simply prevents the transposase from attacking potentially mobile *P* elements. The domain for *P*-element binding is located within the first 88 amino acids of the repressor polypeptide sequence (Lee *et al.*, 1998). Artificially engineered polypeptides with these amino acids are able to repress *P*-element transposition *in vitro*, although not as well as longer polypeptides. Thus, other regions in the KP and 66 kD polypeptides contribute significantly to repressor function.

Both the *TP5* and *TP6* elements could encode polypeptides longer than 88 amino acids, and transgenes designed to express the TP5 polypeptide, but not those designed to express the TP6 polypeptide, have a modest ability to repress transposase-induced sn^{w} mutability (Jensen *et al.*, 2008). However, this repression is not nearly so strong as that seen with the *H*(*hsp*/*KP*) transgenes, and it is very much weaker than that of the native *TP5* element situated in the TAS of the XL telomere. The TP5 polypeptide, which is 113 amino acids long, may, therefore, be a bona fide repressor, but it is not as effective as the KP repressor. Furthermore, unlike the KP and 66 kD repressors, the TP5 polypeptide has no ability to alter the phenotypes of sn^{50e} and sn^{w}. Repressors that alter mutant singed phenotypes may do so because they possess downstream amino acid motifs that augment their ability to bind to *P* elements. For instance, a leucine zipper in the KP polypeptide has been implicated in its ability to form dimers, and dimerization may enhance the ability of KP repressors to bind to *P*-element DNA. Andrews and Gloor (1995) demonstrated that this leucine zipper is important for repression of hybrid dysgenesis. One other fact argues that *TP5* and *TP6* do not produce polypeptides with significant repressor function: Unlike *KP*, neither of these elements is widespread in natural populations (Stuart *et al.*, 2002). If *TP5* and *TP6* encoded effective repressor polypeptides, we would expect natural selection to have brought them to noticeable frequencies in many places in the world.

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Evidence for action of the KP repressor polypeptide in the germ line of female *Drosophila melanogaster* carrying piRNA-generating telomeric *P* elements.

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The transposable *P* elements of *D. melanogaster* are responsible for a syndrome of germ-line abnormalities that includes sterility and high frequencies of mutation and chromosome breakage (Engels, 1989). This syndrome, called hybrid dysgenesis, occurs in the offspring of crosses between paternally contributing (P) and maternally contributing (M) strains. Thus, P male × M female produces dysgenic offspring, but P female × M male usually does not. Hybrid dysgenesis is repressed by small *P*-derived RNAs,

called piRNAs, because they interact with the Piwi family of proteins (Brennecke *et al.*, 2008; Jensen *et al.*, 2008). These RNAs are generated by *P* elements inserted in the telomere associated sequences (TAS) at the left end of the X chromosome. *TP5* (1.8 kb long) and *TP6* (1.9 kb long) are two such elements (Stuart *et al.*, 2002). Analyses of *TP5* and *TP6*—and of other *P* insertions in the TAS as well (Marin *et al.*, 2000)—have shown that piRNAs are the basis of the P cytotype, a maternally transmitted state that regulates *P*-element activity in the germ line.

Although piRNAs are the primary means of repressing hybrid dysgenesis, some *P*-encoded polypeptides may also play a role. For example, complete *P* elements (*CP*, 2.9 kb long) encode a 66 kd repressor polpeptide as well as an 87 kd transposase (Rio, 1990). These two polypeptides are produced through alternate splicing of *CP* mRNA; the transposase is synthesized from mRNA that has lost all three of *CP*'s introns, whereas the repressor is synthesized from mRNA that retails the last of these introns (denoted as the *2-3* intron because it lies between exons *2* and *3*). In the germ line, both polypeptides appear to be made, but in the soma, the 66 kd polypeptide is the only *CP* product. This tissue-specific expression of the *CP* polypeptides explains why *P*-element movement, which is catalyzed by the transposase, is restricted to the germ line (Laski *et al.*, 1986).

