Telomeric Trans-Silencing: An Epigenetic Repression Combining RNA Silencing and Heterochromatin Formation

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The study of P-element repression in Drosophila melanogaster led to the discovery of the telomeric Trans-Silencing Effect (TSE), a repression mechanism by which a transposon or a transgene inserted in subtelomeric heterochromatin (Telomeric Associated Sequence or TAS) has the capacity to repress in trans in the female germline, a homologous transposon, or transgene located in euchromatin. TSE shows variegation among egg chambers in ovaries when silencing is incomplete. Here, we report that TSE displays an epigenetic transmission through meiosis, which involves an extrachromosomal maternally transmitted factor. We show that this silencing is highly sensitive to mutations affecting both heterochromatin formation (Su(var)205 encoding Heterochromatin Protein 1 and Su(var)3–7) and the repeat-associated small interfering RNA (or rasiRNA) silencing pathway (aubergine, homeless, armitage, and piwi). In contrast, TSE is not sensitive to mutations affecting r2d2, which is involved in the small interfering RNA (or siRNA) silencing pathway, nor is it sensitive to a mutation in loquacious, which is involved in the micro RNA (or miRNA) silencing pathway. These results, taken together with the recent discovery of TAS homologous small RNAs associated to PIWI proteins, support the proposition that TSE involves a repeat-associated small interfering RNA pathway linked to heterochromatin formation, which was co-opted by the P element to establish repression of its own transposition after its recent invasion of the D. melanogaster genome. Therefore, the study of TSE provides insight into the genetic properties of a germline-specific small RNA silencing pathway.

Introduction

Repression of transposable elements (TEs) involves complex mechanisms that can be linked to either small RNA silencing pathways or chromatin structure modifications depending on the species and/or the TE family [1–6]. Drosophila species are particularly relevant to the study of these repression mechanisms since some families of TEs are recent invaders, allowing genetic analysis to be carried out on strains with or without these TEs [7,8]. In some cases, crossing these two types of strains induces hybrid dysgenesis, a syndrome of genetic abnormalities resulting from TE mobility [9,10]. In D. virilis, repression of hybrid dysgenesis has been correlated to RNA silencing since small RNAs of the retroelement Penelope, responsible for dysgenesis, were detected in nondysgenic embryos but not in dysgenic embryos [11]. In D. melanogaster, repression of retrotransposons can be established by noncoding fragments of the corresponding element (I factor [12,13], ZAM, and Idefix [14]) and can be in some cases (gypsy [15], mad1 [16], copia [17], Het-A, TART [18,19], and ZAM, Idefix [C. Vaury and S. Desset, personal communication]) sensitive to mutations in genes from the Argonaute family involved in small RNA silencing pathways. In the same species, strong repression of the DNA P TE, by a cellular state that has been called “P cytotype” [10], can be established by one or two telomeric P elements inserted in heterochromatic “Telomeric Associated Sequences” (TAS) at the 1A cytological site corresponding to the left end of the X chromosome [20–24]. This includes repression of dysgenic sterility resulting from P transposition. We have previously shown that this P cytotype is sensitive to mutations affecting both Heterochromatin Protein 1 (HP1) [21] and the Argonaute family member AUBERGINE [25]. P repression corresponds to a new picture of TE repression shown, using an assay directly linked to transposition, to be affected by heterochromatin and small RNA silencing mutants.

In the course of the study of P cytotype, a new silencing phenomenon has been discovered. Indeed, a P-lacZ transgene or a single defective P element inserted in TAS can repress expression of euchromatic P-lacZ insertions in the female germline in trans, if a certain length of homology exists between telomeric and euchromatic insertions [23,26]. This...
Author Summary

The genome of the fruitfly was invaded in the last century by a mobile DNA element called the P element. After a transient period of genetic disorders due to P mobility, the P element established a repressive state for its transposition. We have shown that a major component of this repression comes from P copies inserted close to telomeres, the ends of linear chromosomes. One or two P copies inserted in subtelomeric heterochromatin (the DNA region highly compacted by protein complexes) can stabilize around 80 P copies. This finding allowed the discovery of a more general phenomenon called the ‘‘Trans-silencing effect’’ in which a transgene inserted in this subtelomeric heterochromatin represses, in the female germ-line, a homologous transgene, irrespective of the genetic location of the latter. We show that Trans-silencing requires not only the chromosomal copy of the telomeric silencer, but also a maternally transmitted factor whose influence can persist over generations. We have found that this epigenetic silencing is sensitive to mutations in genes involved in heterochromatin formation and in a recently discovered silencing pathway based on small RNAs. Trans-silencing thus provides a tool for mechanistic analysis of gene repression on the basis of chromatin changes combined with small RNA pathways in the germline.

homology-dependent silencing phenomenon has been termed Trans-Silencing Effect (TSE) [26]. Telomeric transgenes, but not centromeric transgenes, can be silencers and all euchromatic P-lacZ insertions tested can be targets [23,26]. TSE is restricted to the female germline (unpublished data) and has a maternal effect since repression occurs only when the telomeric transgene is maternally inherited [27]. Further, when TSE is not complete, variegating germline lacZ repression is observed from one egg chamber to another, suggesting a chromatin-based mechanism of repression [28]. Recently, an extensive analysis of small RNAs complexed with PIWI family proteins (AUBERGINE, PIWI, and AGO3) was performed in the Drosophila female germline [4]. The latter study showed that most of the RNA sequences associated to these proteins derive from TEs. TSE corresponds likely to such a situation.

Here, we analyze the genetic properties of TSE and show that it has an epigenetic transmission through meiosis, which involves an extrachromosomal maternally transmitted stimulating component. Further, in order to investigate the mechanism behind TSE, we performed a candidate gene analysis to identify genes whose mutations impair TSE. We found that TSE is strongly affected by mutations in genes involved in heterochromatin formation and in the recently discovered small RNA silencing pathway called “repeat-associated small interfering RNAs” (rasiRNA) pathway [3,4,6,29]. In contrast, we show that TSE is not sensitive to genes specific to the classical RNA interference pathway linked to small interfering RNAs (siRNA) or to the micro RNA (miRNA) pathway. This suggests thus that TSE involves a rasiRNA pathway linked to heterochromatin formation and that such a mechanism, working in the germline, may underlie epigenetic transmission of repression through meiosis.

