* (phenotypically non-Curly) and *TP5 sn*^{w}; *Cy Roi/+*; *H(hsp/CP)3/+* (phenotypically Curly) sons were individually tested for *sn*^{w} mutability. The tested males from cross I provide a baseline for comparison in these experiments, those from cross II are expected to show the impaired repression that is characteristic of a *TP5* element from the *TP5 sn*^{w}; *Su(var)205*^{4}*/Cy Roi* stock, and those from cross III could show impaired repression if paternally inherited factors from the *Su(var)205*^{4}*/Cy Roi* stock compromise repression by a maternally inherited *TP5* element. As controls in these experiments, we also tested males lacking the *TP5* element. The results from two replicates of these experiments are summarized in Table 3.

The tests from cross I show that the *H(hsp/CP)3* transgene was an effective inducer of *sn*^{w} mutability (mutation rates, 0.476 and 0.378) and that the *TP5* element repressed this mutability almost completely (mutation rates, 0.002 and 0.003). However, as shown by the tests from cross II, this repression was seriously impaired when the *TP5 sn*^{w} chromosome was maternally derived from the *TP5 sn*^{w}; *Su(var)205*^{4}*/Cy Roi* stock. From this cross, the mutation rate of the non-Curly [*i.e.*, *Su(var)*

TABLE 3

Mutability of *sn*^{w} in sons from crosses between *(TP5) sn*^{w} and *Su(var)205*^{4}*/Cy Roi* stocks

	Non-Curly sons			Curly sons		
X chromosome	No. vials	No. flies	Mutation rate[a]	No. vials	No. flies	Mutation rate[a]
Cross I. *(TP5) sn*^{w} ♀♀ × *H(hsp/CP)3* ♂♂ [no effect of *Su(var)205*^{4}*/Cy Roi* stock]						
sn^{w}	50	1067	0.476 ± 0.018[b]			
	50	1417	0.378 ± 0.013[b]			
TP5 sn^{w}	50	1462	0.002 ± 0.002			
	59	1834	0.003 ± 0.001			
Cross II. *(TP5) sn*^{w}*; Su(var)205*^{4}*/Cy Roi* ♀♀ × *H(hsp/CP)3* ♂♂ [cumulative effect of *Su(var)205*^{4}*/Cy Roi* stock][c]						
sn^{w}	49	1140	0.565 ± 0.018	47	1192	0.672 ± 0.022
	50	1299	0.596 ± 0.018	40	1197	0.589 ± 0.016
TP5 sn^{w}	45	1289	0.462 ± 0.019	47	1229	0.527 ± 0.023
	53	1539	0.460 ± 0.018	57	1667	0.527 ± 0.016
Cross III. *(TP5) sn*^{w} ♀♀ × *Su(var)205*^{4}*/Cy Roi; H(hsp/CP)3* ♂♂ [zygotic effect of *Su(var)205*^{4}*/Cy Roi* stock][d]						
sn^{w}	50	1193	0.478 ± 0.016	50	1514	0.495 ± 0.016
	39	1050	0.339 ± 0.014	33	871	0.426 ± 0.016
TP5 sn^{w}	40	1216	0.014 ± 0.005	47	1774	0.009 ± 0.003
	60	1834	0.027 ± 0.006	57	1662	0.018 ± 0.004

[a] Unweighted average ± standard error.
[b] For each pair of results in the table, the data on the top line were collected in November 2000–January 2001 and the data on the bottom line were collected in July 2003.
[c] Cumulative effect due to *TP5* element being present in the *Su(var)205*^{4}*/Cy Roi* stock for many generations.
[d] Zygotic effect due to *TP5* being present with factors from the *Su(var)205*^{4}*/Cy Roi* stock in the test generation.

205⁴/+] sons was ~0.46, and that of the Curly (*i.e.*, *Cy Roi/+*) sons was ~0.52.

Is repression of *sn^w* mutability also impaired when paternally inherited factors from the *Su(var)205⁴/Cy Roi* stock are combined for a single generation with a maternally inherited *TP5 sn^w X* chromosome? The data from cross III indicate that it is not. Both the non-Curly [*Su(var)205⁴/+*] and Curly (*Cy Roi/+*) classes of males from this cross had mutation rates <0.03, indicating strong repression of *sn^w* mutability. Paternally derived factors from the *Su(var)205⁴/Cy Roi* stock therefore do not significantly compromise repression by a maternally derived *TP5 sn^w X* chromosome during a single generation in males.

***TP5*-mediated repression is impaired in the hybrid grandsons of crosses between *TP5 sn^w* and *Su(var)205⁴/Cy Roi* stocks:** The different results from crosses II and III above suggest that the ability to impair *TP5*-mediated repression may require more than one generation to develop. We therefore investigated repression of *P*-element excisions in the grandsons of crosses between *TP5 sn^w* females and *Su(var)205⁴/Cy Roi* males.

