Cytotype Regulation Facilitates Repression of Hybrid Dysgenesis by Naturally Occurring KP Elements in Drosophila melanogaster

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ABSTRACT P elements inserted in the Telomere Associated Sequences (TAS) at the left end of the X chromosome are determiners of cytotype regulation of the entire P family of transposons. This regulation is mediated by Piwi-interacting (pi) RNAs derived from the telomeric P elements (TPs). Because these piRNAs are transmitted maternally, cytotype regulation is manifested as a maternal effect of the TPs. When a TP is combined with a transgenic P element inserted at another locus, this maternal effect is strengthened. However, when certain TPs are combined with transgenes that contain the small P element known as KP, stronger regulation arises from a zygotic effect of the KP element. This zygotic effect is observed with transgenic KP elements that are structurally intact, as well as with KP elements that are fused to an ancillary promoter from the hsp70 gene. Zygotic regulation by a KP element occurs only when a TP was present in the maternal germ line, and it is more pronounced when the TP was also present in the grand-maternal germ line. However, this regulation does not require zygotic expression of the TP. These observations can be explained if maternally transmitted piRNAs from TPs enable a polypeptide encoded by KP elements to repress P element transposition in zygotes that contain a KP element. In nature, repression by the KP polypeptide may therefore be facilitated by cytotype-mediating piRNAs.

KEYWORDS P element hybrid dysgenesis piRNA telomere ping-pong cycle

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Hybrid dysgenesis is a syndrome of germ line abnormalities found in the offspring of crosses between different types of Drosophila strains (Kidwell et al. 1977). These abnormalities include high frequencies of mutation and chromosome breakage and a temperature-dependent form of sterility characterized by the death of germ line cells, a condition termed gonadal dysgenesis (GD). All these traits are caused by the activity of transposable elements. Although different types of transposons may be involved, here we focus on the P elements of Drosophila melanogaster.

P-induced hybrid dysgenesis occurs in the offspring of crosses between paternally contributing (P) and maternally contributing (M) strains (Kidwell et al. 1977; Engels 1989). P strains possess P elements in their genomes whereas M strains usually do not (Bingham et al. 1982). In a dysgenic cross (P male × M female), paternally contributed P elements are activated in the germ lines of the offspring. This activation is catalyzed by an 87 kDa enzyme, the P transposase, whose 751 amino acids are encoded by structurally complete members of the P element family (Rio 1990). These elements are 2907 bp long and are demarcated by 31 bp long inverted terminal repeats (O’Hare and Rubin 1983), which are the substrates for transposase action. Structurally incomplete P elements cannot produce the transposase, but they can be mobilized by it if they possess the inverted terminal repeats. In nature, P element activity is restricted to the germ line because of the way in which the P element’s primary RNA is spliced. All three of the P element’s introns can be removed in the germ line, but in the soma, the last of the introns remains (Laski et al. 1986). This last intron contains a stop codon, which terminates translation prematurely and prevents the transposase from being made.

In the germ line, P element activity can be repressed by different regulatory factors. Some are P-encoded polypeptides with substantial homology to the P transposase, but without its catalytic activity. One of these repressors is the 66 kDa polypeptide translated from incompletely spliced P RNA (Robertson and Engels 1989; Misra and Rio 1990; Misra et al. 1993; Gloor et al. 1993; Andrews and Gloor 1995). Other repressor polypeptides are translated from the transcripts of the

REFERENCES
structurally incomplete P elements that are too small to encode the transposase. The best known of these small elements is called KP because it was discovered in flies from the Russian city of Krasnodar (Black et al. 1987; Jackson et al. 1988). KP elements are missing base pairs 809–2561 in the canonical P element sequence. Consequently, they encode a polypeptide of only 207 amino acids, 199 of which are identical to the beginning of the transposase. The KP polypeptide binds to P elements and represses transposition (Lee et al. 1996, 1998); it also appears to repress P element expression (Lemaire et al. 1993) and to play a role in heterochromatin formation (Bushey and Locke 2004; Sameny and Locke 2011). Surveys have shown that KP elements are widespread in natural populations (Boussy et al. 1988; Itoh and Boussy 2002; Itoh et al. 2007; C. R. Preston and W. R. Engels, personal communication), a finding consistent with the idea that the KP repressor confers a selective advantage on flies that can make it.