Results

Quantification of TSE

TSE was generated by combining telomeric transgene insertions (from the P-1152 line) as a silencer locus, with various euchromatic P-lacZ transgenes expressed in the germline as targets. Depending on the target, TSE can be almost total (Figure 1C) or intermediate (Figure 1E and 1F). When TSE is incomplete, variegation is observed since “on” and “off” lacZ expression is seen among egg chambers; egg chambers can show strong expression (dark blue) or no expression, but intermediate repression levels are not (or very rarely) found. In addition, a given ovary can present ovarioles showing all possible combinations of on or off egg chambers (Figure 1E). Simple quantification of TSE is thus possible by determining the percentage of repressed egg chambers (Figure 1F). We scored the number of repressed chambers among the first five egg chambers of a given ovariole for ten ovarioles chosen at random per ovary. For a given genotype, more than 1,000 egg chambers were classically counted (Table 1). This measure generally produces very reproducible results among replicate experiments allowing accurate quantification of TSE.

Transmission of TSE over Generations

TSE was previously shown to have a maternal effect since strong repression occurs only in the progeny of crosses involving females carrying the telomeric silencer, whereas no or weak repression occurs if the telomeric silencer comes from the father [27,28]. Conversely, the parental origin of the target does not significantly affect TSE. We have thus tested whether TSE exhibits only a maternal effect (during one generation) or maternal inheritance over several generations as well. Two reciprocal G0 crosses were performed in which the telomeric silencer (P-1152) was maternally introduced (maternal lineage) or paternally introduced (paternal lineage) (Figure S1). Thus, the G1 females of the two lineages have the same genotype. These G1 females were backcrossed with males having the telomeric silencer and the target transgene in order to recover G2 females having one copy of the telomeric silencer locus and one copy of the target transgene. Such backcrosses were repeated for several generations in order to follow lineages with constant genotypes over generations. At each generation, the percentage of TSE was measured. Figure 2 shows that in the maternal lineage, strong TSE was detected in G1 (around 70%) and maintained over generations. By contrast, in G1 of the paternal lineage, the level of TSE was weak (around 10%), a result consistent with the maternal effect previously reported. However, in this paternal lineage, a strong level of repression was not observed in G2 females either (around 24%), despite the fact that G1 females were carrying a telomeric silencer. TSE thus shows a maternal inheritance. However, the level of TSE in G2 is greater than in G1 in the paternal lineage, and in succeeding generations TSE gradually increases. A total of six generations are necessary, however, to reach a repression level close to that of the maternal lineage. Thus, a memory of the initial maternal effect is observed over generations. In conclusion, TSE is characterized by maternal transmission that gets progressively reinforced over successive generations. It is therefore partially epigenetically transmitted through meiosis.

TSE Requires an Extrachromosomally Transmitted Component

In the previous experiment, two main hypotheses can be proposed concerning the molecular basis of the difference
inherited in G1 between the maternal and paternal lineages of TSE. First, maternally inherited telomeric transgenes may be imprinted while paternally inherited transgenes are not, and imprinting may be necessary for TSE to take place in the zygote. Second, a mother carrying telomeric transgenes may deposit an extrachromosomal factor in the oocyte, which is necessary for TSE to occur. This latter hypothesis was tested by using females hemizygous for the P-1152 telomeric silencer locus and a dominant genetic marker on the homologous chromosome to identify transmission of the chromosome carrying the telomeric silencer or the chromosome devoid of silencer (M5 balancer chromosome). Figure 3 shows that crossing these hemizygous females ("A" females) with males carrying a target transgene produced control G1 "B" females, which have inherited from their mother both the cytoplasm and a chromosomal copy of the telomeric silencer: in these females TSE is about 65%. However, sisters having inherited the M5 chromosome do not show any repression ("C" females, TSE = 0%). Thus, the cytoplasm of a P-1152 female without a chromosomal P-1152 copy cannot induce the TSE. Crossing P-1152; P-Z-target males with females devoid of telomeric silencer produces a weak repression in the progeny (2.7%) as shown by "E" females, a result consistent with the maternal effect of TSE reported previously [27]. Finally, crossing "A" females with males carrying a P-1152 telomeric silencer allows recovery of females having maternally inherited only a "P-1152" cytoplasm and paternally inherited a P-1152 chromosomal silencer. In that case, strong repression is observed ("D" females, 75% TSE). Thus the cytoplasmic component (incapable by itself of inducing TSE, as shown with "C" females) combined to a paternally inherited telomeric silencer can establish a strong TSE. Moreover, this repression is as strong as if the telomeric silencer was maternally inherited ("B" females). Consequently, the maternal effect of TSE cannot be attributed to a difference in imprinting between the maternally and paternally transmitted P-1152 telomeric silencers, but rather to an extrachromosomally transmitted factor likely deposited in the cytoplasm of the oocyte, which renders the telomeric

### Table 1. TSE Levels in Mutant Contexts

| Genotype | P-LacZ Silencer; P-lacZ Target |
|----------|--------------------------------|
|          | P-1152; BC69 | P-1152; BQ16 | P-1152; P-Co1 |
| Wild type (TSE control) | 62.9% (2,950) | 87.8% (2,300) | 89.3% (3,250) |
| Su(var)2052; Su(var)3–906 | 15.8% (1,150) | 29.5% (2,550) | 39.5% (1,150) |
| Su(var)3–6R2A8 | 31.0% (1,050) | 31.0% (1,050) | 31.0% (1,050) |
| Su(var)3–7R14/Su(var)3–906 | 3.3% (1,100) | 7.5% (1,100) | 7.5% (1,100) |
| Su(var)3–7R2A8/Su(var)3–906 | 58.5% (1,150) | 58.5% (1,150) | 58.5% (1,150) |
| P-Z-target | 0.0% (1,100) | 0.0% (1,100) | 0.0% (1,100) |
| P-Co1 | 0.0% (1,200) | 0.0% (1,200) | 0.0% (1,200) |
| P-Z-target | 0.0% (1,200) | 0.0% (1,200) | 0.0% (1,200) |
| P-Co1 | 84.7% (3,100) | 84.7% (3,100) | 84.7% (3,100) |
| P-Co1 | 92.3% (1,850) | 92.3% (1,850) | 92.3% (1,850) |
| Su(var)2050/piwi2 | 10.0% (2,150) | 10.0% (2,150) | 10.0% (2,150) |
| Su(var)2050/piwi2 | 5.6% (1,200) | 5.6% (1,200) | 5.6% (1,200) |

Females analyzed carried a maternally inherited P-1152 telomeric silencer locus (from a homozygous P-1152 mother) and a paternally inherited P-lacZ target transgene. They also carried mutant alleles of candidate genes involved in heterochromatin formation or small RNA silencing. For heterozygous or heteroallelic mutant genotypes, the maternally inherited allele is written first. The percentage of TSE is given with the total number of egg chambers assayed in parentheses.

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**Figure 1. Phenotype and Quantification of TSE**

(A, B) Ovary β-galactosidase staining of P-lacZ enhancer-trap transgenes inserted in subtelomeric heterochromatin (line P-1152 [A]) and euchromatin (line BQ16 [B]).

(C) Complete repression of lacZ by TSE in the G1 females produced by the cross between P-1152 females and BQ16 males.

