# *P*-Element Repression in *Drosophila melanogaster* by Variegating Clusters of *P-lacZ-white* Transgenes

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Manuscript received December 12, 2000
Accepted for publication September 24, 2001

ABSTRACT

In Drosophila, clusters of *P* transgenes (*P-lac-w*) display a variegating phenotype for the *w* marker. In addition, X-ray-induced rearrangements of chromosomes bearing such clusters may lead to enhancement of the variegated phenotype. Since *P-lacZ* transgenes in subtelomeric heterochromatin have some *P*-element repression abilities, we tested whether *P-lac-w* clusters also have the capacity to repress *P*-element activity in the germline. One cluster (*T-1*), located on a rearranged chromosome (*T2;3*) and derived from a line bearing a variegating tandem array of seven *P-lac-w* elements, partially represses the dysgenic sterility (GD sterility) induced by *P* elements. This cluster also strongly represses *in trans* the expression of *P-lacZ* elements in the germline. This latter suppression shows a maternal effect. Finally, the combination of variegating *P-lac-w* clusters and a single *P-lacZ* reporter inserted in subtelomeric heterochromatic sequences at the *X* chromosome telomere (cytological site 1A) leads to strong repression of dysgenic sterility. These results show that repression of *P*-induced dysgenic sterility can be elicited in the absence of *P* elements encoding a polypeptide repressor and that a transgene cluster can repress the expression of a single homologous transgene at a nonallelic position. Implications for models of transposable element silencing are discussed.

THE *P*-transposable element is a recent invader of natural populations of *Drosophila melanogaster* (Kidwell 1983). Strains that possess *P* elements are called P strains; strains that do not are called M strains. When P males are crossed to M females, the resulting progeny exhibit a syndrome of germline abnormalities (Kidwell *et al.* 1977) that are caused by *P*-element activity. This syndrome, called hybrid dysgenesis, includes a high mutation rate, chromosomal rearrangements, male recombination, and an agametic thermosensitive sterility called GD sterility (gonadal dysgenesis) that reaches 100% for some P lines, which are thus called strong P lines. The manifestation of hybrid dysgenesis appears to be repressed by various mechanisms (Engels 1989). In some populations, repression is maternally inherited—a condition called the P cytotype (Engels 1979). Of the 30–60 *P* elements present in one P strain genome that has been analyzed, one-third are complete *P* elements (Bingham *et al.* 1982; O'Hare and Rubin 1983; O'Hare *et al.* 1992). These elements can produce an 87-kD polypeptide, the *P* transposase, which is required for *P*-element mobility (Karess and Rubin 1984; Rio *et al.* 1986). The other two-thirds of the *P* elements in this P strain genome are structurally incomplete; however, many of these defective *P* elements can still be mobilized *in trans* by complete *P* elements (Engels 1984, 1989) because they possess the sequences recognized by the *P* transposase.

The P cytotype represses transcription from the *P* promoter (Lemaitre and Coen 1991; Lemaitre *et al.* 1993; Coen *et al.* 1994). Although in the short term cytotype repression is maternally inherited, in the long term it is chromosomally determined by the *P* elements themselves (Engels 1979, 1989). Transformation of an M genome with *P* elements can induce the development of *P* repression over generations (Anxolabéhère *et al.* 1987; Daniels *et al.* 1987; Preston and Engels 1989). Conversely, the removal by segregation of *P* elements from a P genome leads to the loss of *P* repression in the progeny (Sved 1987; Ronsseray *et al.* 1993).

The ability of the *P*-element family to repress its own activity was first interpreted as resulting from the synthesis of polypeptides encoded by various *P* elements. Robertson and Engels (1989) and Misra and Rio (1990) have shown that an artificially modified *P* element that encodes a 66-kD truncated transposase can partially repress *P*-element transposition. In addition, *P* elements with a specific internal deletion, called *KP* elements, are present in high copy number in natural populations and appear to possess repression ability (Black *et al.* 1987; Rasmusson *et al.* 1993). Andrews and Gloor (1995) have shown that single insertions of a *KP* element construct driven by the *actin-5* promoter have a strong ability to repress dysgenic sterility in a position-indepen-

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dent manner. However, insertions of a similar construct in which the *KP* sequence that encodes a leucine zipper motif has been modified do not repress sterility. Thus, at least in some cases, *P* repression appears to involve the production of *P*-encoded repressor polypeptides.

