Homology-Dependent Silencing by an Exogenous Sequence in the Drosophila Germline

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ABSTRACT The study of P transposable element repression in Drosophila melanogaster led to the discovery of the trans-silencing effect (TSE), a homology-dependent repression mechanism by which a P-transgene inserted in subtelomeric heterochromatin (Telomeric Associated Sequences) represses in trans, in the female germline, a homologous P-lacZ transgene inserted in euchromatin. TSE shows variegation in ovaries and displays a maternal effect as well as epigenetic transmission through meiosis. In addition, TSE is highly sensitive to mutations affecting heterochromatin components (including HP1) and the Piwi-interacting RNA silencing pathway (piRNA), a homology-dependent silencing mechanism that functions in the germline. TSE appears thus to involve the piRNA-based silencing proposed to play a major role in P repression. Under this hypothesis, TSE may also be established when homology between the telomeric and target loci involves sequences other than P elements, including sequences exogenous to the D. melanogaster genome. We have tested whether TSE can be induced via lacZ sequence homology. We generated a piggyBac-otu-lacZ transgene in which lacZ is under the control of the germline ovarian tumor promoter, resulting in strong expression in nurse cells and the oocyte. We show that all piggyBac-otu-lacZ transgene insertions are strongly repressed by maternally inherited telomeric P-lacZ transgenes. This repression shows variegation between egg chambers when it is incomplete and presents a maternal effect, two of the signatures of TSE. Finally, this repression is sensitive to mutations affecting aubergine, a key player of the piRNA pathway. These data show that TSE can occur when silencer and target loci share solely a sequence exogenous to the D. melanogaster genome. This functionally supports the hypothesis that TSE represents a general repression mechanism which can be co-opted by new transposable elements to regulate their activity after a transfer to the D. melanogaster genome.

Transposable elements (TEs) are present in all organisms, and their activity can both induce severe deleterious effects by disrupting gene activity and create genetic novelties possibly useful from an evolutionary point of view (Wicker et al. 2007). Various mechanisms exist for repressing TE mobility, including auto-repression by proteins encoded by TEs themselves and host defense mechanisms via DNA methylation, heterochromatin formation, and small RNA silencing (Cam et al. 2008; Girard and Hannon 2008; Slotkin and Martienssen 2007). In a given organism, these mechanisms can vary depending on the cellular context. For example in Drosophila melanogaster, TEs are regulated by different RNA silencing pathways in somatic and germline tissues (Dufourt et al. 2011; Hartig and Forstemann 2011; Li et al. 2009; Malone et al. 2009). In species that have been recently invaded by a particular family of TEs, it is possible to recover strains with or without these TEs. These strains are useful to study the mechanisms of repression since TEs containing strains can be crossed to control strains (devoid of the TEs) to genetically isolate and identify regulatory TE copies. D. melanogaster has been invaded in the last century by three families of TEs: the I factor, the hobo element, and the P element (Anxolabehere et al. 1988; Blackman

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established by telomeric repetitive elements. A P-lacZ transgene carrying the lacZ gene under control of the ovarian tumor (otu) gene promoter, from the PCO plasmids described in Boivin et al. (2003). These two fragments were cloned between the HindIII and EcoRI sites of pXLI-BacII (Li et al. 2001; 2005). The transgene is 9815 bp long and is shown in Figure 1A. Transgenic lines were obtained by microinjection in the w1118 strain (defoet of P elements) performed by the BestGene company. New insertions were further produced by remobilization of a primary insertion using the jumpstarter element encoding piggyBac transposase (Horn et al. 2003).

Characterization of the transgenic lines

The transgenic lines were named “PBoL,” for PiggyBac-based transgenes containing the lacZ gene under control of the otu gene promoter. They carry the mini-white gene as transformation marker. Three PBoL-carrying lines were analyzed: m2a, w1a, and A7.6B. Two m2a and w1a insertions are located on chromosome 2, and the A7.6B insertion is located on chromosome 3. The m2a and A7.6B insertions are homozygous viable, whereas w1a is lethal and maintained over a balancer chromosome (Cy).