(D) Expression control of the euchromatic BC69 P-lacZ enhancer-trap.

(E, F) Incomplete TSE in the G1 females produced by the cross between P-1152 females and BC69 males. Variagation is observed inside ovaries that show individual ovarioles with both off (lacZ repressed) and on (lacZ not repressed) egg chambers (Ea, Ec, Ed) and ovarioles that are completely off (Ee) or completely on (Eb). (F) For the quantification of TSE, the number of repressed egg chambers among the first five egg chambers of a given ovariole is scored per ovariole for more than ten ovaries.

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silencers (paternally or maternally inherited) capable of establishing TSE.

Candidate Gene Analysis

Given the variegating phenotype of TSE and the interaction observed above between a maternally transmitted factor and the chromosomal copy of the telomeric silencer, it seemed possible that heterochromatin formation and RNA silencing could be involved in this repression. A candidate gene analysis was thus performed to identify genes whose mutations affect TSE among genes encoding heterochromatin components and actors of the small RNA silencing pathways. For a given assay, a P-1152 telomeric silencer was combined with a P-lacZ target expressed in the female germline, in the absence (TSE positive control) or presence of mutant alleles of the candidate gene. The P-1152 silencer was inherited in each case from a homozygous P-1152 female. When tested in the heterozygous state, the mutant allele was maternally inherited. The first gene tested was Su(var)205 (Figure 4A–4D), which encodes HP1 [30]. HP1 is a multifunctional protein that binds at centromeres, telomeres, and some scattered sites on the chromosomal arms [31,32]. The TSE positive control produced almost 90% repression (Figure 4B), whereas one copy of the null allele Su(var)205*, corresponding to an almost completely amorphic allele, has a strong negative dominant effect on TSE (15.8% TSE remaining, Figure 4C). The allele Su(var)205* encoding a truncated HP1 protein also behaves as a genetically null allele and also strongly impairs TSE (29.5% TSE remaining, Figure 4D). TSE is therefore sensitive to the dose of HP1. The same analysis was performed for SU(VAR)3-7, another nonhistone heterochromatin protein that binds at centromeres and telomeres and is a partner of HP1 [33,34]. SU(VAR)3-7 contains seven zinc finger motifs, which were shown to bind DNA in vitro [35,36]. Again a dose effect was observed since females heterozygous for the Su(var)3-7R14 null allele showed a reduced TSE (less than 40%, Table 1). Since Su(var)3-7 mutants can be homozygous viable, mutations in this gene were also tested at the homozygous state and an almost complete loss of TSE was observed (3.3% TSE remaining, Table 1). Such a result was also obtained with a second Su(var)3-7 mutant allele (Su(var)3-7R14, Table 1). Su(var)3-7 encodes a histone methyl transferase (HMT) responsible for the methylation of histone H3 on lysine 9 [37]. This protein is also a partner of HP1. The null allele of this gene tested here at the homozygous state had no significant effect on TSE (58.5% in the mutant versus 62.9% in the wild-type control, Table 1).

We further tested genes involved in RNA silencing. Three primary RNA silencing pathways have been discovered so far: miRNA, siRNA (classically termed “RNA interference”), and rasiRNA pathways. These pathways may partially overlap, since they have certain actors in common, but they differ in terms of the biogenesis of short RNAs. miRNAs derive from hairpin RNAs and target numerous essential genes; siRNAs derive from double-strand RNAs and can serve as a defence mechanism against parasites such as viruses; and rasiRNAs derive from interactions between two complementary types of PIWI-interacting RNAs and have numerous targets but are more specific of TEs and heterochromatic sequences [3,4,29]. We tested three genes (aubergine [aub], homeless [hls], and armitage [armi]) involved in both the siRNA pathway [38,39] and the rasiRNA pathway in Drosophila ovaries [3,4,29]. Mutations of these genes can induce defaults in siRNA-guided cleavage or in the production of rasiRNAs depending on the target of the silencing [3,39,40]. These mutations are also responsible for disruption of embryonic axis specification linked to disturbance of microtubule polarisation, but this effect is mediated by a mechanism different from rasiRNA silencing itself [41]. The first gene tested is aub, an RNA binding protein and member of the Argonaute family (Figure 4E–4H) [42]. No significant dose effect of aub was detected (Figure 4G and unpublished data), but the two heteroallelic aub mutant genotypes tested completely abolish TSE (Figure 4H; Table 1). The second one is hls, which encodes an RNA helicase [43]. Again no dose effect was observed (unpublished data), but a heteroallelic hls mutant genotype completely abolishes TSE (Table 1). The third one is armi, which encodes a putative RNA helicase [44,45]. No dose effect was observed (unpublished data), but a complete loss of TSE was observed in a heteroallelic mutant context (Table 1).
another member of the Argonaute family [46] shown to be involved in the rasiRNA pathway [3,4,6,29,47] was tested. This gene is also necessary for germline stem-cell renewal [48] and was shown to be required for post-transcriptional transgene silencing (a phenomenon termed cosuppression) [49]. No dose effect on TSE was observed (unpublished data). Because piwi has deleterious effects on ovary structure at the homozygous state, we tested the effects of piwi mutant alleles at the heterozygous state in combination with a Su(var)205 mutant. In these combinations, piwi mutant alleles aggravate the negative effect of reduction of the dose of HP1: the single Su(var)205 mutant shows 29.5% TSE, whereas the double heterozygotes show 10.0% and 5.6% for piwi1 and piwi2 respectively (Table 1). These results indicate that the siRNA and/or the rasiRNA pathways are involved in TSE. To determine which of these two pathways is responsible for TSE, several other mutations were tested. r2d2 is involved in the siRNA pathway [3,50,51]. R2D2 is a double-stranded RNA (dsRNA) binding protein involved in siRNA loading onto RISC [50]. In ovaries of females homozygous for the r2d2 null allele, the siRNA pathway is severely affected, as shown by a dsRNA-initiated RISC (or RNA-induced silencing complex) assay [52]. These ovaries show somewhat abnormal ovarioles (Figure S2), but quantification of TSE was nonetheless possible. The loss of function for r2d2 does not affect TSE (Table 1). A second protein DICER-2 (DCR-2), which combined with R2D2 is responsible for dsRNA cleavage, is also involved specifically in the siRNA pathway [53]. The loss of function of Dcr-2 is fertile. Unfortunately, we were not able to test the effect of a Dcr-2 mutant allele on TSE. Indeed, the control staining (in absence of a telomeric silencer) of the two different P-lacZ target transgenes that we tested in homozygous Dcr-2L811fsX mutants intriguingly showed almost no staining in ovaries, thus making it impossible to assay any target repression by a telomeric silencer in this context. Finally, a mutant allele of loquacious (loqs), a dsRNA binding protein involved in the miRNA pathway was tested. The loqs f00791 allele corresponds to a hypomorphic allele that alters miRNA-induced silencing in all tissues where it has been tested, including ovaries [54]. This allele is viable, allowing us to test homozygous mutant females. No effect on TSE was detected (Table 1).