Germ line P element activity is also repressed by a maternally transmitted condition called the P cytotype, a hallmark of all P strains (Engels 1979, 1989). Repression by the P cytotype involves small RNAs generated from P elements inserted at special loci in the genome. These RNAs, called Piwi-interacting (or pi) RNAs because they associate with members of the Piwi family of proteins, are deposited in the oocyte to protect the future offspring from the untoward effects of rampant P transposition (Brennecke et al. 2008). On account of this maternal endowment, flies from crosses between P females and M males show much lower levels of dysgenesis than flies from reciprocal crosses; likewise, flies from crosses between P females and P males seldom show dysgenic traits.

Genetic studies indicate that P elements inserted in the TAS at the left end of the X chromosome are determiners of the P cytotype (Ronsseray et al. 1991, 1993, 1996, 1998; Marin et al. 2000; Stuart et al. 2002; Simmons et al. 2004; Niemi et al. 2004; Jensen et al. 2008). The regulatory power of these elements is enhanced when other P elements at nontelomeric loci are added to the maternal genotype, even though the latter have no intrinsic ability to repress dysgenesis (Simmons et al. 2007, 2012, 2014). This synergism is thought to result from a process that amplifies the pool of P-specific piRNAs. In brief, antisense piRNAs derived from the telomeric P elements target and cleave long sense RNAs derived from the nontelomeric P elements, generating sense piRNAs that can target antisense transcripts from the telomeric elements. With repetition, this process—called the ping-pong cycle (Aravin et al. 2007; Gunawardane et al. 2007)—generates a large population of piRNAs, which can then be transmitted maternally to minimize dysgenesis in the next generation. These piRNAs undergo transposase synthesis either by degrading P mRNAs or by preventing their translation (Jensen et al. 2008). They may also repress transposition by influencing the structure of chromatin around P elements (Josse et al. 2007).

A recent study has revealed another dimension of piRNA-based cytotype regulation. Telomeric P elements acting maternally facilitate repression of dysgenesis by the KP element acting zygotically (Simmons et al. 2015). This phenomenon was discovered by combining different X-linked telomeric P elements with hobo-element transgenes designed to express the KP polypeptide. The KP elements within these hobo transgenes were terminally truncated to preclude mobilization by the P transposase. They were also supplied with an ancillary promoter (from the Drosophila hsp70 gene). By themselves, these \( H(\text{hsp}/\text{KP}) \) transgenes could not repress dysgenesis. However, when combined with a cytotype-determining telomeric P element (TP), they could. This emergent property was manifested as a zygotic effect in the progeny of test crosses to induce dysgenesis—that is, it was seen only in the progeny that inherited the \( H(\text{hsp}/\text{KP}) \) transgene, even if those progeny had not inherited the TP. These observations suggest that TP-derived piRNAs enable transgene-encoded KP polypeptides to repress hybrid dysgenesis.

In this paper, we explore this unanticipated connection between the piRNA and polypeptide mechanisms of P element regulation further by determining if cytotype-determining TP elements have the same effect on naturally occurring KP elements as on \( \text{hsp}/\text{KP} \) fusions. Specifically, we show that a TP enables an intact KP element situated within a transgenic genomic fragment to repress GD through a zygotic effect. This finding demonstrates that cytotype regulation empowers naturally occurring KP elements that have not been augmented with an ancillary promoter to repress P element transposition, presumably by facilitating the action of the KP polypeptide.