However, *P* repression may not depend exclusively on the production of *P*-encoded repressor polypeptides (Roche and Rio 1998; Ronsseray *et al.* 1998). In particular, we have shown that *P* elements at the *X* chromosome telomere (cytological site 1A) regulate genome-wide *P*-element activity even though they are expressed at low levels. Indeed, although the *P* elements at 1A are responsible for a detectable level of *P* tranposase (Ronsseray *et al.* 1996), RT-PCR and Northern blot analysis indicate that they are expressed only weakly (Roche *et al.* 1995; Marin *et al.* 2000). These regulatory *P* elements are flanked by subtelomeric noncoding sequences (Ronsseray *et al.* 1996) called telomeric-associated sequences (TAS; Karpen and Spradling 1992), which exhibit some of the properties of heterochromatin, including the ability to silence transgenes inserted within them (Levis *et al.* 1985; Wallrath and Elgin 1995; Cryderman *et al.* 1999; Golubovsky *et al.* 2001). In addition, *P-lacZ* insertions at cytological site 1A and a *P-white-rosy* insertion at an autosomal telomere (100F), also inserted in TAS, have been shown to prevent germline expression of a euchromatic *P-lacZ* insertion, a phenomenon termed *trans*-silencing (Roche and Rio 1998). These telomeric *P* transgenes also stimulate the regulatory properties of other *P* elements in a P strain genome (Ronsseray *et al.* 1998). However, the telomeric *P*-transgene insertions do not by themselves significantly repress the dysgenic sterility induced by a strong P strain (Ronsseray *et al.* 1998).

The repression ability of the telomeric *P* elements at cytological site 1A is sensitive to the dose of HP1 (Ronsseray *et al.* 1996), a nonhistone heterochromatin protein that binds mostly to centromeres and telomeres (James and Elgin 1986; James *et al.* 1989; Wustmann *et al.* 1989; Fanti *et al.* 1998); heterozygosity for a null allele of *Su(var)205*, the gene that encodes this protein, strongly impairs the repression ability of telomeric *P* elements (Ronsseray *et al.* 1996, 1997). Dorer and Henikoff (1994) have found that tandem repeats of *P-lacZ-white* transgenes, inserted in euchromatin, show a variegating phenotype for the *white* marker and that the level of variegation is positively correlated with the number of transgenes. In addition, this variegation is suppressed by a *Su(var)205* mutation. Immunostaining on polytene chromosomes indicates that a cluster with a high number of *P-lacZ-white* transgenes creates a new HP1 binding site despite its location in euchromatin (Fanti *et al.* 1998). Because the *P-lac-w* clusters appear to be heterochromatinized, we hypothesized that they might show some ability to repress *P*-element activity similar to that of *P-lacZ* elements inserted in subtelomeric heterochromatin. In this article, we show that some *P-lac-w* transgene clusters are able to repress the dysgenic sterility induced by a strong P strain and that they repress the germline expression *in trans* of *P-lacZ* elements through a maternal effect.

## MATERIALS AND METHODS

**Drosophila stocks:** Canton$^{y}$ is a typical M strain (Kidwell *et al.* 1977) containing no *P* elements and marked with a spontaneous mutation of *yellow*.

Harwich-2 is a strong P strain. The subline used here shows >80 *P* labels by *in situ* hybridization on polytene chromosomes. It carries an unidentified autosomal recessive marker (sepia-colored eye), which appeared spontaneously in the stock.

*Lk-P(1A):* This line carries two autonomous *P* elements inserted in TAS at the *X* chromosome telomere (cytological site 1A; Ronsseray *et al.* 1996). These elements, derived from a natural population from Azerbaïjan (Biémont *et al.* 1990), were isolated by genetic recombination in a background devoid of other *P* elements (Ronsseray *et al.* 1991); this strain has a strong ability to repress *P* activity in the germline and the repression ability is maternally inherited (P cytotype).

*BQ16/Cy, BC69/Cy, and ABOO/Cy:* These three lines carry a *P-lacZ-rosy* enhancer-trap insertion (*P*[*lac, ry*$^{+}$]A, renamed *P{A92}* in Flybase) at different sites on the second chromosome. The *lacZ* coding sequence is fused in frame with the first 587 bp of *P*, a sequence that includes exon 0 and part of exon 1 of the *P* element. β-Galactosidase staining is mostly nuclear since the 5′ part of the *P* element carries a nuclear localization signal. These insertions express β-galactosidase activity in the germline tissues of the ovaries and testes (J. L. Couderc and F. A. Laski, personal communication). In addition, *ABOO* is expressed in the somatic follicle cells. The genes adjacent to these insertions are still unidentified except for the *BC69* insertion, which is near the gene *vasa*.