Figure 3. TSE Involves a Strictly Maternally Transmitted Component
“A” females, hemizygous for the P-1152 telomeric silencer locus, were established by crossing homozygous P-1152 females and males carrying the Muller-S chromosome (M5) devoid of P sequence and marked by the semidominant Bar marker. These “A” females were crossed with males carrying the BQ16 euchromatic P-lacZ as target in order to recover the “B” and “C” females having inherited or not P-1152. “A” females were also crossed with P-1152; BQ16 males in order to recover the “D” females having inherited P-1152 from the father. P-1152; BQ16 males were also crossed with females devoid of P transgenes producing “E” females genotypically similar to “D” females, with the exception that they have inherited a naive cytoplasm, whereas, “D” females have inherited a P-1152 cytoplasm. “B–E” females were scored for TSE. “F” females show an expression control of the target. doi:10.1371/journal.pgen.0030158.g003
In conclusion, TSE strongly depends on the function of two genes involved in heterochromatin formation and four genes involved in the rasiRNA silencing pathway but is not affected by mutations of genes involved in the siRNA or miRNA pathways.

Discussion

Our study of the regulatory mechanism of a TE that recently invaded the D. melanogaster genome allowed the discovery of a master site for establishing strong transposon repression in the germline; indeed, one or two P elements inserted in TASs are sufficient to repress the mobility of the whole P family [21]. TSE was further discovered [26], a mechanism by which a transgene inserted in subtelomeric heterochromatin has the capacity to repress a homologous transgene located in euchromatin. This phenomenon appears general since it can be induced by transgenes inserted at the telomere of the X chromosome as well as autosomal telomeres, and the targets can be located on all major chromosomes [26,28]. TSE corresponds thus to a useful tool to investigate the properties of the telomeric master site of P repression. Here, we show that TSE requires genes encoding proteins involved in heterochromatin formation (including HP1, a major component of heterochromatin) and proteins involved in the rasiRNA silencing pathway. Another TE master regulatory locus has been described in Drosophila close to the centromere of the X chromosome. It was first shown to regulate the gypsy retrotransposon and referred to as the flamenco locus [55]. This locus was further characterized as regulating together two other retrotransposons, ZAM and Idefix, and proposed as a repression center for multiple TEs, known as “COM” (Centre Organisateur de Mobilisation) [14]. Repression by this locus is based on RNA silencing, but differs from TSE in that repression of gypsy, ZAM, and Idefix occurs in the somatic follicle cells of the ovary. This point is important since in somatic follicle cells, AUBERGINE, a major actor of the rasiRNA pathway, is not expressed [4]. Conversely, in the germline, where TSE is active, all known components of the rasiRNA machinery (AUBERGINE, PIWI, and AGO3) are present. Consequently, TSE is particularly appropriate to investigate the genetic properties of a complete rasiRNA-based repression machinery.

What Is the Molecular Support of Epigenetic Transmission of TSE?

Here, we show that TSE exhibits a maternal memory that can be detected for six generations and has thus a partial
epigenetic transmission through meiosis (Figure 2). The genetic analysis indicates that potentialization of the telomeric silencer (“potentialization step”) appears to be a prerequisite for repression of the target transgene by the telomeric silencer (“target repression step”). For the potentialization of the telomeric silencer, both an extrachromosomal maternally transmitted factor, produced by females carrying a telomeric silencer, and a chromosomal copy of the telomeric silencer are necessary (Figure 3). The molecular nature of this extrachromosomal maternally transmitted factor, responsible for the maternal effect, is not known at the moment but it may correspond to rasiRNAs since TAS homologous rasiRNAs have been reported (see Table S1 of [4]). We have attempted to identify short RNAs made by telomeric transgenes by northern blot on ovaries but did not detect them; this may be due to a low concentration of such short RNAs. We propose two nonmutually exclusive mechanisms. In the first, which derives from the “rasiRNA positive loop model” proposed by Brennecke et al. (“ping-pong model”) [4] and Gunawardane et al. [29], females deposit PIWI-interacting RNAs (piRNAs) in the oocyte produced by telomeric transgenes inserted in tandemly repeated TAS heterochromatic sequences. As proposed in their model, these RNAs would be primarily small antisense RNAs associated with PIWI or AUBERGINE. These piRNAs would interact in the embryo with sense RNAs produced by the telomeric transgenes and result in the production of small sense RNAs associated with AGO3, which in turn would promote the production of a new antisense piRNA, resulting in a positive feedback loop. Such a loop can explain the two-component interaction shown in Figure 3 (see below).

A second mechanism can be proposed for the potentialization step. Indeed, RNA silencing and heterochromatin formation have been shown to be connected since “short RNA-dependent heterochromatin formation” pathways have been described in several species (fission yeast [56], ciliates [57], plants [58], and Drosophila [59], for a review see [60–62]). According to this model, maternally transmitted small RNA molecules may modify the chromatin structure of the chromosomal copy of the (paternally or maternally transmitted) telomeric transgenes in the embryo and thereby confer to the telomeric transgene the capacity to be a silencer. Recently, RNA-dependent heterochromatin formation was observed at Drosophila telomeres since mutations of 

For the potentialization step of TSE, the silencing of a telomeric silencer is driven by a heat-shock promoter [29]. The TSE decision appears to be established at the cystoblast stage and stably maintained since the 15 deriving nurse cells are roughly identical in regard to their repression state. Another important point in understanding the repression mechanism is that the telomeric silencer can lack the lacZ gene, i.e., the gene repressed by TSE in the target transgene. For example, a P-white-yellow transgene [66] inserted at 1A can repress the euchromatic P-otu-lacZ-white (pCo) transgene used in this study (unpublished data). In this case, the homology between the telomeric and the euchromatic transgenes comes from the white transformation marker. This rules out the hypothesis that silencing of the target occurs via an interaction between rasiRNAs deriving from the telomeric silencer and the target transgene transcript; indeed, the targeted transcript in TSE corresponds to the lacZ sequence and no lacZ rasiRNAs can be produced by the silencer. Therefore, TSE would not occur by post-transcriptional gene silencing.