Precise localization of PBoL insertions was performed using inverse polymerase chain reaction (PCR) and the following oligonucleotides as primers: i2PCR3’ Ph (GGTCTCAGTCGAGTCTCCGTT), i2PCR5’ Ph (GTGCTTCCTGTGACCGCCCATG), i2PCR5’ Ph (GGACCGGCTAGTCTCTG), icP5’ Ph (ACTGAGATGTCGATCCTCC), and icP5’ Ph (ACTGAGATGTCGATCCTCC).
All lines apparently carry a single PBoL transgene insertion. The m2a insertion is located between the mir-8 and the Ugt37c1 genes at cytological site 53D. The w1a insertion is located in the Arc-p20 gene at cytological site 26B. The A7.6B insertion is located in the Alhambra gene at cytological site 84B.

**P-element – derived transgenes and Drosophila lines**

**P-lacZ fusion enhancer trap transgenes:** P-1152, P-1155 are enhancer-trap transgenes and contain an in-frame translational fusion of the *E. coli* lacZ gene to the second exon of the *P-transposase* gene. They carry rosy+ as a transformation marker (P[ArB] transgene) (O’Kane and Gehring 1987). P-1152 (FBti0005700) comes from the stock previously known as #11152 in the Bloomington Stock Center and was mapped to the telomere of the X chromosome (site 1A); this stock carries two P-lacZ insertions in the same TAS unit and in the same orientation (Josse et al. 2007). P-1155 (FBti0005691) comes from the stock previously known as #11155 of the Bloomington Stock Center. It contains a single P-lacZ insertion in TAS at the 3R chromosome arm telomere (site 100F). P-1152 and P-1155 are homozygous viable and fertile. P-1152 shows no lacZ expression in the ovary, whereas P-1155 shows weak and nonuniform lacZ staining in follicle cells but no staining in the germline. The T-1 line carries a cluster of P-lacZ-white elements (P{lacW} transgene) located at cytological site 50C on the second chromosome (Dorer and Henikoff 1994, 1997). The cluster contains seven transgene copies, including a defective copy, all inserted in direct orientation. In addition, the T-1 line has complex chromosomal rearrangements, including translocations between the second and the third chromosomes due to X-ray treatment. After overnight staining, weak lacZ expression is detected in follicle cells of T-1 ovaries, presumably because of a position effect at 50C, but no staining is observed in the germline. P-1152, P-1155, and T-1 have a strong capacity to induce TSE which is maternally inherited (Josse et al. 2008; Roche and Rio 1998; Ronsseray et al. 2001).
Three strong mutant alleles of *aubergine* induced by EMS were used. All of them are homozygous female sterile. *adf P31* (Schupbach and Wieschaus 1991) comes from the Bloomington Stock Center (stock #4968) and has not been characterized at the molecular level. *adf P32* (Schupbach and Wieschaus 1991) has an amino acid substitution. *adf P73* (Wilson et al. 1996) has a 154-bp deletion, resulting in a frameshift that is predicted to add 16 novel amino acids after residue 740 (Harris and MacDonald 2001).

All stocks used carrying transgenic insertions have a *M* genetic background (devoid of **P** transposable elements), as do the multi-marked balancer stocks and those carrying *aubergine* mutations. The *Canton* and *w 1118* lines were used as control lines, and these are completely devoid of any **P** element or **P** element−derived transgene (M lines) and of any piggyBac-derived transgene.

**Experimental conditions**

All crosses were performed at 25°C and involved three to five couples in most of the cases. Ovary *lacZ* expression assays were performed using X-gal overnight staining as described in Josse et al. 2007, except experiments involving *aubergine* mutants for which 24-hr staining was conducted because weaker *lacZ* expression required these conditions to facilitate scoring of TSE.

**Quantification of TSE**

When TSE is incomplete, variegation is observed because “on/off” *lacZ* expression occurs between egg chambers (Josse et al. 2007). TSE was quantified by determining the percentage of egg chambers with no expression among ovarian stages 9-10 because *lacZ* expression of *PBoL* insertions was intense and reproducible at these stages.