MATERIALS AND METHODS

Drosophila stocks and husbandry

Information on genes and mutant alleles can be found on the Flybase website, in Lindsley and Zimm (1992), or in references cited in the text. The P elements TP5 and TP6 were isolated in the telomere of the left arm of X chromosomes from the wild-type strains \( v_y \) and Mt. Carmel, respectively (Stuart et al. 2002). The KP element known as KP1 was isolated in cytological region 2F in the X chromosome from the wild-type strain Sex1 (Rasmussen et al. 1993). Maps of the TP5, TP6, and KP elements are presented in Jensen et al. (2008) and in Simmons et al. (2015). The standard M strain used in the experiments carried the markers \( y \) and \( w^{67C} \) (hereafter, denoted simply as \( w \)) and is devoid of P elements. The standard P strain was Harwich \( y \ w \), which was previously derived from the Harwich \( w \) strain of Kidwell et al. (1977) by incorporating the \( y \) and \( w^{67C} \) markers (Simmons et al. 2012). Harwich \( y \ w \) carries a plethora of P elements, including some that encode the P transposase; however, it does not carry any KP elements (Simmons et al. 2015). Drosophila cultures were reared in vials on a standard cornmeal-molasses-yeast medium. Stock cultures were incubated at 18–21°C. Strains are available upon request.

\( H(\text{hsp}/\text{KP}) \) transgenic stocks

The \( H(\text{hsp}/\text{KP}) \) transgene contains a KP element truncated before base pair 39 in the 5′ end and after base pair 2871 in the 3′ end of the canonical P element sequence (Simmons et al. 2002); the 5′ end includes the native P element promoter. A cassette containing the promoter from \( D. \ melanogaster \)’s \( \text{hsp70} \) gene is situated immediately upstream of the KP element. This transgene was constructed from the \( \text{hawN hobo} \)-element vector, which carries the eye-coloring mini-white gene as a marker. It was introduced into \( y \ w \) Drosophila by injection into embryos along with pHBL1, a plasmid that encodes the hobo transposase (Calvi and Gelbart 1993). Among the transgenic stocks that were obtained, we used \( H(\text{hsp}/\text{KP})/7 \), which carries two loosely linked insertions of the transgene on chromosome 2. When combined with the telomeric P elements TP5 or NA (Marin et al. 2000), the transgenes in this stock enhance repression of dysgenesis through zygotic effects; when combined with the telomeric P element TP6, they enhance repression mainly through maternal effects (Simmons et al. 2015).

Cloning of KP1 and creation of \( H(w^+, \text{KP1}) \) transgenic stocks

Genomic DNA was isolated from 40 flies carrying the X-linked KP element known as KP1 (Rasmussen et al. 1993), which was the only P element in these flies. This DNA was digested with EcoRI and then recombined with the AZapI bacteriophage vector purchased from Stratagene. Recombinant phages were plated on lawns of XL1
**Table 1** Intrinsic inability of KP transgenes to repress gonadal dysgenesis

| Transgenic Stock | No. of Vials | No. of Flies | %GD ± SE\(^a\) | No. of Flies | %GD ± SE\(^a\) |
|------------------|-------------|-------------|----------------|-------------|----------------|
| H(hsp(KP)\(^b\)) | 24          | 316         | 100 ± 0        | 365         | 94.8 ± 1.7    |
| H(w\(^d\), KP1)C | 25          | 279         | 100 ± 0        | 269         | 100 ± 0       |
| H(w\(^d\), KP1)\(^b\) | 25     | 180         | 100 ± 0        | 367         | 98.6 ± 0.6    |

GD, gonadal dysgenesis

\(^a\) Unweighted average percentage GD ± SE.

\(^b\) Two loosely linked insertions of the transgene are present in these stocks.