Target repression could thus occur via transcriptional gene silencing. This would fit with the variegated phenotype of repression observed. Such variegation strongly suggests that repressed targets undergo heterochromatinization. Two hypotheses can be proposed to explain such heterochromatinization. First, rasiRNAs produced by the telomeric silencer would interact with nascent transcripts on the target, and this above requires a number of generations to reach a sufficient concentration to establish strong repression. Under the “rasiRNA positive loop model” proposed by Brennecke et al. [4] and Gunawardane et al. [29], when males carrying a telomeric silencer are crossed with females bearing no silencer, the cytoplasm of the oocytes would lack piRNAs homologous to the transgene. However, synthesis of these RNAs would begin in these G1 females stimulating this pathway and increasing the piRNA concentration. At each generation, females would transmit to their daughters a higher concentration of rasiRNA molecules than at the previous generation. At generation six, a sufficient level would be reached to allow strong TSE. Under the “RNA-dependent heterochromatin formation” model, when males carrying a telomeric silencer are crossed with females bearing no silencer, again the cytoplasm of the oocytes would lack small RNAs homologous to the transgene. However, synthesis of these RNAs would begin in these G1 females stimulating heterochromatinization of the telomeric silencer. This change in the chromatin state would stimulate the production of rasiRNAs at the locus also establishing a positive loop, i.e., reciprocal stimulation between heterochromatin formation and production of small RNAs. Such a reciprocal positive loop between heterochromatin formation and small RNA silencing has been proposed for gene silencing in S. pombe [64,65]. Again, at each generation, females would receive from their mother and transmit to their daughters a higher concentration of RNA molecules than at the previous generation until a sufficient level is reached at generation six.

**How Does the “Target Repression Step” Occur?**

Repression of the target transgene by the telomeric silencer was shown to be homology dependent [23,26,28]. TSE however does not require homology at the level of the promoters of the telomeric and euchromatic insertions since a defective telomeric P element lacking the P promoter can repress a P-lacZ transgene in which lacZ is driven by a heat-shock promoter [29]. The TSE decision appears to be established at the cystoblast stage and stably maintained since the 15 deriving nurse cells are roughly identical in regard to their repression state. Another important point in understanding the repression mechanism is that the telomeric silencer can lack the lacZ gene, i.e., the gene repressed by TSE in the target transgene. For example, a P-white-yellow transgene [66] inserted at 1A can repress the euchromatic P-otu-lacZ-white (pCo) transgene used in this study (unpublished data). In this case, the homology between the telomeric and the euchromatic transgenes comes from the white transformation marker. This rules out the hypothesis that silencing of the target occurs via an interaction between rasiRNAs deriving from the telomeric silencer and the target transgene transcript; indeed, the targeted transcript in TSE corresponds to the lacZ sequence and no lacZ rasiRNAs can be produced by the silencer. Therefore, TSE would not occur by post-transcriptional gene silencing.

Target repression could thus occur via transcriptional gene silencing. This would fit with the variegated phenotype of repression observed. Such variegation strongly suggests that repressed targets undergo heterochromatinization. Two hypotheses can be proposed to explain such heterochromatinization. First, rasiRNAs produced by the telomeric silencer would interact with nascent transcripts on the target, and this
interaction could provoke a local heterochromatinization of the target [64,65]. Heterochromatinization may then spread along the target as suggested by the repression of a P-otu-lacZ-white target by a telomeric P-white-yellow transgene. In this case heterochromatinization would start on the white sequence of the target and subsequently reach the lacZ sequence. HP1 and SU(VAR)3–7 could be involved in this spreading since centromeric heterochromatin spreading has been shown to be sensitive to the dose of these proteins [35,67]. An alternative hypothesis for target heterochromatinization is a DNA–DNA interaction between the telomeric and the euchromatic transgenes, leading to their pairing and to trans-heterochromatinization of the target following this pairing. Indeed, telomeric silencers are themselves in a heterochromatic state due to the heterochromatic nature of the TAS [68–71]. This is illustrated by a phenomenon referred to as telomeric position effect, in which a transgene inserted in TAS shows variegation in the eye for the transformation marker [72–75]. Again HP1 and SU(VAR)3–7 could play a role in this trans-heterochromatinization.

What Is the Role of the Telomere Cap in TSE?

*Drosophila* telomeres can be divided into three domains with respect to chromatin structure [71]: (1) the subtelomeric cluster of heterochromatic TAS repeats [68–70] in which the tested P elements or P transgenes are inserted; (2) distal to the TAS, a telomeric retrotransposon array repeatedly in the same orientation [76–80], the retrotransposon array is partially euchromatic [71], and transgenes located inside this domain do not show variegation for an eye marker [81]; and (3) at the extremity of the retrotransposon array, the telomere protein cap that prevents telomere fusion and regulates telomeric retrotransposon transcription and transposition [82–85]. Interestingly, HP1 (Su(var)205) and SU(VAR)3–7, both implicated in TSE, are part of the cap and therefore could be required for the telomeric locus to be a silencer. This hypothesis is supported by the analysis of the different Su(var)205 alleles. In addition to the two null alleles of Su(var)205 tested here, which showed a strong effect on TSE (Table 1), we also tested a deficiency and the Su(var)205 allele corresponding to a mutation in the chromodomain (involved in the histone H3 K9-methylated binding), which leaves the capping activity intact [85,86]. The deletion of Su(var)205 as expected has a strong effect on TSE but Su(var)205 has no effect (unpublished data). This suggests that HP1 effect can be mediated (at least partially) via its capping activity. Therefore, HP1 and SU(VAR)3–7 could affect the telomeric transgenes in TAS indirectly via such capping activity, for example, by affecting the localisation of the telomere inside the nucleus.

Finally, it could appear paradoxical that an amorphic mutant allele of the Su(var)3–9 HMT has no effect on TSE since RNA-dependent heterochromatin formation in most cases involves the histone H3 methylation transition from lysine 4 to lysine 9 to establish the link between short RNAs and formation of heterochromatin. However, this result can be explained by the fact that this HMT has been recently shown not to be responsible for histone H3 methylation on lysine 9 at *Drosophila* telomeres [85]; another unidentified HMT likely plays this role. We are therefore pursuing the candidate gene analysis in order to identify a HMT involved in TSE.

Conclusions

Telomeric TSE appears to be a complex repression mechanism that requires genes involved in heterochromatin formation and in RNA silencing. In *Drosophila*, interaction between small RNA silencing pathways and transcriptional repression was previously shown to exist in somatic tissues for cosuppression phenomena or for variegation phenotypes with the white marker in the eye [49,50]. TSE shows that interaction between RNA silencing and heterochromatin formation can also occur in the germline. This type of silencing appears to be the basic mechanism for P-element repression [28], although some P-encoded repressors encoded by euchromatic copies may contribute in some cases to P repression [10,87]. TSE has the same complex inheritance as the P cytotype [10,88,89]. The subtelomeric heterochromatin thus represents a piRNA producing “platform” [4] available for recent invaders of the genomes to establish their own repression. The study of TSE illustrates the genetic properties of such a platform.