An element called *KP* (1.15 kb long) also encodes a repressor polypeptide (Rio, 1990; Andrews and Gloor, 1995; Lee *et al.*,1996, 1998). This repressor is 207 amino acids long and shares 199 amino acids with the transposase. It is able to bind to *P* elements and may also interact with other *P*-encoded polypeptides, including itself and the transposase. Because *KP* elements are widespread in natural populations, they appear to confer a selective advantage (Boussy *et al.*, 1988; Itoh and Boussy, 2002; Itoh *et al.*, 2007). A recent report (Simmons *et al.*, 2015) indicates that repression of hybrid dysgenesis by *KP* elements is enhanced by cytotype-anchoring telomeric *P* elements such as *TP5* and *TP6*. This *KP* effect is zygotic rather than maternal and presumably results from the action of the KP polypeptide. Models of transposon regulation have assumed that piRNAs act by destroying transposon mRNAs or by blocking their translation. However, the *KP* effect implies that some *P* mRNAs survive and are translated, which in turn implies that piRNA-mediated regulation is more complicated than simply destroying transposon mRNAs or blocking their translation.

This report provides additional evidence for the action of the KP repressor polypeptide in the face of piRNA-mediated cytotype regulation. First, it confirms that the telomeric elements *TP5* and *TP6* confer the P cytotype in males and females. Second, it demonstrates that the KP repressor polypeptide is active in flies that have inherited these elements from their mothers.

Two assays were used to demonstrate that *TP5* and *TP6* confer the P cytotype. The strains used in these assays were homozygous for one of the telomeric *P* elements (*TP*), a null allele of the closely linked *white* (*w*) locus, and a double *P*-element insertion allele of the X-linked *singed* (*sn*) locus. This last allele, called *weak singed* (sn^w), causes a mild malformation of the bristles in hemizygous males but has little or no effect on the bristles of homozygous females.

The first assay measured the incidence of sterility in females from testcrosses between *TP w* sn^w or *w* sn^w (control) females and males from a strong P strain (Harwich). This sterility results from the failure of the ovaries to develop properly, a condition called gonadal dysgenesis (GD). It is induced specifically by the action of the Harwich-derived *P* elements in the germ lines of testcross offspring, and is easily recognized in females by their inability to produce eggs. GD was induced by crossing females from the *TP* and control strains *en masse* to Harwich males at 21°C. After three days, each female was individually placed in a fresh culture vial, which was incubated at 29°C, a temperature that maximizes the occurrence of GD. On day 11, the offspring were transferred to a holding vial, where they matured for two days at 21°C. The females were then screened for GD by squashing them between two glass slides. Females that did not extrude eggs were classified as dysgenic. A solution of green food coloring was placed between the slides to make the eggs easier to see. The data on the left side of Table 1 show that both *TP5* and *TP6* strongly repressed Harwich-induced GD.

The second assay measured the frequency of *P*-element excisions from sn^w in the male germ line. Transposase-catalyzed excision of one or the other of the *P* elements in the sn^w allele creates alleles with a phenotype more or less severe than that of the parent allele (Roiha *et al.*, 1988). Germ-line excisions of these elements can be detected by scoring for the extreme singed (sn^e) or pseudo-wild phenotypes ($sn^{(+)}$) in the next generation. To induce the excisions, homozygous sn^w females were crossed to males that carried *P*(ry^+, Δ*2*-

3)*99B*, a stable *P* transgene that makes the P transposase in the soma as well as in the germ line, because the *2-3* intron has been deleted by construction (Robertson *et al.*, 1988). These crosses were incubated at 18°C, and the sn^w; Δ*2-3*/+ sons from them were crossed at 25°C to *C*(*1*)*DX, y w* females with attached-X chromosomes to enforce patroclinous transmission of the sn^w allele or its sn^e or $sn^{(+)}$ derivatives. These attached-X females came from a P strain to prevent transposase activity in their offspring's soma (Robertson and Engels, 1989). The sons of these crosses were scored for the three singed phenotypes and the proportion that were sn^e or $sn^{(+)}$ was calculated to measure the mutability of the sn^w allele. The data on the right side of Table 1 show that both *TP5* and *TP6* strongly repressed *P* excisions from sn^w in the male germ line. \

Table 1. Repression of gonadal dysgenesis (GD) and sn^w-mutability by strains with telomeric *P* elements.

	Gonadal dysgenesis in females			Mutability of sn^w in males		
Strain	No. vials	No. daughters	%GD ± SE[a]	No. vials	No. sons	Mutability ± SE[b]
w sn^w	25	344	98.4 ± 0.7	30	819	0.340 ± 0.029
TP5 w sn^w	29	455	2.2 ± 1.4	28	577	0.041 ± 0.012
TP6 w sn^w	28	245	37.3 ± 6.2	29	1186	0.059 ± 0.014

[a] Unweighted average percentage of GD among daughters ± standard error.
[b] Unweighted average frequency of sn^e and $sn^{(+)}$ among sons ± standard error.