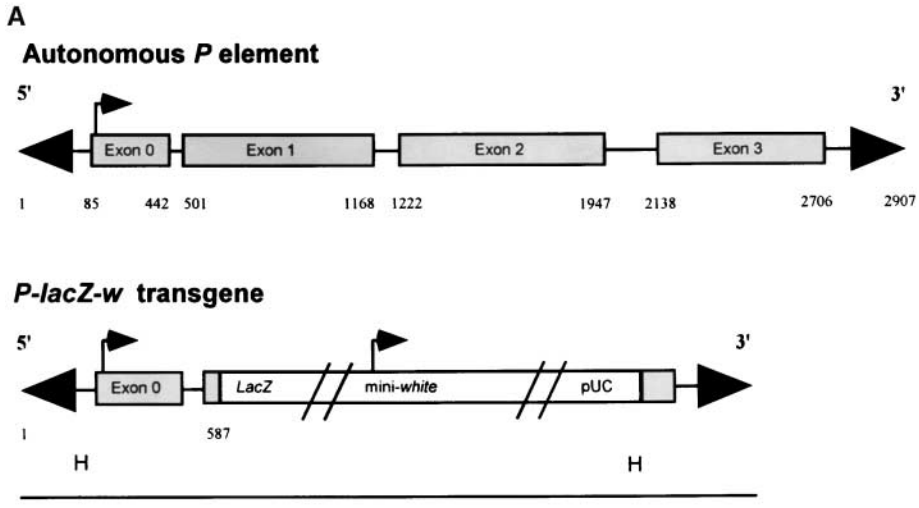
*R3-29:* This *P-lacZ* insertion, located in 1A-B on the *X* chromosome, is expressed only in late stage nurse cells. The genes adjacent to this insertion are unidentified.

*P-Co-1:* This line carries a homozygous viable insertion of pCo (*P-white-otu-lacZ*; see below) on the third chromosome (87A-B, according to an inverse PCR cloning procedure; DDBL/EMBL/GenBank accession no. AC007889). β-Galactosidase expression in this transgene is driven by the *otu* promoter and is therefore strongly detected in both nurse cells and the mature oocyte. The staining is cytoplasmic because the β-galactosidase encoded by this construct does not possess a nuclear localization signal.

*P-1039: cn*$^{1}$ *P*[*ry*$^{+t7.2}$ = PZ]elF-5A[01296]/*CyO*; *ry*$^{506}$.This line, provided by Allan Spradling, carries a *P-lacZ-ry* transgene inserted at 60B. This transgene is strongly expressed in the female germline (nurse and follicle cells).

*P-1152: P*[*ry*$^{+t7.2}$ = lArB]A171.1F1; *ry*$^{506}$ (synonym *WG-1152*). This line, from Walter Gehring, carries a *P-lacZ-ry-adh* construct at cytological site 1A. It is inserted in TAS (Roche and Rio 1998). No LacZ expression is detected in the female germline, even after overnight staining.

*P-lac-w clusters:* Seven lines with different numbers of *P-lacZ-white* elements (*P*[*lacZ*$^{P/I.W}$ *w*$^{+mC}$ *ampR ori = lacw*]; Bier *et al.* 1989) located at cytological site 50C on the second chromosome (Dorer and Henikoff 1994, 1997). The *P-lacZ-white* construct is 10,691 bp long, contains the *P-lacZ* translational fusion at position 587 of the *P* sequence, and is marked by the mini-*white* gene (Figure 1A). The *white* gene, used initially as a transformation marker, is also used as a reporter for the level of repression of the transgenes. Variegating clusters of transgenes were first generated at cytological site 92E (Dorer and Henikoff 1994). Mobilization of a tandem duplication at this site, by the *P*-element transposase, led to the recovery