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erichia coli cells and the resulting plaques were screened for the KP1 element by hybridization with a \(^32\)P-labeled probe, produced by random priming from a PCR-amplified complete P element. A KP1-containing phage clone was isolated and the plasmid cassette (pBlue-script + EcoRI genomic fragment) within the phage vector was obtained by \textit{in vivo} excision. The cloned EcoRI fragment within this plasmid was transferred into another plasmid (pMartini), which has NolI sites flanking the EcoRI cloning site. After digestion with NolI, the KP1-containing genomic fragment was inserted into the unique NolI site of the \textit{hobo} transformation vector pHawN. The resulting construct, denoted H(w\(^d\), KP1), was injected into \textit{y w Drosophila} embryos along with pHBL1 to obtain transgenic stocks, and insertions were localized to specific chromosomes by segregation against dominant markers. The KP1 clone was analyzed by DNA sequencing using P element-specific primers, and the genomic position of the KP1 element was determined by comparing the sequence data to the \textit{D. melanogaster} genome using the BLAST analytic.

**Assay for GD**

GD was induced by mass mating test females to males from the Harwich \textit{y w P} strain at 21\(^\circ\). After 3 d, the mated females were individually transferred to fresh cultures, which were reared at 29\(^\circ\). On day 11, the offspring that had hatched from each culture were transferred to a holding vial, where they matured for 2 d at 21\(^\circ\). The frequency of GD was determined by squashing samples of the females among these offspring between two glass slides to see if they had any eggs. Females with GD do not produce eggs. Green food coloring was placed between the slides to facilitate the scoring of this egg-less phenotype. When the test crosses segregated different types of females, each type was scored separately, with a maximum of 20 females per type. The females that were used in the test crosses to Harwich \textit{y w} males were obtained from different types of crosses, which are described in Results. These initial crosses were incubated at 25\(^\circ\).

**Statistical analyses**

The frequency of GD was calculated independently for each type of female in each test vial. Unweighted average frequencies and empirical standard errors (SE) among all the vials in a test group were then computed for each type. Statistical differences between averages were evaluated by performing \(t\) or \(z\) tests. GD frequencies were also computed by pooling data across types of females in each vial. Differences between pooled groups (females carrying a transgene vs. females not carrying it) were summarized by the number of vials in which one group had less GD than the other group. This “comparison score” between the two groups was evaluated using the nonparametric sign test.

**Data availability**

The authors state that all data necessary for confirming the conclusions presented in the article are represented fully within the article.

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**RESULTS**

**The H(w\(^d\), KP1) hob transgene contains an intact KP element**

Individual KP elements had previously been isolated in X chromosomes derived from a wild-type strain known as Sexi (Kidwell 1985). One of these elements, denoted KP1, was localized to region 2F of the X chromosome, and genetic tests had shown that it could repress hybrid dysgenesis in some assays (Rasmussen et al. 1993). We cloned this KP element from a recombinant DNA library and used it to create a \textit{hobo} transgene marked with the mini-white gene (here symbolized w\(^\dagger\)), which served as a convenient visible marker for subsequent genetic analyses. The transgene is denoted H(w\(^d\), KP1).

The KP1 element within H(w\(^d\), KP1) is situated 1.3 kb from the distal end of a 7 kb EcoRI fragment derived from cytological region 2F5 in the X chromosome of the Sexi strain. Sequence analysis showed that the element is intact, that it is oriented 5\(^'\) to 3\(^'\) from distal to proximal in the cloned fragment, and that the 8 bp target site duplication created when the element inserted into this region begins at nucleotide 2,197,975 of the reference \textit{D. melanogaster} genome sequence. This insertion site is located well within the long intron of the \textit{pole hole (phil)} gene.

**H(w\(^d\), KP1) transgenes do not repress Harwich-induced GD**

We first assessed whether or not the H(w\(^d\), KP1) transgenes in two different stocks, denoted C and J, have an intrinsic ability to repress GD induced by the strong P strain Harwich \textit{y w}. This assessment was accomplished by crossing \textit{y w} H(w\(^d\), KP1)/+ females to Harwich \textit{y w} males and scoring the incidence of GD in their two types of daughters—those carrying the H(w\(^d\), KP1) transgene (identified by their colored eyes) and those not carrying it (identified by their white eyes). The \textit{y w} H(w\(^d\), KP1)/+ females for these tests were obtained by crossing homozygous \textit{y w} H(w\(^d\), KP1) females to \textit{y w} males from an M strain. In stock C, the H(w\(^d\), KP1) transgene is located on chromosome 2; in stock J, two loosely linked insertions of this transgene are located on chromosome 3. Along with these stocks, we also tested a stock that carries two insertions of \textit{H(hsp(KP))}, a transgene that has been studied previously (Simmons et al. 2015).