Together these two assays show that both *TP5* and *TP6* repress *P*-element activity in the germ line—that is, they confer the P cytotype. This activity is catalyzed by the P transposase. The telomeric *P* elements are thought to repress *P* activity by targeting *P*-specific piRNAs to the transposase mRNA, which is then either degraded or blocked from serving as a template for polypeptide synthesis. Either way, transposase activity would be reduced, although, as the data in Table 1 show, it is not completely abolished. It is worth noting that RT-PCR data have shown that the transposase mRNA, though diminished, is not entirely eliminated in females that inherit a *TP* from their mothers (Jensen *et al.*, 2008).

A different assay was used to see if *TP5* and *TP6* could undercut germ-line activity of the KP repressor polypeptide. This assay makes use of mutant alleles of the *singed* gene that impair female fertility. Females carrying these alleles produce ill-formed eggs that are unlikely to develop normally after fertilization; thus, they produce few viable offspring (Robertson and Engels 1989). This "singed sterility" results from defects in the ring canals, which contain the singed protein and which normally allow materials to pass from the nurse cells into the oocyte during oogenesis (Cant *et al.*, 1994). *P*-insertion alleles such as sn^w cause singed sterility, but only when P repressor polypeptides are present, presumably because the repressors interfere with the expression of the mutant *singed* gene by binding to its inserted *P* elements. These "repressor-sensitive" alleles are, therefore, useful detectors of P repressor polypeptides.

The issue is whether or not a *KP* element can produce the KP repressor in flies that have inherited a *TP* element maternally. To resolve this issue, females that were heterozygous for sn^{x2} (a null mutation) and sn^w were tested for fertility in the presence and absence of *H(hsp/KP)3*, an *hobo* transgene that contains a *KP* element fused to an ancillary promoter from the *hsp70* gene (Simmons *et al.*, 2002). These females were obtained by crossing *FM7*, w^{81g} sn^{x2} *B*; *H(hsp/KP)3*/+ males to *TP w* sn^w females; w^{81g} is a null mutation of the *white* gene that arose spontaneously in the *FM7* balancer chromosome. Both types of daughters—those that inherited the *H(hsp/KP)3* transgene (recognized by their colored eyes) and those that did not (white eyes)—were allowed to mate with their brothers for 3-4 days, and then placed individually in 13 × 100 mm culture tubes supplied with a sugar-dried yeast medium (Simmons *et al.*, 1980); these tubes were then incubated at 25°C. Each female's fertility was quantified by counting the number of pupae present in the tube 9 days later. Previous work had established that the *H(hsp*/*KP)3* transgene induces singed sterility by acting zygotically, presumably because it produces the KP repressor polypeptide (Paterson *et al.*, 2007). If the telomeric *P* elements undercut *KP* expression, they would be expected to suppress singed sterility in sn^w/sn^{x2} females that carry *H*(*hsp*/*KP*)*3*.

Table 2. Effect of maternally inherited telomeric *P* elements on *H*(*hsp*/*KP*)*3*-induced singed sterility in sn^{w}/sn^{x2} females.

	H(*hsp*/*KP*)*3* present					*H*(*hsp*/*KP*)*3* absent				
			No. of Progeny per fertile female					No. of Progeny per fertile female		
Strain	No. of females	Prop. sterile	Median	Mean	SD[a]	No. of females	Prop. sterile	Median	Mean	SD[a]
w sn^{w}	91	0.86	2	2.5	1.5	83	0.01	23	23.2	9.2
TP5 w sn^{w}	64	0.55	2	5.0	5.7	75	0	39	37.5	13.8
TP6 w sn^{w}	83	0.48	4	5.5	5.2	72	0	35	35.4	13.2

[a] Standard deviation

The data (Table 2) show that many of the sn^{w}/sn^{x2} females that carried this transgene were completely sterile, and of those that were not, the number of progeny was meager. The telomeric elements did seem to alleviate complete sterility in the *KP*-bearing females somewhat (55% for *TP5* and 48% for *TP6*, compared to 86% for the control), but they did not improve progeny numbers significantly. By contrast, almost all the sn^{w}/sn^{x2} females that did not carry the *KP* transgene were fertile. These females produced many offspring, especially when a *TP* was present. The increased number of progeny when a *TP* was present suggests that the sn^{w} allele has an intrinsic fertility-reducing effect, and that this effect is mitigated by piRNAs generated from *TP5* and *TP6*. Perhaps these piRNAs interact with the *P* elements inserted in sn^{w} and boost the expression of the *singed* gene, thereby bringing about more nearly normal levels of singed protein. However, no such boosting occurs in sn^{w}/sn^{x2} females that carry the *KP* transgene. In these females, the KP repressor polypeptide dramatically impairs singed expression, even when the telomeric *P* element is present.