The experiments were initiated by crossing homozygous *TP5 sn^w* females to *y; Su(var)205⁴/Cy Roi* males. The *y* (*yellow*) body mutation carried by the males is tightly linked to the left end of the *X* chromosome, and the *TP5 sn^w* chromosome carried by their mates is marked with the y^+ allele. Thus, we could readily identify *X* chromosomes that carried *TP5* by scoring for the y^+ marker. In the F_1, *TP5 sn^w/y; Su(var)205⁴/+* (denoted genotype SV) and *TP5 sn^w/y; Cy Roi* (denoted genotype CR) females were crossed to *y; H(hsp/CP)3* males and their phenotypically y^+ sn^w sons (assumed to carry *TP5*) were tested for *sn^w* mutability. The tested sons comprise three classes: (1) non-Curly males from the SV F_1 females, (2) non-Curly males from the CR F_1 females, and (3) Curly males from the CR F_1 females. Any of these types of males might show impaired repression of *sn^w* mutability if the P cytotype associated with the grand-maternally inherited *TP5* element was compromised by factors from the *Su(var)205⁴/Cy Roi* stock.

We also tested males from two other sets of crosses. In one set, homozygous *TP5 sn^w* females were crossed to *y* males and their *TP5 sn^w/y* daughters were crossed to *y; H(hsp/CP)3* males to obtain phenotypically y^+ sn^w sons. Repression in these males could not be affected by factors from the *Su(var)205⁴/Cy Roi* stock. In the other set of crosses, females from the *TP5 sn^w; Su(var)205⁴/Cy Roi* stock were crossed to *y* males and their *TP5 sn^w/y; Su(var)205⁴/+* (genotype SV) and *TP5 sn^w/y; Cy Roi/+* (genotype CR) F_1 daughters were crossed to *y; H(hsp/CP)3* males to obtain y^+ sn^w sons for the *sn^w* test. These males would be expected to show impaired repression due to a long-term effect of the *Su(var)205⁴/Cy Roi* genotype on regulation by the *TP5* element. For all sets of crosses, we carried out a parallel control experiment using *sn^w* females that lacked the *TP5* element.

The results of all the *sn^w* mutability tests are summarized in Table 4. The data from cross I, which did not involve the *Su(var)205⁴/Cy Roi* genotype, show that the *H(hsp/CP)3* transgene induced a high level of *sn^w* mutability in the control flies (mutation rate, 0.492); however, this mutability was strongly repressed by a *TP5* element inherited grand-maternally from the *TP5 sn^w* stock (mutation rate, 0.050). The data from cross II show that, as expected, this repression was severely compromised when the *TP5* element was grand-maternally inherited from the *TP5 sn^w; Su(var)205⁴/Cy Roi* stock. The mutation rates for the control flies of cross II ranged from 0.558 to 0.627, and those for the *TP5* flies ranged from 0.490 to 0.532. The closeness of these numbers indicates that the long-term presence of the *Su(var)205⁴/Cy Roi* genotype seriously impairs regulation by the *TP5* element.

The results of cross III show the effect of factors from the *Su(var)205⁴/Cy Roi* stock acting for two generations on a *TP5* element that was inherited grand maternally from the *TP5 sn^w* stock. Among the control flies, the mutation rates ranged from 0.467 to 0.538, which are consistent with the control rate observed in cross I. Among the flies carrying the *TP5* element, the mutation rates ranged from 0.138 to 0.275; all these rates are significantly less than the control rates. Thus, a *TP5* element from the *TP5 sn^w* stock that has passed for two generations through flies with factors from the *Su(var)205⁴/Cy Roi* stock can repress *sn^w* mutability; however, it does so much less effectively than a *TP5* element that has not been exposed to such factors (see the results of cross I). Factors from the *Su(var)205⁴/Cy Roi* stock therefore impair *TP5*-mediated repression of *sn^w* mutability in the course of two generations.