Table 1 summarizes the results of these tests for repression of GD by the two kinds of KP transgenes. All the testcross offspring that did not inherit a transgene were dysgenic. Among the offspring that did inherit a transgene, only those from the H(hsp(KP))/\textit{7} stock showed a statistically significant reduction in GD frequency (\(P < 0.05\)), and it was slight. Thus, the H(w\(^d\), KP1) and H(hsp(KP)) transgenes have, at best, a very weak intrinsic ability to repress GD induced by the Harwich \textit{y w P} strain.

**The P cytotype enables the KP transgenes to repress GD**

We next investigated whether or not the P cytotype could enhance the ability of the two kinds of KP transgenes to repress GD. In these tests,
the P cytotype was determined by P elements inserted in the TAS of the XL telomere. In one experiment, the telomeric P element was TP5; in another, it was TP6. These two elements are similar in size and are inserted in the same site within one of the repeating DNA units within the TAS (Stuart et al. 2002). The TP5 element shares less DNA sequence with the KP element than the TP6 element does. Previous work has shown that both TP5 and TP6 interact synergistically with the transgenes in the H(hsp/kp)/ stock to repress GD, and with the H (hsp/kp) transgenes in other stocks as well (Simmons et al. 2015). With TP5, this synergism is due mainly to a zygotic effect of the transgenes, whereas with TP6 it is due mainly to a maternal effect of the TP6-H (hsp/kp)7 transgene combination. Thus, the H(hsp/kp)7 transgenes served as positive controls in these experiments.

The experiments began by reciprocally crossing flies from the two TP and the three transgenic KP strains. The transgenic strains were homozygous for the markers y and w, which are tightly linked to the XL telomere; the TP strains were homozygous for the w marker only. The crosses using TP females are denoted with the letter A; those using transgenic females are denoted with the letter B. The TP y′ w y w; H(KP)/+ F1 females from these reciprocal crosses were then mated to Harwich y w males, and their F2 daughters with different body and eye color phenotypes were scored for GD. Daughters with wild-type body color carried the TP, and daughters with colored eyes carried the KP transgene. This design allowed us to determine if repression of GD involved maternal or zygotic effects of the telomeric and transgenic P elements. Because the tested F1 females were obtained from reciprocal crosses, we could also determine if repression depended on the parental origin of the telomeric P element. Each of the experiments also included controls without transgenes to determine the intrinsic repression abilities of the telomeric P elements.

Table 2 summarizes the results of the experiment involving TP5. A solitary TP5 element derived maternally from cross A repressed GD moderately (63.6% GD overall), but when paternally derived from cross B, it repressed GD very weakly (92.8% GD overall). Thus, as expected from previous studies (Simmons et al. 2012, 2014, 2015), the parental origin of the cytotype-determining TP5 element has a significant effect on repression ability. However, among the F2 flies that were scored for GD, there was no significant difference between those that inherited TP5 and those that did not. Thus, as previously reported (Thorpe et al. 2009), repression of GD in these kinds of control crosses is mediated by a strictly maternal effect of the TP5 element.

When a KP transgene was present in the tested females from cross A, repression of GD was markedly enhanced, but only among the offspring that inherited the transgene. We observed this zygotic effect with all three of the stocks that were tested, but most strongly with H(hsp/KP)7. The frequency of GD among the F2 females that inherited an H(hsp/KP) transgene was 21.3%, whereas among the F2 females that did not, it was 62.3%. As judged by the comparison score computed from the GD frequencies within replicate test cultures, this difference is highly significant (P < 0.01). Thus, when combined with a TP5 element derived from cross A, the H(hsp/KP) transgenes strongly repress GD through a zygotic effect in the F2 testcross offspring.