**RESULTS**

**Production of *PBoL* transgenic lines**

A transgene was designed to test whether “non **P**-element” homology between a telomeric transgene and a target euchromatic transgene allows trans-silencing to take place. More precisely, we asked whether a telomeric *P-lacZ* transgene can repress, in *trans*, a transgene carrying the *lacZ* sequence in a TE other than the **P** element. The piggyBac-based transgenic system was used. The *Trichoplusia ni* piggyBac element is absent from the *Drosophila melanogaster* genome and shows no significant sequence similarity with the **P** element, as tested by BLAST analysis (data not shown). Because TSE is restricted to the female germline, expression of the *lacZ* gene in piggyBac was placed under the control of the germline-specific promoter of the *otta* (Figure 1A). After transformation of embryos and mobilization, six transgenic lines were recovered (named *PBoL*). All insertions but one showed β-galactosidase expression restricted to germline cells of the ovary. However, *lacZ* expression levels varied from one *PBoL* insertion to another, likely because of position effects. The three lines showing the strongest *lacZ* expression were selected for further analysis. Details about these lines are given in Materials and Methods. These lines are called *m2a* and *w1a* (chromosome 2) and *A7.6B* (chromosome 3). For all *PBoL* insertions, *lacZ* expression in ovaries was assessed in two different genetic backgrounds (Canton and *w 1118*) to take into account possible background effects on transgene expression. No significant difference was observed between the two backgrounds (data not shown). The *m2a*, *w1a*, and *A7.6B* insertions produced strong *lacZ* expression in nurse cells, especially at late stages of oogenesis and in mature oocytes (Figure 1B). Scoring egg chambers at stages 9-10 allowed detection of *lacZ* expression in all (but one) egg chamber among more than 4700 egg chambers assayed for the three *PBoL* insertions tested (Figure 1B).

**LacZ homology between a telomeric and an euchromatic locus allows trans-silencing to take place in germline cells of the ovary**

*P-lacZ* insertions located in subtelomeric heterochromatin (TAS) of the *X* chromosome induce strong repression of any *P-lacZ* transgene inserted in euchromatin expressed in the female germline (Josse et al. 2008; Roche and Rio 1998; Ronsseray et al. 2001). In addition, incomplete repression results in variegation for X-gal staining from one egg chamber to another (Josse et al. 2007). This repression shows a strong maternal effect because strong repression is observed only when the telomeric locus is maternally inherited (Josse et al. 2008; Ronsseray et al. 2001). For example, crossing *P-1152* females with males carrying an euchromatic *P-lacZ* transgene resulted in *G1* females showing 80% to 95% of egg chambers with repressed *lacZ* expression, whereas the reciprocal cross resulted in only 15% to 30% repression in *G1* females (Josse et al. 2007, 2008). When *P-1152* females were crossed with males carrying any one of the three *PBoL* insertions tested, *G1* females showed strong *lacZ* silencing in all cases (Figure 1C). 98% for *m2a* and *A7.6B*; 90% for *w1a*). In addition, in each case, incomplete repression resulted in variegating *lacZ* expression characterized by *on/off* egg chamber *lacZ* expression (Figure 1C). Finally, the reciprocal cross was performed, and no repression was detected with any of the three *PBoL* insertions tested (Figure 1D). Therefore, *lacZ* homology allows trans-silencing to take place in the female germline and repression shows phenotypic and genetic properties of TSE.