What factors from the *Su(var)205⁴/Cy Roi* stock cause this impairment of repression ability? Among the *TP5* males from cross III, those with the highest *sn^w* mutation rate (and therefore the lowest repression ability) were the non-Curly males from the SV F_1 females. Half these males were expected to carry the *Su(var)205⁴* mutation. Thus, the *Su(var)205⁴* mutation could be responsible for compromising repression ability. However, the Curly males from the CR F_1 females of cross III had a mutation rate of 0.203, and their non-Curly brothers had a mutation rate of 0.138. Both of these rates are significantly greater than the rate for the *TP5* flies from cross I (0.050), yet neither of these types of males carried the *Su(var)205⁴* mutation—nor did their mothers. Thus, impairment of *TP5*-mediated repression in the offspring of cross III is not due strictly to the presence of the *Su(var)205⁴* mutation in the males that were tested or in their mothers. Rather, it appears to involve factors present in the *Su(var)205⁴/Cy Roi* stock that are transmitted independently of the *Su(var)205⁴* mutation. These factors evidently interfere with the inheritance of the P cytotype through the F_1 females that were produced by crossing *TP5 sn^w* (P cytotype) females with

TABLE 4

Mutability of *sn*^{w} in the grandsons of crosses between *(TP5) sn*^{w} and *Su(var)205*^{4}*/Cy Roi* stocks

	Non-Curly sons				Curly sons			
Genotype of mother[a]	Class[b]	No. vials	No. flies	Mutation rate[c]	Class[b]	No. vials	No. flies	Mutation rate[c]
Cross I. *(TP5) sn*^{w} ♀♀ × *y* ♂♂ [no effect of factors in *Su(var)205*^{4}*/Cy Roi* stock]								
Control flies								
sn^{w}*/y*		56	1726	0.492 ± 0.015				
TP5 flies								
TP5 sn^{w}*/y*		60	1951	0.050 ± 0.007				
Cross II. *(TP5) sn*^{w}*; Su(var)205*^{4}*/Cy Roi* ♀♀ × *y* ♂♂ [cumulative effect of factors in *Su(var)205*^{4}*/Cy Roi* stock]								
Control flies								
(SV) *sn*^{w}*/y; Su(var)205*^{4}*/+*	1	58	1584	0.558 ± 0.017[d]				
(CR) *sn*^{w}*/y; Cy Roi/+*	2	60	1560	0.580 ± 0.017	3	49	1447	0.627 ± 0.017
TP5 flies								
(SV) *TP5 sn*^{w}*/y; Su(var)205*^{4}*/+*	1	58	1780	0.522 ± 0.016[d]				
(CR) *TP5 sn*^{w}*/y; Cy Roi/+*	2	59	1798	0.490 ± 0.020	3	42	1240	0.532 ± 0.019
Cross III. *(TP5) sn*^{w} ♀♀ × *y; Su(var)205*^{4}*/Cy Roi* ♂♂ [two-generation effect of factors in *Su(var)205*^{4}*/Cy Roi* stock]								
Control flies								
(SV) *sn*^{w}*/y; Su(var)205*^{4}*/+*	1	59	1562	0.467 ± 0.018[d]				
(CR) *sn*^{w}*/y; Cy Roi/+*	2	37	1060	0.501 ± 0.017	3	42	946	0.538 ± 0.017
TP5 flies								
(SV) *TP5 sn*^{w}*/y; Su(var)205*^{4}*/+*	1	60	1720	0.275 ± 0.026[d]				
(CR) *TP5 sn*^{w}*/y; Cy Roi/+*	2	51	1415	0.138 ± 0.018	3	30	842	0.203 ± 0.020

[a] Mothers were obtained from the crosses indicated and then crossed to homozygous *H(hsp/CP)3* males. See text for details.
[b] See text for explanation of classes.
[c] Unweighted average ± standard error.
[d] Half the sons tested were expected to carry the *Su(var)205*^{4} mutation.

Su(var)205^{4}*/Cy Roi* (M cytotype) males. It remains possible, of course, that the presence of these factors in the *Su(var)205*^{4}*/Cy Roi* stock is ultimately due to some effect of the *Su(var)205*^{4} mutation.

Impairment of *TP5*-mediated repression persists when *TP5 sn*^{w} *X* chromosomes are extracted from a *TP5 sn*^{w}*; Su(var)205*^{4}*/Cy Roi* stock: *TP5*-mediated repression is severely compromised in stocks with the *Su(var)205*^{4}*/Cy Roi* genotype. Does this impairment persist when the *TP5 sn*^{w} chromosome is extracted from a *Su(var)205*^{4}*/Cy Roi* stock and made homozygous? To answer this question we crossed individual *TP5 sn*^{w}*; Su(var) 205*^{4}*/Cy Roi* males to females with attached-*X* chromosomes to obtain *TP5 sn*^{w}*; Cy Roi/+* males. These males were individually double mated, first to attached-*X* females and then to females heterozygous for the *FM7* balancer *X* chromosome. Non-Curly *TP5 sn*^{w} sons from the former mating were crossed to non-Curly *FM7/TP5 sn*^{w} daughters from the latter mating to obtain homozygous and hemizygous *TP5 sn*^{w} progeny, which were then crossed *inter se* to establish *TP5 sn*^{w} lines free of the *Su(var)205*^{4} and *Cy Roi* chromosomes. In the next generation, *TP5 sn*^{w} females from these lines were crossed to males homozygous for the *H(hsp/CP)2* transgene and their *TP5 sn*^{w}*; H(hsp/CP)2/+* sons were individually tested for *sn*^{w} mutability. We also tested males in which the *TP5 sn*^{w} chromosome had been extracted from the basic *TP5 sn*^{w} stock according to these same procedures. As controls, we tested males derived directly from crosses between males from the *H(hsp/CP)2* stock and females from the *sn*^{w} (M cytotype), *TP5 sn*^{w}, and *TP5 sn*^{w}*; Su(var)205*^{4}*/Cy Roi* stocks. The results of all these tests are summarized in Table 5.