The H(w′, KP1) transgenes also repressed GD through zygotic effects in cross A. For stock C, 37.2% of the F2 flies that inherited the H(w′, KP1) transgene were dysgenic, compared to 76.2% of the F2 flies that did not inherit it; for stock J, the corresponding frequencies were 73.6% and 91.8%. For each stock, these differences are statistically significant (P < 0.05). Thus, as with the H(hsp/KP) transgenes, a TP5 element derived from Cross A enables the H(w′, KP1) transgenes to repress GD through a zygotic effect.

| Table 2: Repression of gonadal dysgenesis in the daughters of TP5 y′ w y w; hobo+ females from reciprocal crosses between TP5 y′ w y w; hobo+ and the three transgenic KP strains. |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Stock                           | Cross                         | Transgenic Only                 | Transgenic Absent                |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
|                                | No. of Flies | %GD ± SE | No. of Flies | %GD ± SE | No. of Flies | %GD ± SE |
|---------------------------------|----------------|----------|--------------|----------|--------------|---------|
| None                            | 23             | 92 ± 3   | 82 ± 4     | 25 ± 3   | 68 ± 5     | 25 ± 3   |
| H(hsp/KP)7                      | 26             | 74 ± 2   | 52 ± 3     | 63 ± 2   | 69 ± 4     | 51 ± 3   |
| H(w′, KP1)                      | 25             | 71 ± 3   | 39 ± 4     | 78 ± 3   | 48 ± 4     | 64 ± 4   |
| H(w′, KP1)/+                     | 24             | 74 ± 3   | 35 ± 4     | 81 ± 4   | 43 ± 5     | 69 ± 4   |
| H(w′, KP1)/+ w y w              | 23             | 72 ± 3   | 33 ± 4     | 84 ± 5   | 42 ± 5     | 67 ± 4   |
| H(w′, KP1)/+ y w                | 24             | 79 ± 4   | 30 ± 5     | 87 ± 5   | 49 ± 6     | 74 ± 4   |
| H(w′, KP1)/+ y w y w            | 23             | 80 ± 4   | 27 ± 5     | 89 ± 6   | 51 ± 6     | 76 ± 4   |
| H(w′, KP1)/+ y w y w y w         | 24             | 81 ± 5   | 24 ± 6     | 91 ± 7   | 53 ± 7     | 80 ± 5   |
| H(w′, KP1)/+ y w y w y w y w     | 23             | 82 ± 6   | 21 ± 7     | 93 ± 8   | 55 ± 8     | 83 ± 6   |
| H(w′, KP1)/+ y w y w y w y w y w| 24             | 83 ± 7   | 18 ± 8     | 94 ± 9   | 57 ± 9     | 85 ± 7   |

Indicates numbers that are statistically significant by the sign test. GD of the daughters of the transgenic females was scored for GD. The data in the rightmost column were scored for GD of the daughters of the transgenic females that did not inherit the transgene. The results indicate that the presence of the transgene in the F2 females that were scored for GD.

Note that the TP5 and the H(hsp/KP) transgenes interact synergistically with the transgenic H(hsp/KP)7 transgenes in the F2 females that were scored for GD.

The four categories of data have been pooled into two categories to assess the effect of the transgene in the F2 females that were scored for GD.

**Table 2 Repression of gonadal dysgenesis in the daughters of TP5 y′ w y w; hobo+ females from reciprocal crosses between TP5 y′ w y w; hobo+ and the three transgenic KP strains.**
In cross B, the offspring that carried either a H(hsp/KP) or a H(w\(^+\), KP1) transgene had slightly lower frequencies of GD than those that did not carry a transgene. This pattern suggests that, as in cross A, the H(hsp/KP) and H(w\(^+\), KP1) transgenes repressed GD through zygotic effects. However, none of these effects were statistically significant. The absence of significant repression in cross B underscores the importance of the parental origin of the cytotype-determining TP5 element.