**PBoL repression by autosomal silencers**

Previous studies of TSE allowed the identification of several silencers located on autosomes (Josse et al. 2008; Roche and Rio 1998). First, *P-lacZ* transgenes inserted in subtelomeric heterochromatin of chromosomes 2 and 3 were found to be able to establish strong repression of a *P-lacZ* target transgene (Josse et al. 2008). This repression is also maternally inherited and shows variegation: for example, the *P-1155* telomeric *P-lacZ* transgene, located in the TAS of the *3R* chromosomal arm, was shown to repress a *P-lacZ* transgene located in euchromatin of chromosome 3 (named *P-Co1*). This repression is however weaker (TSE = 65%) than that induced by X chromosome telomeric insertion *P-1152* [TSE = 88% (Josse et al. 2008)]. Second, complete trans-silencing of *P-lacZ* was found to be induced by the *T-1* line (Ronsseray et al. 2001), which carries a cluster of *P-lacZ* transgenes (Dorner and Henikoff 1994, 1997) inducing local heterochromatin formation (Fanti et al. 1998) and which has complex chromosomal rearrangements induced by X-rays. Again trans-silencing was maternally inherited (Ronsseray et al. 2001).

The capacity of these two silencer loci to repress *PBoL* insertions was tested. The *P-1155* telomeric transgene induced repression of the three *PBoL* insertions tested (Table 1). *P-1155*-mediated repression is weaker than that obtained for *P-1152* (Figure 1) with *m2a* (56% vs. 98%) and *w1a* (81% vs. 90%), whereas strong repression was observed for both *P-1155* and *P-1152* with *A7.6B* (93% and 98%). Table 1 also shows that *T-1* induced complete silencing of the three *PBoL* tested. Note that in this case, repression can result from both *lacZ* and white homology. Autosomal *P-lacZ* silencers can thus strongly repress *PBoL* transgenes. In addition, a maternal effect was found for both the *P-1155* and *T-1* autosomal silencers because no repression was observed in the progeny of reciprocal crosses (Table 1).

Further, we tested whether single transgenes located in euchromatin and heterochromatin (pericentromeric heterochromatin and fourth chromosome) can repress *PBoL* insertions. Indeed, previously such transgenes were shown to be unable to repress a *P-lacZ* transgene expressed in the female germline (Josse et al. 2008). Similarly, of five
Table 1 PBol transgenes are repressed by autosomal TSE silencers

| Line name | m2a | w1a | A7.6B |
|-----------|-----|-----|-------|
| Canton†  |     |     |       |
| ♀ x♂     | 0% (1344) | 0% (1077) | 0% (1457) |
| ♀ x♂     | 0% (420) | 0% (429) | 0% (368) |
| P-1155    |     |     |       |
| ♀ x♂     | 56.3% (448) | 81.2% (399) | 93.2% (382) |
| ♀ x♂     | 0% (409) | 0.3% (344) | 0% (305) |
| T-1       |     |     |       |
| ♀ x♂     | 100% (769) | 100% (465) | 100% (544) |
| ♀ x♂     | 0% (332) | 0% (303) | 0% (353) |

Reciprocal crosses were performed between individuals indicated in column 1 and in line 1. TSE was measured in G_1 females. In each case, the first line (♀ x ♂) gives the percentage of TSE (total number of egg chambers scored in parenthesis) in progeny produced by crossing females indicated in column 1 with males indicated in line 1. The second line (♂ x ♀) gives the percentage of TSE observed in progeny of the reciprocal cross (total number of egg chambers scored in parenthesis). P-1155 carries a P-lacZ-rosy transgene, similar to P-1152, located at the telomere of the 3R chromosomal arm. The T-1 line carries a tandem array of seven P-lacZ-white transgenes located in the middle of the 2R chromosomal arm. The Canton† reference line is devoid of any P-transgene or P-element. PBol, PgygBac-based transgenes containing the lacZ gene under control of the cyt gene promoter; TSE, trans-silencing effect.

euchromatic P-lacZ insertions, and three pericentromeric chromosome insertions tested, located on chromosomes X, 2 and 3, none repressed m2a, w1a or A7.6B (Table S1). In addition, a P-lacZ transgene located on the heterochromatic fourth chromosome, previously shown to be unable to repress a P-lacZ transgene (Josse et al. 2008), did not repress the PBol insertions. Therefore, P-lacZ and PBol target transgenes respond in the same way (repressed or not repressed) to all silencer/nonsilencer loci tested.