Materials and Methods

Experimental conditions. All crosses were performed at 25 °C and involved three to five couples in most cases. All ovary lacZ expression assays were carried out using X-gal overnight staining as described in Lemaitre et al. 1993 [90], except that ovaries were fixed for 6 min.

Transgenes and strains. P-lacZ fusion enhancer trap transgenes (P-1152, BQ16, and BC69) contain an in-frame translational fusion of the *E.coli* lacZ gene to the second exon of the P transposase gene and contain *roy* as a transformation marker [91]. The P-1152 insertion comes from stock number 11152 of the Bloomington Stock Center (http://flystocks.bio.indiana.edu) and was mapped at the telomere of the X chromosome (site 1A); this stock was previously described to carry a single P-lacZ insertion in TAS [26]. However, in our number 11152 stock, we have mapped two P-lacZ insertions in the same TAS unit and in the same orientation that might have resulted from an unequal recombination event duplicating the P-lacZ transgene. P-1152 is homozygous viable and fertile. BQ16 is located at 64C in euchromatin of the third chromosome and is homozygous viable and fertile. BC69 is inserted on Chromosome 2 in the first exon of the vasa gene and results in a vasa loss of function; it is consequently homozygous female sterile. P-1152 shows no lacZ expression in the ovary (Figure 1A), whereas BQ16 and BC69 are strongly expressed in the nurse cells and in the oocyte (Figure 1B and 1D, respectively). P-Cel is an insertion of the Pco transgene (P-otu-lacZ) on the third chromosome (85A-B), which is homozygous viable and fertile. β-galactosidase expression of the Pco transgene is driven by the otu promoter and is therefore strongly detected in both nurse cells and the mature oocyte [27]. This transgene contains a white gene as a transformation marker.

Lines carrying transgenes have M genetic backgrounds (devoid of P TEs), as well as the multimarked balancer stocks used in genetic experiments (yw; Cyo; TM3B / T(2;3)apXa, M5; CyO / T(2;3)apXa and M5; TM3B/T(2;3)apXa and the strains carrying mutations used for the candidate gene analysis (see below).

Mutations used for the candidate gene analysis. Su(var)205, aub, psi, z2d, and lops genes are located on Chromosome 2, whereas Su(var)3–7, Su(var)3–9, hbs (synonym of spindle-E), and armi are located on Chromosome 3. Loss of function is lethal in the case of Su(var)205 and lops, female sterile in the case of aub, psi, hbs, z2d, and armi, and is viable and fertile in the case of Su(var)3–9. Loss of function of Su(var)3–7 is viable when the mother is heterozygous mutant but lethal when the mother is homozygous mutant [92]. Su(var)3–2505 was x-ray induced and corresponds to a null allele since it only encodes the first ten amino acids of the HP1 protein [67]. Su(var)2–504 was EMS induced and encodes a truncated HP1 protein, ~85% of wild type size [67], missing a domain necessary for targeting the nucleus [93]. Lines carrying these mutations were kindly provided by Gunter Reuter.

Su(var)3–7265 and Su(var)3–7214 were generated by homologous recombination [92,94]. Su(var)3–7214 produces a chimeric protein and Su(var)3–7265 deletes most of the protein-coding sequence of the
Three strong mutant alleles of abu induced by EMS were used. All of them were homozygous female sterile, abu2 [CG12479] [96] comes from the Bloomington Stock Center (stock number 4968), abu2[269] [96] has an amino acid substitution, abu2[1429] [97] has a 134-hp deletion, resulting in a frameshift that is predicted to add 16 novel amino acids after residue 740 [98]. abu2[269] and abu2[1429] were kindly provided by Paul Macdonald.

\[ p\textit{fus}1 \text{ and } p\textit{fus}2 \text{ are P-element-induced mutations; } p\textit{fus}1 \text{ contains a P transgene inserted in the first exon [46]. The orientation of the transgene is opposite to that of the gene, } p\textit{fus}2 \text{ contains a P transgene inserted in the same orientation as the gene. In both cases, homozygous females are sterile. Lines carrying these mutations were kindly provided by Alain Bucheton.}\]

\[ hls6315 \text{ (synonym of } spn-E^{-} \text{)} \text{ was generated by EMS [43,99], } hls1215 \text{ (synonym of } spn-E^{\text{a2c5}} \text{)} \text{ was generated by P-element excision resulting in deletion of coding sequences and adjacent sequences. Deletion may extend into other genes [43]. Lines carrying these mutations were kindly provided by Utpal Bhadra.}\]

\[ \text{arm}1 \text{ and } \text{arm}2 \text{ are P-element-induced mutations; } \text{arm}1 \text{ has an insertion of a P transgene in the 5' UTR of the armitage gene [44]. } \text{arm}2 \text{ derives from } \text{arm}1 \text{ by an imprecise excision of the P element, resulting in a deletion of } \text{arm} \text{ sequences in the 5' untranslated region [39]. These two alleles are female sterile and come from the Bloomington Stock Center (stock numbers 8513 and 8544). } \text{r2d}2 \text{ results from a deletion induced by the remobilization of a P transgene located in 5' of the } \text{r2d}2 \text{ gene [50]. The 4.9-kb deletion removes the entire } \text{r2d}2 \text{ open reading frame [29]. This allele is an amorphic allele and homozygous females are sterile due to abnormal ovaries. The siRNA pathway is severely affected as shown by a dsRNA-initiated RISC assay in ovaries of homozygous mutant females [52]. This line comes from the Bloomington Stock Center (stock number 18371).}\]

\[ \text{loqs} \text{ from a flybase transgene insertion 57 bp upstream of the transcription start site of } \text{loqs} [54], \text{a gene necessary for miRNA-directed silencing, } \text{loqs} \text{ is a hypomorphic allele that is homozygous viable. At the homozygous state, this allele has a strong effect on miRNA processing in ovaries. It also has a negative effect on siRNA silencing [54]. This allele comes from the Bloomington Stock Center (number 18371).}\]

All the alleles described above are maintained over a balancer chromosome. Additional information about mutants and stocks are available at Flybase (http://flybase.bio.indiana.edu).
35. Reuter G, Giarre M, Farah J, Gausz J, Spierer A, et al. (1990) Dependence of 
Drosophila melanogaster. Gene 164: 501–509.

34. Delattre M, Spierer A, Tonka CH, Spierer P (2000) The genomic silencing 
in retrotransposons in the Drosophila male germline. Mol Genet Genomics 263: 205–209.

33. Vagin VV, Klenov MS, Kalmykova AI, Stolyarenko AD, Kotelnikov RN, et al. (2005) RNA interference proteins and Vasa locus are involved in the silencing of retrotransposons in the female germline of Drosophila melanogaster. RNA Biol 2: 54–58.

32. Savitsky M, Kwon D, Georgiev P, Kalmykova A, Gvozdev V (2006) Telomere elongation is under the control of the RNAi-based mechanism in the Drosophila germine. Genes Dev 20: 345–354.