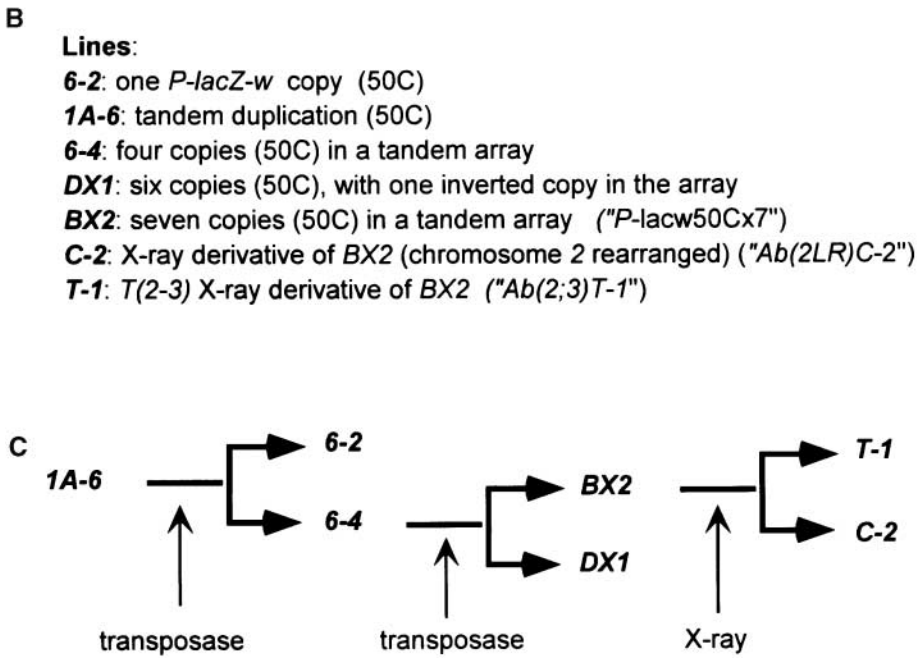




FIGURE 1.—Nomenclature, structure, and origin of the transgene clusters. (A) The autonomous *P* element is shown as well as the *P-lac-w* transgene present in the clusters. The arrowheads indicate the 31-bp inverted terminal repeats of the *P* element. The *Hin*dIII restriction sites used in Southern blot analysis are indicated as H. (B) The nomenclature and description of the lines is given. (C) The relationships among the different lines is given along with the agents used to modify them.

of a tandem transgene duplication at cytological position 50C in the middle of the euchromatic region of the right arm of a nonrearranged second chromosome (line *1A-6*; DORER and HENIKOFF 1994). Flies hemizygous for the 50C duplication showed wild-type red eye pigmentation with no mottling at any temperature. Secondary mobilization of the tandem duplication step with transposase activity (Figure 1C) led to the excision of one of the two elements, thereby creating a strain with a single insertion at 50C (line *6-2*, hemizygous with orange eye pigmentation). In the same mobilization experiment, increased transgene copy number at 50C was also recovered (four copies, line *6-4*). At 20°, the *6-4* hemizygous flies showed red eye pigmentation with some white spots. Further mobilization led to strains with six or seven transgene copies at 50C (lines *DX1* and *BX2*, respectively). At 20°, these strains also showed red pigmentation at 20° with unpigmented spots. Finally, X-ray mutagenesis was performed on *BX2* males to induce chromosomal rearrangements (DORER and HENIKOFF 1997). The *C-2* and *T-1* lines thus derived showed large unpigmented sectors in otherwise red eyes, indicating very strong enhancement of the variegating repression on the *white* gene in the *P-lacZ-white* transgenes. *C-2* has a rearrangement involving the second chromosome (*2R* arm), whereas *T-1* has complex chromosomal rearrangements, including translocations between the second and the third chromosomes. The single insertion at 50C (*6-2*) is fully fertile and can be maintained in the homozygous state. In contrast, all clusters of insertions are homozygous lethal or sterile and must be balanced with a *Cy*-marked second chromosome. After overnight staining, LacZ expression is detected in the follicle cells of all lines, presumably because of a position effect at 50C (see the RESULTS and Figure 3L). No staining is detected in nurse cells or in oocytes. The main properties of these lines are summarized in Figure 1, B and C.

*P-605: y w; P{w[+mC] = lac-w}/CyO.* This line carries an insertion of a *P-lacZ-white* enhancer trap at 39E on chromosome *2*.

*w; T-1/Cy Roi; P-Co-1:* This line carries both the *T-1* cluster

on the second chromosome and the *P-Co-1* insertion on the third chromosome. The balancer of the second chromosome carries two dominant markers, *Curly* and *Roi*.