The control data shown at the top of Table 5 were obtained at two different times: at the beginning of the extraction process (pretest data) and during testing of the extracted lines (main test data). The two sets of data are remarkably consistent. The mutation rates for the M controls were 0.416 and 0.449, indicating vigorous transposase activity; those for the *TP5 sn*^{w} stock were 0.015 and 0, indicating strong repression of transposase activity; and those for the *TP5 sn*^{w}*; Su(var)205*^{4}*/Cy Roi* stock were 0.397 and 0.445, indicating severe impairment of *TP5*-mediated repression of transposase activity.

Data from 10 lines derived from the basic *TP5 sn*^{w} stock are shown in the middle of Table 5 (denoted as N lines) and data from 11 lines derived from the *TP5 sn*^{w}*; Su(var)205*^{4}*/Cy Roi* stock are shown at the bottom of Table 5 (denoted as SV lines). Within these two groups, the entries are listed from highest mutation rate (least repression ability) to lowest mutation rate (most repression ability). Among the 10 N lines, the mutation rates ranged from 0.058 to 0.013, and among the 11 SV lines, they ranged from 0.289 to 0.045. Because only one of

TABLE 5

Repression of *sn*^{w} mutability by *X* chromosomes extracted from the *TP5 sn*^{w} and *TP5 sn*^{w}*; Su(var)205*^{4} stocks

Stock	No. vials	No. flies	Mutation rate[a]
Basic *sn*^{w} M stock			
Pretest	48	1137	0.416 ± 0.017
Main test	29	742	0.449 ± 0.023
Basic *TP5 sn*^{w} stock			
Pretest	45	1170	0.015 ± 0.005
Post-test	30	859	0.000 ± 0.000
Basic *TP5 sn*^{w}*; Su(var)205*^{4}*/ Cy Roi* stock			
Pretest	41	1133	0.397 ± 0.020
Main test	28	812	0.445 ± 0.024
Lines derived from basic *TP5 sn*^{w} stock			
N-4	28	1052	0.058 ± 0.012
N-10	29	724	0.056 ± 0.016
N-1	28	1057	0.051 ± 0.017
N-8	29	914	0.051 ± 0.020
N-5	30	741	0.043 ± 0.011
N-2	30	975	0.031 ± 0.008
N-9	30	840	0.026 ± 0.017
N-6	30	535	0.020 ± 0.009
N-3	28	859	0.018 ± 0.006
N-7	29	1109	0.013 ± 0.005
Lines derived from basic *TP5 sn*^{w}*; Su(var)205*^{4}*/ Cy Roi* stock			
SV-10	30	896	0.289 ± 0.045
SV-7	29	800	0.238 ± 0.043
SV-11	28	678	0.196 ± 0.045
SV-2	27	596	0.194 ± 0.030
SV-9	29	884	0.192 ± 0.032
SV-1	30	936	0.144 ± 0.036
SV-4	30	713	0.129 ± 0.023
SV-6	29	975	0.112 ± 0.029
SV-8	30	1109	0.097 ± 0.029
SV-3	28	848	0.096 ± 0.028
SV-5	29	1037	0.045 ± 0.017

[a] Unweighted average ± standard error.

the SV lines had a mutation rate within the range of the N lines, the two sets of mutation rates are significantly different by the Mann-Whitney rank sum test. Thus, compared to a group of *TP5 sn*^{w} lines derived from the basic *TP5 sn*^{w} stock, lines derived from a *TP5 sn*^{w}*; Su(var) 205*^{4}*/Cy Roi* stock show impaired repression of *sn*^{w} mutability four generations after both the *Cy Roi* and *Su(var) 205*^{4} chromosomes were removed from the genotype.

Several of these lines were retested after 15 more generations of culture. The mutation rate for the M controls at the time of these tests was 0.463. All six of the N lines that were tested showed mutation rates <0.023, indicating that they retained strong repression ability. Among the seven SV lines that were tested, two were strong repressors (mutation rates, 0.003 and 0.009), two were weaker repressors (mutation rates, 0.081), and three were only moderate repressors (mutation rates, 0.162, 0.209, and 0.272). Thus, at the time of these tests, cytotype regulation had largely been restored in four of the lines, but in the other three it remained compromised.