Table 3 summarizes the results of the experiment involving TP6. Unlike TP5, a solitary TP6 element had little ability to repress GD, even in the tests from cross A (97.7% GD). However, when TP6 was combined with a KP transgene through either cross A or cross B, GD was repressed in the offspring from some of the test crosses. The strongest repression was seen in the F2 flies derived from the TP6 +/w y w H(hsp/KP)7+/ F1 females from cross A. In this case, only 18.5% of the F2 flies that inherited an H(hsp/KP) transgene were dysgenic, compared to 74.9% of those that did not. When compared to the control with TP6 alone (97.7% GD), these frequencies indicate that in cross A GD was repressed through a combination of weak maternal effects of the TP6-H(hsp/KP)7 F1 genotype and strong zygotic effects of the H(hsp/KP)7 transgenes. In cross B, 35.7% of the F2 flies that inherited an H(hsp/KP)7 transgene were dysgenic, compared to 98.3% of those that did not. Thus, in cross B, GD was repressed exclusively by the zygotic effects of the H(hsp/KP)7 transgenes.

When combined with TP6, the H(w\(^+\), KP1) transgenes from stocks C and J repressed GD through zygotic effects only: moderately in cross A, the H(w\(^+\), KP1) transgene had little ability to repress GD, even in the tests from cross A (97.7% GD). However, when TP6 was combined with a KP transgene through either cross A or cross B, GD was repressed in the offspring from some of the test crosses. The strongest repression was seen in the F2 flies derived from the TP6 +/w y w H(hsp/KP)7+/ F1 females from cross A. In this case, only 18.5% of the F2 flies that inherited an H(hsp/KP) transgene were dysgenic, compared to 74.9% of those that did not. When compared to the control with TP6 alone (97.7% GD), these frequencies indicate that in cross A GD was repressed through a combination of weak maternal effects of the TP6-H(hsp/KP)7 F1 genotype and strong zygotic effects of the H(hsp/KP)7 transgenes. In cross B, 35.7% of the F2 flies that inherited an H(hsp/KP)7 transgene were dysgenic, compared to 98.3% of those that did not. Thus, in cross B, GD was repressed exclusively by the zygotic effects of the H(hsp/KP)7 transgenes.

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DISCUSSION

For many years after their discovery, P elements were thought to be regulated exclusively by P-encoded polypeptides, including the 66 kDa repressor produced by complete P elements and the smaller repressor produced by KP elements (Rio 1990). This idea was supported by considerable evidence, but it failed to account for the maternal inheritance of cytotype that was seen in all the classic genetic analyses. Cyto-

type regulation is now understood to be mediated by piRNAs rather than by repressor polypeptides (Brennecke et al. 2008; Jensen et al. 2008).

P-specific piRNAs are generated by P elements inserted in the TAS of chromosome XL. These RNAs can be deposited in oocytes, where they provide a defense against P element activity in future embryos. Biochemical and genomic analyses have suggested that interactions between these primary piRNAs and long RNA molecules transcribed from transposons at other loci boost overall piRNA abundance. This repetitive process—called the ping-pong cycle—can provide a robust defense against P element activity in the next generation (Brennecke et al. 2008). Genetic analyses with combinations of telomeric and nontelomeric P elements support this idea. The nontelomeric P elements have no regulatory abilities of their own, but they can significantly enhance repression of dysgenesis by the telomeric P elements (Simmons et al. 2007). A key finding is that the enhanced repression is manifested in all the progeny of a test cross, even those that do not inherit any P elements (Simmons et al. 2012). Enhanced repression is therefore manifested as a strictly maternal effect, which at the molecular level implies that the amplified piRNA pool (or the raw material to produce it) is deposited into oocytes regardless of their genotype.

Genetic analysis of specific combinations of telomeric and nontelomeric P elements indicates that the ability to repress hybrid dysgenesis depends on the amount of DNA sequence shared by the
participating elements, presumably by influencing the efficacy of ping-pong cycling (Simmons et al. 2012; Jessen et al. 2013). Too little shared sequence may prevent the cycle from sustaining itself, but extensive shared sequence may also impair the cycle by allowing the formation of long double-stranded RNAs, which may be shunted into a pathway that does not produce piRNAs. Ping-pong cycling may also be influenced by the efficiency of RNA transport from the nucleus into the nuage, the perinuclear organelle in which piRNA production apparently occurs.