*$w^{m4}$; Su(var)(205)$^5$/Cy; +/+:* This stock carries a *Su(var)205* mutant allele, which encodes only the first 10 amino acids of the HP1 protein (Eissenberg *et al.* 1992). This allele strongly impairs the repression ability of *Lk-P*(1A) (Ronsseray *et al.* 1996, 1997).

**Making of the pCo plasmid:** The *otu* promoter was amplified by PCR from the pCOG plasmid (Robinson and Cooley 1997) with oligonucleotide primers containing additional restriction sites at the 5′ end (*Eco*RI and *Bam*HI, underlined) for subcloning purposes. Primers used were 5′-CGGAATTC ATAGTCGTTGCG-3′ and 5′-CGGGATCCGTTAACAATTAA ATTAAC-3′. The PCR-amplified product was then digested with *Eco*RI and *Bam*HI and cloned into pCaSpeR-AUG-β-gal (Thummel *et al.* 1988) digested with the same enzymes. This new construct was named pCo and will be figured in the results section. Transgenic lines were obtained as previously described in Boivin and Dura (1998).

**Southern blot analysis:** Genomic DNA was extracted as described previously (Boivin and Dura 1998). A total of 25 μl of the DNA preparation was digested to completion with *Hin*dIII. The DNA was then size fractionated on a 1% agarose gel and transferred to a reinforced cellulose nitrate membrane. The blot was hybridized with the 650-bp *Bcl*I fragment from the 5′ region of the *white* locus, [$^{32}$P-dCTP]-labeled by random priming [Boehringer Mannheim (Indianapolis) High Prime kit] in 6× SSC, 5× Denhardt's solution, 0.5% SDS at 65° for 12 hr, and then washed three times for 10 min with 2× SSC, 0.1% SDS at 65°. The autoradiogram was visualized on RX Fuji medical X-ray film.

**Gonadal dysgenesis assay:** The ability of lines to repress the occurrence of GD sterility was measured by the A* assay (Kidwell *et al.* 1977). Females of the tested line were crossed with strong P males (Harwich-2). For each testcross, 3–10 pairs were mated *en masse* and placed at 29°. Parents were discarded after 3 days of egg laying. Approximately 2 days after the onset of eclosion, $G_1$ progeny were collected and allowed to mature for 2 days at room temperature. A total of 25 to 50 $G_1$ females were then taken at random for dissection. Dissected ovaries were scored as unilaterally dysgenic (S1 type) or bilaterally dysgenic (S0 type; Schaeffer *et al.* 1979). The frequency of gonadal dysgenesis was calculated as %GD = %S0 + ½%S1 and will be referred to as percentage of GD A* (%GD A*). The M cytotype, which allows *P* elements to be active, results in a high percentage of GD A*, whereas the P cytotype, which represses *P*-element activity, results in a low percentage of GD A* (<5%). An intermediate percentage indicates incomplete repression.

**Repression of *P-lacZ* expression in ovaries:** $G_1$ females derived from a cross between individuals from a tested line and individuals from lines bearing a *P-lacZ* transgene were examined by staining for their ability to repress *P-lacZ* expression in the ovaries. Staining of ovaries to detect *lacZ* expression was performed as described in Lemaitre *et al.* (1993). High lacZ activity indicates an absence of *P* repression and low activity indicates strong *P* repression (Lemaitre *et al.* 1993).

**Assay of the *Su(var)205* mutant allele effect on the ability of *T-1* to repress a transgene in the germline:** Females with the genotype *w; T-1/Cy Roi; P-Co-1* were crossed with *$w^{m4}$; Su(var)205$^5$/Cy; +/+* males at 25°. $G_1$ females bearing both the *T-1* and the *P-Co-1* transgenes (phenotype [Roi$^+$]) as well as the *Su(var)205$^5$* mutant allele (phenotype [Cy$^+$]) were compared to their sisters [Cy Roi$^+$] for lacZ expression in ovaries. As a positive control of lacZ expression, $G_1$ females that inherited the *P-Co-1* insertion but not the cluster (phenotype [Cy Roi]) were also stained.


Figure 2.—Southern blot analysis of transgene clusters. *Hin*dIII digestion of genomic DNA. The blot was probed with a 650-bp PCR-amplified fragment corresponding to the *Bcl*I fragment located 5′ to *white* (Boivin and Dura 1998).

**Statistical analysis:** The repression abilities as measured by GD sterility percentages were compared using the nonparametric Mann-Whitney test performed on A* assay replicates.