31. Jensen S, Gassama MP, Dramard X, Heidmann T (2002) Regulation of I- 
26S proteasome in Drosophila. Cell 113: 4253–4261.

30. James TC, Elgin SC (1986) Identification of a nonhistone chromosomal 
position-effect variegation in Drosophila melanogaster. Genetica 117: 135–147.

29. James TC, Eissenberg JC, Craig C, Dietrich V, Hobson A, et al. (1989) The distribution of heterochromatin protein 1 (HP1) in Drosophila. Nature 338: 189–195.

28. Ronseray S, Boivin A, Anxolabehere D (2001) P-Element repression in 
Drosophila melanogaster: A mammalian transmitted regulatory state of the germ line associated with telomeric P Elements. Genes Dev 15: 232–243.

27. Ronseray S, Boivin A, Anxolabehere D (2003) Telomeric P elements associated with cytotypic regulation of the P transposon family in Drosophila. Genes Dev 17: 1611–1615.

26. Reiss D, Josse T, Anxolabehere D, Ronseray S (2004) Aubergine mutations in Drosophila melanogaster impair P cytotypic elements inserted in heterochromatin. Mol Gen Genomics 272: 336–345.

25. Sarot E, Payen-Groschene G, Bucheton A, Pelisson A (2004) Evidence for a 
role of SU(VAR)3–9 in histone H3-K9 methylation and heterochromatin assembly defects in the X chromosome. Genetics 167: 489–500.

24. Stuart JR, Haley KJ, Swedzinski D, Lockner S, Kocian PE, et al. (2002) The P 
locus governing the control of independent endogenous retroviruses from Drosophila melanogaster. Genetics 155: 1841–1854.

23. Van Eventer AJ, Moseley DF, Weller JS (1983) Hypomethylation of DNA in 
the Drosophila X chromosome. Genetics 105: 501–512.

22. Simmons MJ, Raymond JD, Niemi JB, Stuart JR, Merriman PJ (2004) The P 
transposon family in Drosophila melanogaster: A maternally transmitted regulatory state of the germ line associated with telomeric P Elements. Genes Dev 15: 232–243.

21. James TC, Eissenberg JC, Craig C, Dietrich V, Hobson A, et al. (1989) The distribution of heterochromatin protein 1 (HP1) in Drosophila. Nature 338: 189–195.

20. Ronseray S, Boivin A, Anxolabehere D (2001) P-Element repression in 
Drosophila melanogaster: A mammalian transmitted regulatory state of the germ line associated with telomeric P Elements. Genes Dev 15: 232–243.

19. Marin I, Lehmann M, Nouna D, Izabel H, Anxolabehere D, et al. (2000) 
P-Element repression in Drosophila melanogaster by a naturally occurring defective telomeric P copy. Genetics 155: 1597–1608.

18. Cook HA, Koppetsch BS, Wu J, Theurkauf WE (2004) The RNAi machinery. Science 303: 669–672.

17. Vagin VV, Klenov MS, Kalmykova AI, Stolyarenko AD, Kotelnikov RN, et al. (2005) RNA interference proteins and Vasa locus are involved in the silencing of retrotransposons in the female germline of Drosophila melanogaster. RNA Biol 2: 54–58.

16. Kalmykova AI, Klenov MS, Gvozdev VA (2005) Argonaute protein PIWI controls mobilization of retrotransposons in the Drosophila male germline. RNA 11: 393–403.

15. Sarot E, Payen-Groschene G, Bucheton A, Pelisson A (2004) Evidence for a 
role of SU(VAR)3–9 in histone H3-K9 methylation and heterochromatin assembly defects in the X chromosome. Genetics 167: 489–500.

14. Reuter G, Giarre M, Farah J, Gausz J, Spierer A, et al. (1990) Dependence of 
Drosophila melanogaster. Gene 164: 501–509.

13. Jensen S, Gassama MP, Dramard X, Heidmann T (2002) Regulation of I- 
26S proteasome in Drosophila. Cell 113: 4253–4261.

12. Ronseray S, Bosse T, Baudin I, Boivin A, Anxolabehere D (2001) P-Element repression in 
Drosophila melanogaster: A mammalian transmitted regulatory state of the germ line associated with telomeric P Elements. Genes Dev 15: 232–243.

11. Reiss D, Josse T, Anxolabehere D, Ronseray S (2004) Aubergine mutations in Drosophila melanogaster impair P cytotypic elements inserted in heterochromatin. Mol Gen Genomics 272: 336–345.

10. Roche SE, Rio DC (1998) Trans-silencing by P elements inserted in 
Drosophila melanogaster by a naturally occurring defective telomeric P copy. Genetics 155: 1597–1608.

9. Ronsseray S, Boivin A, Anxolabehere D (2001) P-Element repression in 
Drosophila melanogaster: A mammalian transmitted regulatory state of the germ line associated with telomeric P Elements. Genes Dev 15: 232–243.

8. Ronseray S, Boivin A, Anxolabehere D (2003) Telomeric transgenes and 
trans-silencing in Drosophila. Gene 317: 327–335.

7. Guarnawardane LS, Saito K, Nishita KM, Miyoshi K, Kawamura Y, et al. (2007) A slicer-mediated mechanism for repeat-associated siRNA 5′ end formation in Drosophila melanogaster. Science 315: 1857–1859.

6. Ronseray S, Bosse T, Baudin I, Boivin A, Anxolabehere D (2001) P-Element repression in 
Drosophila melanogaster: A mammalian transmitted regulatory state of the germ line associated with telomeric P Elements. Genes Dev 15: 232–243.

5. James TC, Elgin SC (1986) Identification of a nonhistone chromosomal 
protein associated with heterochromatin in Drosophila melanogaster and its gene. Mol Cell Biol 6: 3862–3872.

4. James TC, Eissenberg JC, Craig C, Dietrich V, Hobson A, et al. (1989) Distribution patterns of HP1, a heterochromatin-associated nonhistone 
protein and companion of HP1 in the genomic silencing of retrotransposons in the female germline of Drosophila melanogaster. Gene 117: 135–147.

3. Fanti L, Belfoco M, Piacentini L, Pimpinelli S (2003) Chromosomal 
distribution of heterochromatin protein 1 (HP1) in Drosophila: A cytological map of euchromatic HP1 binding sites. Genetics 167: 157–167.

2. Cleard F, Delattre M, Spierer P (1997) SU(VAR)3–7, a Drosophila 
heterochromatin-associated protein and companion of HP1 in the genomic silencing of position-effect variegation. Embry 16: 5280–5288.