A telomeric P-lacZ locus can repress two PBol transgenes inserted on different chromosomes

A single telomeric P-1152 locus was previously shown to be able to strongly repress two P-lacZ targets located at allelic or nonallelic positions (Josse et al. 2008). We tested whether a single P-1152 locus can similarly repress two PBol insertions. Females having maternally inherited P-1152 and carrying two PBol insertions presented more than 80% of repressed egg chambers (Table 2). This result was obtained for flies homozygous for the m2a insertion or for flies carrying two nonallelic PBol insertions located on the same or on different chromosomes (m2a and w1a located on chromosome 2 and A7.6B located on chromosome 3). Therefore, a single P-1152 locus can repress simultaneously two PBol insertions located at allelic or nonallelic positions. In addition, we found that a single maternally inherited P-1152 locus established strong lacZ repression in females carrying both a hemizygous P-lacZ insertion located on chromosome 3 ([BQ16 (Josse et al. 2007)]) and a hemizygous PBol insertion (m2a; 93.8% of TSE, n = 241).

LacZ homology–dependent silencing is sensitive to mutations affecting the piRNA pathway gene aubergine

TSE has been shown to be highly sensitive to mutations affecting the piRNA silencing pathway (Josse et al. 2007; Todeschini et al. 2010). Repression of a P-lacZ target transgene by a P-lacZ telomeric locus was tested in aubergine mutant contexts (Table 3). aubergine is one of the main actors involved in the piRNA amplification mechanism termed “ping-pong” which functions in the germline (Brennecke et al. 2007; Li et al. 2009; Malone et al. 2009). TSE between P-lacZ transgenes was found previously to be null in aubergine heteroallelic mutant contexts (Josse et al. 2007). Similarly, P-1152 repression of the PBol A7.6B insertion was almost completely (2.6%) or completely abolished in the two aubergine heteroallelic mutant contexts tested (Table 3). Therefore, lacZ homology-based trans-silencing is dependent on aubergine.

DISCUSSION

The genome of natural populations of Drosophila melanogaster has been invaded by three TE families during the last century: I, hobo, and P (Blackman 1989; Engels 1989; Finnegan 1989). For all three, a repression mechanism was established, and for two of them, P and I, repression has been shown to involve a maternal effect and complex epigenetic transmission over generations (Bucheton et al. 1976; Engels 1979; Picard et al. 1978). P and I repression involves regulatory copies of these TEs located on all chromosomes (Engels 1979; Picard et al. 1978), but some master regulatory sites corresponding to copies inserted in heterochromatin have been identified (Jensen et al. 2002; Picard 1978; Ronsseray et al. 1991). Both P and I repression mechanisms are sensitive to mutations affecting heterochromatin formation and RNA silencing (Bucheton et al. 2002; Chambeyron et al. 2008; Klenov et al. 2007; Reiss et al. 2004; Ronsseray et al. 1996). For P element repression (P cytotype), the existence of a maternally transmitted cytoplasmic component (pre-P cytotype), coupled with chromosomally inherited P copies, was shown to be necessary to establish strong repression in the zygote (Niemi et al. 2004; Ronsseray et al. 1993). However, the maternally inherited component is not an autoreproducible component (Ronsseray et al. 1993; Sved 1987). Furthermore, upon discovery of the piRNA silencing pathway (Brennecke et al. 2007; Gunawardane et al. 2007) sequence analysis of piRNAs suggested that a high proportion of TEs are repressed in the gonads by this homology-dependent silencing mechanism. In particular, deep-sequencing of ovarian small RNAs allowed detection of piRNAs derived from I and P elements whose maternal transmission is correlated with repression of hybrid dysgenesis induced by massive transposition of these TEs