***TP5*-mediated repression is impaired in a stock with a deficiency encompassing the *Su(var)205* gene:** To investigate whether a mutation in the *Su(var)205* gene could be indirectly responsible for the impairment of *TP5*-mediated repression of *sn*^{w} mutability seen in the previous experiments, we constructed a *TP5 sn*^{w} stock that carried *Df(2L)TE29Aa-11*, a recessive lethal deficiency with breakpoints in bands 28E4-7 and 29B2-C1 in the left arm of chromosome *2*, balanced with the *Cy Roi* chromosome. This deficiency deletes the *Su(var) 205* locus. We also constructed a *TP5 sn*^{w} stock that carried *Df(2L)Trf-C6R31*, a recessive lethal deficiency within sections 28D-E in the left arm of chromosome *2*, balanced with the *Cy Roi* chromosome. This deficiency does not delete the *Su(var)205* locus. After many generations of laboratory culture, *TP5 sn*^{w}*; Df(2L)/Cy Roi* females from these two stocks were crossed to males homozygous for the *H(hsp/CP)2* transgene and their *TP5 sn*^{w}*; Df(2L)/H(hsp/CP)2* sons were individually tested for *sn*^{w} mutability. For controls, we tested the *sn*^{w}; *+/H(hsp/CP)2* sons of crosses between homozygous *sn*^{w} females that lacked the *TP5* element and homozygous *H(hsp/CP)2* males.

Among a total of 907 sons from 29 control cultures, the unweighted mutation rate was 0.432 ± 0.017; thus, as expected, *sn*^{w} mutability was vigorously induced by the *H(hsp/CP)2* transgene. Among 828 sons from 27 cultures involving the *Trf-C6R31* deficiency, the unweighted mutation rate was 0.046 ± 0.014, which indicates repression of *sn*^{w} mutability by the maternally inherited *TP5* element. By contrast, among 545 sons from 20 cultures involving the *TE29Aa-11* deficiency, the unweighted mutation rate was 0.206 ± 0.032, which indicates significant impairment of *TP5*-mediated repression. The large difference between the last two rates implies that *Df(1)TE29Aa-11*, which is the deficiency that removes the *Su(var)205* gene, plays a role in the impairment of *TP5*-mediated repression of *sn*^{w} mutability. Furthermore, this difference rules out a causative role for the *Cy Roi* balancer chromosome, which was common to both of the deficiency stocks.

***TP5*-mediated repression is impaired in stocks with different point mutations in the *Su(var)205* gene:** To extend this analysis, we tested four other *Su(var)205* mutant alleles, all independently induced, for effects on *TP*-mediated repression of *sn*^{w} mutability. The data were collected in two experiments performed 2 years apart. *TP sn*^{w}; *Su(var)205*^{x}*/Cy Roi* females were crossed to

TABLE 6

Effects of different *Su(var)205* alleles on repression of germ-line sn^w mutability by *TP5* and *TP6*

TP stock and *Su(var)205* allele[a]	Experiment 1 (October 2001, January 2002)[b]			Experiment 2 (January 2004)		
	No. vials	No. flies	Mutation rate[c]	No. vials	No. flies	Mutation rate[c]
sn^w Control (no *TP*)						
Basic stock				29	803	0.427 ± 0.019
$Su(var)205^1$	44	928	0.539 ± 0.025			
$Su(var)205^2$	44	953	0.535 ± 0.029			
$Su(var)205^{03}$	36	850	0.657 ± 0.027			
$Su(var)205^4$	43	875	0.473 ± 0.032			
$Su(var)205^5$	47	1218	0.651 ± 0.024			
TP5 sn^w						
Basic stock	28	778	0.018 ± 0.007			
$Su(var)205^1$	43	1080	0.074 ± 0.021	29	1023	0.439 ± 0.041
$Su(var)205^2$	34	851	0.022 ± 0.017	25	870	0.116 ± 0.029
$Su(var)205^{03}$	50	1589	0.455 ± 0.028	29	862	0.417 ± 0.037
$Su(var)205^4$	44	1245	0.318 ± 0.025	25	713	0.492 ± 0.022
$Su(var)205^5$	48	1185	0.428 ± 0.036	25	861	0.298 ± 0.039
TP6 sn^w						
Basic stock	30	1005	0.096 ± 0.020			
$Su(var)205^1$	46	1283	0.013 ± 0.006	27	764	0.029 ± 0.020
$Su(var)205^2$	32	1061	0.066 ± 0.019			
$Su(var)205^{03}$	47	1223	0.302 ± 0.022	29	825	0.265 ± 0.033
$Su(var)205^4$	42	1028	0.089 ± 0.017	27	791	0.139 ± 0.025
$Su(var)205^5$	46	1225	0.056 ± 0.012	29	825	0.042 ± 0.015