Though they are small, KP elements appear to have sufficient sequence overlap with the telomeric element TP6 to sustain ping-pong cycling and enhance cytotype regulation. Females that carried TP6 and an H(hsp/KP) transgene moderately repressed GD in daughters that inherited neither TP6 nor H(hsp/KP) (Table 3; Simmons et al. 2015). Repression through this strictly maternal effect presumably reflects the transmission of P-specific piRNAs that were amplified by ping-pong cycling in the mother’s germ line. However, intact KP elements within H(w°, KP1) transgenes did not enhance maternal regulation by TP6. This shortcoming may be due to differences in KP expression in the two types of transgenic stocks.

When either H(hsp/KP) or H(w°, KP1) transgenes were combined with the telomeric element TP5, enhanced repression of dysgenesis was seen only in flies that carried a transgene—that is, it was manifested as a strictly zygotic effect of the transgene rather than as a maternal effect of the TP5 and KP elements in the mother’s genotype. The absence of a maternal effect suggests that KP has insufficient sequence overlap with TP5 to sustain ping-pong amplification of P-specific piRNAs. A maternal effect was also not observed in tests where H(hsp/KP) transgenes were combined with NA, a telomeric P element that has even less sequence overlap with KP than TP5 does (Simmons et al. 2015).

Our data show that cytotype-determining Tp6 enabled H(hsp/KP) and H(w°, KP1) transgenes to repress GD through a zygotic effect in testcross offspring that inherited the transgene, even those that did not inherit the telomeric P element. This zygotic repression by the transgenic KP element is an emergent property not seen with other transgenic P elements, and it clearly depends on the P cytotype. We see it strongly in offspring derived from cross A, which have an unbroken maternal lineage of cytotype regulation, but less so in offspring from cross B, which do not. We also see this repression no matter which telomeric P element—TP5, TP6, or NA—determines the P cytotype (Simmons et al. 2015), although the repression is most pronounced with TP5 and NA, which share less sequence with KP than TP6 does. Zygotic repression by the KP element depends on the P cytotype, but it does not require a telomeric P element to be present in the females at risk for dysgenesis. Nor does it require the KP element to have been associated with a telomeric P element in the maternal germ line; KP elements derived paternaly from a Harwich y w strain can repress GD in the female offspring of testcrosses, as long as a telomeric element was present in their mothers (Simmons et al. 2015).

All these observations indicate that cytotype regulation mediated by maternally transmitted piRNAs enables KP elements to repress dysgenesis through the zygotic expression of a KP-derived factor, presumably the KP repressor polypeptide. Of course, it is formally possible that the zygotic effect of the KP element is mediated by a KP-derived RNA, but the overall pattern of results with different TPS and different KP transgenes suggests otherwise (Simmons et al. 2015). In addition, the repressor function of the KP polypeptide has been well documented in vitro (Lee et al. 1996, 1998). The mechanism by which maternally transmitted piRNAs derived from a telomeric P element enable the expression and/or action of the KP repressor polypeptide is unknown. One possibility is that the piRNAs enhance the ability of the KP polypeptide to bind to P elements throughout the genome and repress their movement. In fission yeast, piRNAs appear to be involved in chromatin organization (Grewal 2010), and in Drosophila, there is some evidence that they play a role in heterochromatin formation (Jesse et al. 2007).

The peculiar way in which piRNAs facilitate repression of hybrid dysgenesis by KP elements indicates that transposon regulation involves more than the simple depletion or destruction of transposase-encoding mRNAs, and the finding that intact KP elements, as well as hsp/KP fusions, respond to this facilitation indicates that piRNA-repressor polypeptide interactions may be important for transposon regulation in natural populations.

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