1. Cleard F, Spierer A, Tonka CH, Spierer P (2000) The genomic silencing of position-effect variegation in Drosophila melanogaster: Interaction between the heterochromatin-associated proteins Su(var)3–7 and HP1. J Cell Sci 113: 4293–4291.
dosage-dependent effects on position-effect variegation. Genetics 131: 345–352.

68. Levis R, Hazelrigg T, Rubin GM (1985) Effects of genomic position on the expression of transduced copies of the white gene of Drosophila. Science 229: 558–561.

69. Karpen GH, Spradling AC (1992) Analysis of subtelomeric heterochromatin in the Drosophila minichromosome Dp(1)87 by single P element insertional mutagenesis. Genetics 132: 757–753.

70. Walter MF, Jiang C, Kasravi B, Donath J, Mechler BM, et al. (1995) DNA organization and polymorphism of a wild-type Drosophila telomere region. Chromosoma 104: 229–241.

71. Andreyeva EN, Belyaeva ES, Semeshin VF, Pokholkova GV, Zhimulev IF (2005) Three distinct chromatin domains in telomere ends of poltynote chromosomes in Drosophila melanogaster Tel mutants. J Cell Sci 118: 5465–5477.

72. Wallrath LL, Elgin SC (1995) Position effect variegation in Drosophila is associated with an altered chromatin structure. Genes Dev 9: 1263–1277.

73. Cryderman DE, Morris EJ, Biessmann H, Elgin SC, Wallrath LL (1999) Silencing at Drosophila telomeres: Nuclear organization and chromatin structure play critical roles. Embo J 18: 3724–3735.

74. Golubovsky MD, Konev AY, Walter MF, Biessmann H, Mason JM (2001) Terminal retrotransposons activate a subtelomeric white transgene at the 2L telomere in Drosophila. Genetics 158: 1111–1123.

75. Mason JM, Konev AY, Golubovsky MD, Biessmann H (2003) Cis- and trans-acting influences on telomeric position effect in Drosophila melanogaster detected with a subterminal transgene. Genetics 165: 917–930.

76. Biessmann H, Champion LE, O’Hair M, Ikenna K, Kasravi B, et al. (1992) Frequent transpositions of Drosophila melanogaster He-TA transposable elements to receding chromosome ends. Embo J 11: 4459–4469.

77. Levis RW, Ganesan R, Houitschens K, Tolar LA, Sheen FM (1993) Transposons in place of telomeric repeats at a Drosophila telomere. Cell 75: 1083–1093.

78. Sheen FM, Levis RW (1994) Transposition of the LINE-like retrotransposon TART to Drosophila chromosome termini. Proc Natl Acad Sci U S A 91: 12510–12514.

79. Pardue ML, DeBaryshe PG (2003) Retrotransposons provide an evolutionary advantage by stabilizing telomeres. Annu Rev Genet 37: 485–511.

80. Abad JP, De Pablos B, Osoegawa K, De Jong PJ, Martin-Gallardo A, et al. (2004) TAHRRE, a novel telomeric retrotransposon from Drosophila melanogaster, reveals the origin of Drosophila telomeres. Mol Biol Evol 21: 1620–1628.

81. Biessmann H, Prasad S, Semeshin VF, Andreyeva EN, Nguyen Q, et al. (2005) Two distinct domains in Drosophila melanogaster telomeres. Genetics 171: 1767–1777.

82. Biessmann H, Carter SB, Mason JM (1990) Chromosome ends in Drosophila without telomeric DNA sequences. Proc Natl Acad Sci U S A 87: 1758–1761.

83. Savitsky M, Kravchuk O, Melnikova L, Georgiev P (2002) Heterochromatin protein 1 is involved in control of telomere elongation in Drosophila melanogaster. Mol Cell Biol 22: 3204–3218.

84. Cenci G, Siriaci G, Raffa GD, Kellum R, Gatti M (2003) The Drosophila HOAP protein is required for telomere capping. Nat Cell Biol 5: 82–84.

85. Perrini B, Piacentini L, Fanti L, Aliferi F, Chiaciarelli S, et al. (2004) HP1 controls telomere capping, telomere elongation, and telomere silencing by two different mechanisms in Drosophila. Mol Cell 15: 467–476.

86. Fanti L, Giovinazzo G, Berloco M, Pimpinelli S (1998) The heterochromatin protein 1 prevents telomere fusions in Drosophila. Mol Cell 2: 527–538.

87. Rio DC (1991) Regulation of Drosophila P element transposition. Trends Genet 7: 282–287.

88. Ronseray S, Lemaire B, Coen D (1995) Maternal inheritance of P cytotype in Drosophila melanogaster: A “pre-P cytotype” is strictly extra-chromosonally transmitted. Mol Genet Genom 241: 115–123.

89. Coen D, Lemaire B, Delattre M, Queeneville H, Ronseray S, et al. (1994) Drosophila P element: Transposition, regulation and evolution. Genetics 135: 61–78.

90. Lemaire B, Ronseray S, Coen D (1995) Maternal repression of the P element promoter in the germline of Drosophila melanogaster: A model for the P cytotype. Genetics 135: 149–160.

91. O’Kane CJ, Gehring WJ (1987) Detection in situ of genomic regulatory elements in Drosophila. Proc Natl Acad Sci U S A 84: 9123–9127.

92. Seum C, Pauli D, Delattre M, Jaquet Y, Spierer A, et al. (2002) Isolation of Su(var)3–7 mutations by homologous recombination in Drosophila melanogaster. Genetics 161: 1125–1136.

93. Kellum R, Raff JW, Alberts BM (1995) Heterochromatin protein 1 distribution during development and during the cell cycle in Drosophila embryos. J Cell Sci 108: 1407–1418.

94. Spierer A, Seum C, Delattre M, Spierer P (2005) Loss of the modifiers of variegation Su(var)3–7 or HP1 impacts male X polytene chromosome morphology and dosage compensation. J Cell Sci 118: 5047–5057.

95. Tschiersch B, Hofmann A, Krauss V, Dorn R, Korge G, et al. (1998) The HOAP protein encoded by the Drosophila HET-E element promoter in the germline of Drosophila melanogaster. II. Mutations blocking oogenesis or altering egg morphology. Genetics 129: 1119–1136.

96. Wilson JE, Connell JE, Schlenker JD, Macdonald PM (1996) Novel genetic screen for genes involved in posterior body patterning in Drosophila. Development 123: 1651–1662.

97. Harris AN, Macdonald PM (2001) Aubergine encodes a granule component required for pole cell formation and related to eIF2C. Dev Genet 19: 199–209.

98. Tschiersch B, Hofmann A, Krauss V, Dorn R, Korge G, et al. (1995) Maternal inheritance of P cytotype in Drosophila melanogaster: A model for the P cytotype. Genetics 135: 149–160.

99. Tearle RG, Nusslein-Volhard C (1987) Tubingen mutants and stock list. Development 128: 2823–2832.