[a] Stocks were established by August 2000.
[b] Data for alleles *1*, *2*, *03*, and *4* were obtained in October 2001; data for allele *5* were obtained in January 2002.
[c] Unweighted average ± standard error.

homozygous *H(hsp/CP)2* males and their *TP* sn^w; *Su(var) 205*^{x}*/H(hsp/CP)2* sons were individually tested for germline sn^w mutability. To check for a generalized effect of each *Su(var)205* mutation on sn^w mutability *per se*, in the first experiment we also performed the same crosses with stocks that lacked a telomeric *P* element. The results of all these tests are summarized in Table 6.

In the absence of a telomeric *P* element, sn^w mutability ranged from 0.435 to 0.657. The two highest values, obtained from tests with alleles *03* and *5*, suggest a generalized enhancement of sn^w mutability reminiscent of the effect seen with allele *4* in a previous experiment (see Table 2); however, in the present experiment, allele *4* did not enhance sn^w mutability; neither did alleles *1* nor *2*.

The results from the crosses with the basic stocks in the second experiment show, as expected, that *TP5* and *TP6* are strong repressors of sn^w mutability (mutation rates of 0.018 for *TP5* and 0.096 for *TP6*). These results are consistent with those from similar crosses in other experiments (see Tables 2, 3, 5, and 7; Stuart *et al.* 2002, Tables 4 and 6; and Niemi *et al.* 2004, Table 1). Compared to these control values, the data in Table 6 indicate that repression by *TP5* was profoundly impaired in stocks with four of the mutant alleles of *Su(var) 205* (*1*, *03*, *4*, and *5*; mutation rate >0.3 in at least one experiment) and that it was moderately impaired in the stock with allele *2* (mutation rate was 0.116 in the second experiment). For *TP6*, only the stock with allele *03* impaired repression significantly. The stock with allele *4* showed an increase in sn^w mutability in the second experiment, but this increase was not statistically significant. Thus, all the mutant stocks of *Su(var)205* appear to disrupt cytotype regulation by *TP5*, but only one of them appears to disrupt cytotype regulation by *TP6*. The consistency of the results with *TP5* strongly suggest that the disruption of cytotype regulation associated with this element is due to an effect of the *Su(var)205* mutations.

***TP5*-mediated repression is impaired in a stock with the Gaiano genetic background:** Savitsky *et al.* (2002) and Perrini *et al.* (2004) have shown that telomeres of Drosophila chromosomes are elongated by the addition of *HeT-A* and *TART* retrotransposons in stocks that are heterozygous for *Su(var)205* mutations. This finding raises the possibility that elongated telomeres are the factors responsible for the impairment of repression in the *TP5; Su(var)205/Cy Roi* stocks. To test this possibility, we introduced *TP5* and sn^w into a stock with elongated telomeres. This stock, called Gaiano, is derived from a natural population, and its elongated telomeres are due to an accumulation of retrotransposons at the ends of its

TABLE 7

Effect of the Gaiano genetic background on repression of germ-line sn^w mutability by *TP5* and *TP6*

Stock[a]	Test 1 (August 2000)			Test 2 (November 2001)			Test 3 (December 2002)		
	No. vials	No. flies	Mutation rate[b]	No. vials	No. flies	Mutation rate[b]	No. vials	No. flies	Mutation rate[b]
sn^w control stocks									
Basic	49	1273	0.564 ± 0.019	50	1447	0.565 ± 0.014	50	1817	0.511 ± 0.015
Gaiano	50	1583	0.512 ± 0.013	50	1448	0.532 ± 0.020	49	2004	0.516 ± 0.014
TP5 sn^w stocks									
Basic	50	1486	0.005 ± 0.004	49	1309	0.014 ± 0.005	50	2028	0.013 ± 0.006
Gaiano	49	1578	0.121 ± 0.023	45	1233	0.194 ± 0.025	49	1933	0.202 ± 0.023
TP6 sn^w stocks									
Basic	49	1457	0.046 ± 0.012	50	1288	0.052 ± 0.011	50	2064	0.085 ± 0.014
Gaiano	50	1619	0.042 ± 0.011	41	1070	0.034 ± 0.009	47	1912	0.009 ± 0.003

[a] Gaiano stocks were created in February 2000.
[b] Unweighted average ± standard error.