Table 2 A telomeric P-lacZ locus can repress two PBol transgenes inserted at allelic or nonallelic positions

| Row | Parental Cross | Genotype of G_1 Females Analyzed | % TSE | n   |
|-----|---------------|---------------------------------|-------|-----|
| 1   | ♀ m2a x ♂ m2a | + / + ; m2a / m2a ; + / +       | 0.0   | 381 |
| 2   | ♀ m2a x ♂ w1a | + / + ; m2a / w1a ; + / +       | 0.0   | 844 |
| 3   | ♀ m2a x A7.6B | + / + ; m2a / + ; + / A7.6B     | 0.0   | 316 |
| 4   | ♀ P-1152 ; m2a x ♂ P-1152 ; m2a | P-1152 / P-1152 ; m2a / m2a ; + / + | 95.8 | 406 |
| 5   | ♀ P-1152 ; m2a x ♂ w118 | P-1152 / + ; m2a / + ; + / + | 84.2 | 310 |
| 6   | ♀ P-1152 ; m2a x ♂ m2a | P-1152 / + ; m2a / m2a ; + / + | 87.3 | 512 |
| 7   | ♀ P-1152 ; m2a x ♂ w1a | P-1152 / + ; m2a / w1a ; + / + | 90.2 | 877 |
| 8   | ♀ P-1152 ; m2a x A7.6B | P-1152 / + ; m2a / + ; + / A7.6B | 97.4 | 381 |

The parental crosses shown in column 2 were performed in order to generate G_1 females whose genotype is given in column 3. In each case, G_1 females carrying P-1152 were homozygous for this locus. LacZ staining of G_1 female ovaries was performed and TSE was measured. Columns 4 and 5 give the TSE percentage and the total number of egg chambers counted, respectively.
The functional assay indicates that repression by a telomeric element is dependent on the maternal or paternal aub aut element allele, which cannot be discriminated. TSE, trans-silencing effect.

(Brennecke et al. 2008). In the case of P, these maternally transmitted small RNAs very likely correspond to the pre-P cytotype.

All these data suggest a model in which the genome harbors several “traps” for invasive mobile DNA sequences which constitutively produce piRNAs and allow repression by a homology-dependent silencing mechanism. Some families (gypsy, ZAM and Idefix) are regulated by a heterochromatic locus called flanc-COM located close to the centromere of the X chromosome (Desset et al. 2003; Mevel-Ninio et al. 2007; Pelisson et al. 1994; Prud’homme et al. 1995). This repression takes place in the somatic follicle cells of the ovary and, therefore, is mediated by a functionally different piRNA pathway (Desset et al. 2008; Malone et al. 2009; Pelisson et al. 2002). It is noteworthy that this regulation presents different genetic properties because gypsy repression, for example, does not exhibit a maternal effect nor trans-generational epigenetic transmission (A. Pelisson, personal communication). For the I factor, repression appears to involve homology-dependent silencing in the germline (Jensen et al. 1999, 2002; Malinsky et al. 2000; Robin et al. 2003) and major repressive loci appear to be located in pericentromeric heterochromatin (Jensen et al. 2002) and in the intercalary heterochromatin 42AB locus which contains numerous fragments of TEs (Brennecke et al. 2008). For the P element, Telomeric-Associated Sequences appear to be a major trap. Indeed, numerous P strains deriving from natural populations having various geographical origins have been found to carry P elements located at the telomere of the X chromosome (Ajioka and Eanes 1989; Ronsseray et al. 1989). Telomeric P elements inserted in TAS deriving from seven natural populations have been further isolated in a genomic background devoid of other P copies and were shown to have repressive capacities (Marin et al. 2000; Ronsseray et al. 1996, 1998; Stuart et al. 2002). The combination of a telomeric defective P element with various target P-transgenes showed that repression induced by the telomeric P-element is dependent on P-element homology between the telomeric and target loci (Marin et al. 2000; Roche and Rio 1998).

To validate and generalize this model, it remained, however, important to determine via a functional assay whether trans-silencing can be established via sequence homology other than that derived from the P element. In the present article, we show that the lacZ gene located inside a piggyBac-derived transgene is strongly repressed by a maternally inherited telomeric P-lacZ transgene, this repression exhibiting variegation, which is a typical phenotype of TSE. The parallel between P-lacZ and PBol repression by a telomeric P-lacZ also includes the capacity to be repressed by autosomal silencers and sensitivity to aubergine mutations. Therefore, this functional assay indicates that repression by sequences inserted in TAS is not a P-element restricted property but rather a more general repression system that may function for other TEs inserted in TAS, including those recently introduced in the genome. Thus, TSE not only allows us to address the nature of P cytotype but also corresponds to a sensitive or appropriate tool to investigate phenotypic and genetic properties of a piRNA silencing pathway functioning in nurse cells.