The paramount effect of the KP repressor polypeptide in the flies that carry the *H(hsp/KP)3* transgene is *prima facie* evidence that cytotype-anchoring telomeric *P* elements do not abolish the production of P polypeptides; the mRNAs that encode these polypeptides must survive and be translated despite attacks by *TP*-generated piRNAs. The piRNA-mediated repression of hybrid dysgenesis must, therefore, involve something more than the destruction or translational arrest of *P*-element mRNAs. One possibility is that piRNAs alter the chromatin in and around *P* insertions in such a way that these insertions can no longer be mobilized by the P transposase. It is known that chromatin can be reorganized by piRNA action (Grewal 2010), and there is some evidence that *TP*-generated piRNAs affect the status of the chromatin that contains *P* elements (Josse *et al.*, 2007).

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Oscillations in the ERG of the *Drosophila trpl*^{302} mutant are caused by an additional mutation in the *inebriated* gene.

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Introduction

Light-absorption in the *Drosophila* compound eye triggers a G protein- and PLCβ-mediated signalling cascade that finally leads to the opening of the cation channels TRP and TRPL. The influx of cations causes a depolarisation of the photoreceptor cells and, via histamine-dependant synaptic transmission, an activation of downstream neurons. Electroretinogram recordings (ERGs) reveal the depolarization of photoreceptor cells as a prominent voltage change while electrical responses of laminar neurons are recorded as on- and off-transients. Mutations in the *inebriated* (*ine*) gene, that encodes a putative neurotransmitter transporter, induce defects at the photoreceptor synapse and result in electrical oscillations superimposed on the depolarizing receptor potential (Gavin *et al.*, 2007). Gavin *et al.* have proposed a mechanism for the oscillations in the *ine* mutant. Histamine stored in the photoreceptor synapse is released into the synaptic cleft. Here, it activates postsynaptic Cl^- channels as well as presynaptic H_3 receptors. The activation of the H_3 receptor inhibits presynaptic Ca^{2+} channels and down-regulates histamine release. In the glia cells, histamine is converted to carcinine, which is transported into the photoreceptor cell by Inebriated. If Inebriated is missing, carcinine accumulates in the synaptic cleft and competes with histamine binding to the H_3 receptor. This leads to fluctuations of the current through the presynaptic Ca^{2+} channel. The resulting Ca^{2+} fluctuations finally cause oscillations in the response of downstream neurons. Similar oscillations were observed in the *trpl*^{302} mutant (Leung *et al.*, 2000), giving rise to speculations whether TRPL, in addition to its function in the phototransduction cascade, might have a function at the photoreceptor synapse. Interestingly, the *arrestin1*^{1} mutant also displays ERG oscillations. Here we show that oscillations observed in the ERG of *trpl*^{302} or *arr1*^{1} mutant flies are due to a secondary mutation in the *ine* gene.

Material and Methods

Drosophila stocks

Oregon R *w*^{1118} (here referred to as wild type), *y*; *ine*^{MI05077} [*y*$^{+}$], and the deletion strain *w*^{1118}; Df(2R)BSC131/CyO (46A1-46B4 covering trpl at 46B2) were obtained from the Bloomington *Drosophila* Stock Center. *trpl*^{302} *cn bw* (Niemeyer *et al.*, 1996), *arr1*^{1} *cn bw* (Dolph *et al.*, 1993) were obtained from C.S. Zuker. For the generation of a pure *trpl* mutant, *trpl*^{302} *cn bw ine* flies were crossed with *yw;* + mutant flies and female *trpl*^{302} *cn bw ine* / + flies were crossed with a *CyO*-balancer stock. Single male offspring flies carrying a recombined second chromosome and the *CyO* balancer were crossed with a *Sco*/*CyO* balancer stock. In the next generation, flies carrying the recombined second chromosome and *CyO* were crossed *inter se* and finally stocks carrying the recombined second chromosome homozygously were established. The mutant carrying the *trpl*^{302} mutation but lacking the *ine* second site mutation is now called *trpl*^{302NO} (No Oscillations) and still carries the *cn* and *bw* mutations (*trpl*^{302NO} *cn bw*). Flies were raised on standard cornmeal food at 25°C and crossings were carried out using standard *Drosophila* genetics.