chromosomes. This accumulation is caused by a dominant mutation, *Tel*, located on chromosome *3* (Siriaco *et al.* 2001). The *TP5* element and the sn^w mutation from the basic *TP5* sn^w stock were introduced into the Gaiano background by a series of backcrosses (see materials and methods) and the resulting *TP5* sn^w Gaiano stock was tested for repression of sn^w mutability three times over a period of >2 years. Each set of tests was initiated by crossing *TP5* sn^w Gaiano females to males homozygous for the *H(hsp/CP)2* transgene; the *TP5* sn^w*; H(hsp/CP)2/+* sons from these crosses were then mated to attached-*X* females to obtain progeny, which were scored for sn^w mutability. *TP6* sn^w Gaiano and sn^w Gaiano stocks were similarly constructed and tested. As controls, we tested flies from the basic sn^w, *TP5* sn^w, and *TP6* sn^w stocks. Table 7 summarizes the results of all these tests.

The sn^w and sn^w Gaiano stocks had comparable mutation rates, which ranged from 0.511 to 0.565. Thus, the Gaiano genetic background did not seem to have any effect on sn^w mutability *per se*. As expected, the basic *TP5* sn^w and *TP6* sn^w stocks repressed sn^w mutability effectively; for *TP5*, the mutation rates ranged from 0.005 to 0.014, and for *TP6*, they ranged from 0.046 to 0.085. However, in the Gaiano genetic background repression by one of the telomeric elements was significantly impaired; for the *TP5* sn^w Gaiano stock the mutation rates ranged from 0.121 to 0.202, whereas for the *TP6* sn^w Gaiano stock they ranged from 0.009 to 0.042. Thus, repression by *TP5*, but not by *TP6*, was compromised in a genetic background derived from a stock with elongated telomeres.

DISCUSSION

The P cytotype is a maternally transmitted condition that strongly represses *P*-element activity in both the male and female germ lines. In some stocks, this condition is associated with *P* elements inserted near the left telomere of the *X* chromosome (Ronsseray *et al.* 1991; Stuart *et al.* 2002), but it is effective only when these elements are transmitted from a female. When they come from a male, all regulatory ability is lost. However, such loss can be overcome if the telomeric *P* elements subsequently pass through a female germ line (Stuart *et al.* 2002; Niemi *et al.* 2004; Simmons *et al.* 2004). Previous studies have indicated that regulation by the P cytotype seems to be disrupted by mutations in the *Su(var)205* gene (Ronsseray *et al.* 1996,1998; Marin *et al.* 2000). Mutations in a handful of other genes, including *aubergine*, *Enhancer of variegation 205*, *Polycomb*, *polyhomeotic*, *Posterior sex combs*, *Suppressor of variegation 2-1*, *Suppressor of zeste 2*, and *trithorax*, have also been tested, but only one—*aubergine*—has been found to impair the P cytotype (Ronsseray *et al.* 1996; Reiss *et al.* 2004).

Our studies of the P cytotype employed stocks in which telomeric *P* elements were maintained along with *Su(var)205* mutations for many generations. Thus, we were able to investigate the long-term effects of these mutations on cytotype regulation. The data indicate that this regulation is profoundly impaired in most *TP5* stocks heterozygous for *Su(var)205* mutations and that it is also impaired in a *TP5* stock with the genetic background of Gaiano, a strain with elongated telomeres. The data show that regulation by *TP6* is impaired in some stocks carrying *Su(var)205* mutations, but that it is not impaired in a stock with the Gaiano genetic background.

How might these observations be explained? Savitsky *et al.* (2002) have shown that in stocks heterozygous for *Su(var)205* mutations, the end of a terminally deleted *X* chromosome accumulates telomere-specific retrotransposons. These authors conjecture that abnormal types or quantities of HP1, the polypeptide product of the *Su(var)205* gene, deregulate the processes that control the

addition of *HeT-A* and *TART* retrotransposons to the ends of chromosomes. Consequently, a stock with a *Su(var)205* mutation acquires abnormally long telomeres, and some of these elongated telomeres might persist even after the *Su(var)205* mutation is removed from the genotype. We propose that elongated telomeres engendered by *Su(var)205* mutations are responsible for the impairment of cytotype regulation documented in this study.

The mechanism of cytotype regulation is unknown. One hypothesis is that it is mediated by the products of telomeric *P* elements—either RNAs or polypeptides that repress transposase activity or synthesis. It is not known if either *TP5* or *TP6* is transcribed into RNA in either the sense or antisense directions. Antisense RNA from these elements might repress *P* activity through an RNA interference mechanism. However, studies with antisense *P* transgenes have indicated that repression by this mechanism is not nearly as strong as regulation by telomeric *P* elements (Simmons *et al.* 1996; Stuart *et al.* 2002). Sense RNA transcribed from telomeric *P* elements might be translated into polypeptide repressors of *P* activity. Complete *P* elements and some incomplete *P* elements are known to produce such repressors (Simmons *et al.* 2002a,b). However, the absence of the *TP5* and *TP6* elements in a survey of >90 P strains argues that they probably do not produce polypeptide repressors; otherwise, natural selection would have favored their spread in Drosophila populations (Stuart *et al.* 2002).