Taking into account the epigenetic trans-generational effects of TSE and its variegating phenotype (Josse et al. 2007), predictive assumptions can be proposed for properties of a germline-specific piRNA pathway. For example, variation between egg chambers inside ovaries resembles position effect variegation in the eye observed for genes located close to heterochromatin (Girton and Johansen 2008), and this suggests that target repression by piRNAs may involve heterochromatin formation. In addition, long-term memory of the maternal effect observed over generations with TSE (Josse et al. 2007) indicates that piRNA-based repression functioning in the germline undergoes amplification that can transcend a single fly generation to reach its maximum level. Note that P is not the only TE displaying long-term inheritance of its repressive properties because I factor regulation also shows such trans-generational effects (Bucheton 1979; Jensen et al. 1999; Picard et al. 1978). It will be interesting to investigate whether such a long-term trans-meiosis epigenetic inheritance exists for other piRNA producing loci in the genome.

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LITERATURE CITED

Ajioka, I. W., and W. F. Eanes, 1989 The accumulation of P-elements on the tip of the X chromosome in populations of Drosophila melanogaster. Genet. Res. 53: 1–6.

Anzalohere, D., M. G. Kidwell, and G. Periquet, 1988 Molecular characteristics of diverse populations are consistent with the hypothesis of a recent invasion of Drosophila melanogaster by mobile P elements. Mol. Biol. Evol. 5: 252–269.

Blackman, R. K., R. Grimaila, M. M. Koehler, and W. M. Gelbart, 1987 Mobilization of hobo elements residing within the decapeptideg gene complex: suggestion of a new hybrid dysgenesis system in Drosophila melanogaster. Cell 49: 497–505.

Blackman, R. K., and W. M. Gelbart, 1989 The transposable hobo of Drosophila melanogaster, pp. 523–529 in Mobile DNA, edited by D. E. Berg and M. M. Howe. American Society for Microbiology, Washington, DC.
O’Kane, C. J., and W. J. Gehring, 1987 Detection in situ of genomic regulatory elements in Drosophila. Proc. Natl. Acad. Sci. USA 84: 9123–9127.

Pelisson, A., S. U. Song, N. Prud’homme, P. A. Smith, A. Bucheton, et al., 1994 Gypsy transposition correlates with the production of a retroviral envelope-like protein under the tissue-specific control of the Drosophila flamenco gene. EMBO J. 13: 4401–4411.

Pelisson, A., L. Meljumian, V. Robert, C. Terzian, and A. Bucheton, 2002 Drosophila germline invasion by the endogenous retrovirus gypsy: involvement of the viral env gene. Insect Biochem. Mol. Biol. 32: 1249–1256.

Picard, G., 1978 Non mendelian female sterility in Drosophila melanogaster: sterility in the daughter progeny of SF and RSF females. Biol. Cell. 31: 235–244.

Picard, G., J. C. Bregliano, A. Bucheton, J. M. Lavige, A. Pelisson et al., 1997 Non-mendelian female sterility and hybrid dysgenesis in Drosophila melanogaster. Genet. Res. 32: 275–287.

Prud’homme, N., M. Gans, M. Masson, C. Terzian, and A. Bucheton, 1995 Flamenco, a gene controlling the gypsy retrovirus of Drosophila melanogaster. Genetics 139: 697–711.

Reiss, D., T. Josse, D. Anxolabehere, and S. Ronsseray, 2004 Aubergine mutations in Drosophila melanogaster impair P cytotype determination by telomeric P elements inserted in heterochromatin. Mol. Genet. Genomics 272: 336–343.

Rio, D. C., 2002 in P transposable elements in Drosophila melanogaster, pp. 484–518 in Mobile DNA II, edited by N. L. Craig, R. Craige, and Gellert et al., American Society for Microbiology, Washington, DC.

Robin, S., S. Chambeiryon, A. Bucheton, and I. Busseau, 2003 Gene silencing triggered by non-LTR retrotransposons in the female germline of Drosophila melanogaster. Genetics 164: 521–531.

Roche, S. E., and D. C. Rio, 1998 Trans-silencing by P elements inserted in subtelomeric heterochromatin involves the Drosophila Polycomb group gene, Enhancer of zeste. Genetics 149: 1839–1855.

Ronsseray, S., M. Lehmann, and D. Anxolabehere, 1989 Copy number and distribution of P and I mobile elements in Drosophila melanogaster populations. Chromosoma 98: 207–214.

Ronsseray, S., M. Lehmann, and D. Anxolabehere, 1991 The maternally inherited regulation of P elements in Drosophila melanogaster can be elicited by two P copies at cytological site 1A on the X chromosome. Genetics 129: 501–512.

Ronsseray, S., B. Lemaître, and D. Coen, 1993 Maternal inheritance of P cytotype in Drosophila melanogaster: a "pre-P cytotype" is strictly extra-chromosomally transmitted. Mol. Gen. Genet. 241: 115–123.

Ronsseray, S., M. Lehmann, D. Nousaud, and D. Anxolabehere, 1996 The regulatory properties of autonomous subtelomeric P elements are sensitive to a Suppressor of variegation in Drosophila melanogaster. Genetics 143: 1663–1674.

Ronsseray, S., L. Marin, M. Lehmann, and D. Anxolabehere, 1998 Repression of hybrid dysgenesis in Drosophila melanogaster by combinations of telomeric P-element reporters and naturally occurring P elements. Genetics 149: 1857–1866.

Ronsseray, S., A. Boivin, and D. Anxolabehere, 2001 P-Element repression in Drosophila melanogaster by variegating clusters of P-lacZ-white transgenes. Genetics 159: 1631–1642.

Ronsseray, S., T. Josse, A. Boivin, and D. Anxolabehere, 2003 Telomeric transgenes and trans-silencing in Drosophila. Genetics 117: 327–335.

Schupbach, T., and E. Wieschaus, 1991 Female sterile mutations on the second chromosome of Drosophila melanogaster. II. Mutations blocking oogenesis or altering egg morphology. Genetics 129: 1119–1136.

Simmons, M. J., D. F. Ryzek, C. Lamour, J. W. Goodman, N. E. Kummer et al., 2007 Cytotype regulation by telomeric P elements in Drosophila melanogaster: evidence for involvement of an RNA interference gene. Genetics 176: 1945–1955.

Slotkin, R. K., and R. Martienssen, 2007 Transposable elements and the epigenetic regulation of the genome. Nat. Rev. Genet. 8: 272–285.

Stuart, J. R., K. J. Haley, D. Swedzinski, S. Lockner, P. E. Kocian et al., 2002 Telomeric P elements associated with cytotype regulation of the P transposon family in Drosophila melanogaster. Genetics 162: 1641–1654.

Sved, J. A., 1987 Hybrid dysgenesis in Drosophila melanogaster: evidence from sterility and southern hybridization tests that P cytotype is not maintained in the absence of chromosomal P factors. Genetics 115: 121–127.

Todeschini, A. L., L. Teysset, V. Delmarre, and S. Ronsseray, 2010 The epigenetic trans-silencing effect in Drosophila involves maternally-transmitted small RNAs whose production depends on the piRNA pathway and HP1. PLoS ONE 5: e11032.

Wicker, T., F. Sabot, A. Hua-Van, J. L. Bennetzen, P. Capy et al., 2007 A unified classification system for eukaryotic transposable elements. Nat. Rev. Genet. 8: 973–982.

Wilson, J. E., J. E. Connell, J. D. Schlenker, and P. M. Macdonald, 1996 Novel genetic screen for genes involved in posterior body patterning in Drosophila. Dev. Genet. 19: 199–209.

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