Another hypothesis is that cytotype regulation involves the silencing of *P* elements scattered throughout the genome by interactions between these elements and telomeric *P* elements (Roche and Rio 1998; Ronsseray *et al.* 1998,2001; Stuart *et al.* 2002). Telomeric *P* elements might pair with other *P* elements and transfer to them some aspect of the repressive chromatin organization that is associated with the ends of chromosomes. Nontelomeric *P* elements that receive this telomeric chromatin imprint might thereby be inactivated.

How might telomere length disrupt the P cytotype mediated by telomeric *P* elements? Elongated telomeres might reduce or abolish transcription through such elements and thereby limit or block the production of repressor P polypeptides or antisense *P* RNAs. However, studies with telomeric *P* transgenes indicate that the addition of retrotransposons to a chromosome's end typically increases the expression of a transgene, possibly through the influence of transcriptional enhancers located in the retrotransposons (Golubovsky *et al.* 2001). Telomere elongation might therefore be expected to increase the abundance of telomeric *P*-element products—just the opposite of the decrease hypothesized to account for impairment of the P cytotype. Furthermore, telomeric retrotransposons are vigorously transcribed in stocks with *Su(var)205* mutations (Perrini *et al.* 2004); yet, cytotype regulation associated with telomeric *P* elements is significantly impaired in these stocks.

If elongated telomeres do not impair cytotype by lessening the expression of telomeric *P* elements, perhaps they interfere with the ability of these elements to interact with other *P* elements and silence them. Telomeric *P* elements might have difficulty pairing with other *P* elements in a genome with abnormally long telomeres because an accumulation of retrotransposons at the ends of chromosomes might favor pairing between the telomeres themselves. Such telomere-telomere associations are observed in the polytene chromosomes of the Gaiano stock. If pairing between telomeres is favored, the silencing power of the telomeric *P* elements would be reduced. This effect might be observed if either the chromosome bearing a telomeric *P* element had acquired an abnormally long telomere or other chromosomes in the genome had done so.

Whatever the mechanism of cytotype regulation, we hypothesize that elongated telomeres impair it. *Su(var) 205* mutations are responsible for this impairment only in so far as they cause telomeres to become elongated. Thus, their effect is construed to be indirect. This distinction explains why a stock can continue to show impaired regulation even after a *Su(var)205* mutation has been removed from its genotype. We also propose that elongated telomeres impair cytotype regulation by acting in the female germ line, where this regulation is established and through which it must be transmitted (Niemi *et al.* 2004). F_1 males produced by crossing females from a telomeric *P* stock to males from a *Su(var)205*^{4}*/Cy Roi* stock do not show impaired cytotype regulation even though they presumably have elongated telomeres on at least some of their chromosomes (Table 3), most likely because these males have inherited the repressive P cytotype from their mothers. By contrast, F_2 males from these crosses do show some impairment of cytotype regulation even when they do not carry a *Su(var)205* mutation (Table 4). The difference between the F_1 and F_2 males from these crosses is that the mothers of the latter may have elongated telomeres on some of their chromosomes and these elongated telomeres may interfere with the establishment and maintenance of the P cytotype.

Our data show that cytotype regulation by *TP5* is impaired in stocks with any of five different *Su(var)205* mutations, although not so dramatically in a stock with mutant allele *2*, and that it is also impaired in a stock with the Gaiano genetic background. In contrast, cytotype regulation by *TP6*, a slightly larger telomeric *P* element inserted in the same position in a TAS repeat as *TP5*, is impaired only in stocks with two of the five mutant *Su(var)205* alleles (*03* and *4*), and it is not impaired in a stock with the Gaiano genetic background. The different results with *TP5* and *TP6* suggest that another factor, perhaps the size of the telomeric *P* element, its DNA sequence, or its position within the array of elements and repeats at the end of the *X* chromosome, influences the susceptibility of cytotype

regulation to the effects of elongated telomeres in these stocks. Indeed, cytological examination of polytene chromosomes hybridized *in situ* with a *P*-element probe suggests that *TP6* is closer than *TP5* to the end of the chromosome (Todd R. Laverty, personal communication). Thus, the *TP5 X* chromosome may naturally have a longer telomere than the *TP6 X* chromosome, and this longer telomere may predispose *TP5* to lose regulatory ability more easily than *TP6* when it is placed in a mutant *Su(var)205* or a Gaiano genetic background.

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