## RESULTS

**Molecular comparison of the clusters:** X-ray irradiation, performed on the line *BX2*, led to the recovery of the *T-1* line (Dorer and Henikoff 1997). This line carries chromosomal rearrangements that are thought to be responsible for the enhancement of the variegated repression of the *white* marker. We compared the structure of the clusters in the *BX2* and *T-1* lines to determine whether X-ray irradiation had also altered the structure of the cluster. Genomic DNA was digested by *Hin*dIII, which cuts at position 40 inside the 5′ *P*-element sequence and at positions 10,272 and 10,453 in the pUC sequence of the *P-lac-w* transgene. The Southern blot was probed with a 650-bp fragment from the 5′ side of the *white* gene. This is expected to hybridize to a 10.2-kb internal fragment from an intact *P-lac-w* transgene. Figure 2 shows that *6-2* and *6-4* DNAs produce the full-size *Hin*dIII fragment. However, *6-4* also produces a second shorter fragment corresponding to a deleted transgene. This deletion is known to remove most of the pUC sequences and the 3′ polylinker from one *P-lac-w* element, but the *lacZ* and *white* sequences appear intact (D. Dorer, personal communication). *BX2* shows the same pattern as *6-4*, indicating the presence of both intact transgenes and of transgenes deleted in the same way. Finally, *T-1* shows the same pattern as *BX2*. Thus, at this level of resolution, this indicates that the X rays used on *BX2* to induce the chromosomal rearrangements present in *T-1* did not alter the internal structure of the transgene cluster in *T-1*.

***P* repression capacities of the clusters:** The ability of the lines to repress *P*-element activity was tested with four different assays.

*(a) Repression of GD A* sterility (Table 1, left):* $G_1$ females reared at 29° and generated from the cross of M females (Canton$^y$) with strong P males (Harwich-2) are sterile due to atrophy of the gonads (100% gonadal dysgenesis). Conversely, females with the P cytotype [Harwich-2 or *Lk-P*(1A)] crossed with strong P males produce $G_1$ females with trivial percentages of GD sterility (0.3 and 1.1%, respectively) due to the repressive component

**TABLE 1**

**Repression of *P*-induced gonadal dysgenesis**

| | ♀ Tested × ♂ Harwich-2 ↓ 29° (%GD) | | | ♀ *P-1152* × ♂ Tested ↓ 20° ♀ $G_1$ × ♂ Harwich-2 ↓ 29° (%GD) | | |
|---|---|---|---|---|---|---|
| Tested line | *m* | *s* | *n* | *m* | *s* | *n* |
| Canton$^y$ | 100 | 0.0 | 10 | 99.6 | 0.7 | 13 |
| Harwich-2 | 0.3 | 0.6 | 5 | 5.3 | 4.8 | 8 |
| *Lk-P*(1A) | 1.1 | 2.0 | 35 | 33.1 | 20.3 | 14 |
| *P-1152* | 96.3 | 3.5 | 13 | 96.3 | 3.5 | 13 |
| *P-605* | 100 | 0.0 | 9 | 99.5 | 0.9 | 10 |
| *6-2* | 100 | 0.0 | 10 | 99.2 | 1.4 | 15 |
| *1A-6* | 100 | 0.0 | 8 | 93.5 | 4.7 | 11 |
| *6-4* | 99.8 | 0.7 | 9 | 56.1 | 27.6 | 12 |
| *DX1* | 100 | 0.0 | 9 | 78.4 | 14.2 | 12 |
| *BX2* | 100 | 0.0 | 9 | 73.5 | 12.9 | 12 |
| *C-2* | 100 | 0.0 | 8 | 84.8 | 8.2 | 12 |
| *T-1* | 74.3 | 16.0 | 20 | 21.0 | 13.0 | 12 |

(Left) Ability of lines to repress *P*-induced gonadal dysgenesis: replicate sets of 3–10 females of the tested lines were crossed with 5 Harwich-2 males (A* assay). Progeny were allowed to develop at 29°. For each replicate test cross, 50 to 100 ovaries were examined in the progeny. *m*, *s*, and *n* are, respectively, the mean percentage of GD sterility, the standard deviation among replicates, and the number of replicates performed. (Right) Combination effect between the tested lines and a *P-lacZ* telomeric insertion. The *P-1152* line carries a *P-lacZ* insertion at 1A. *P-1152* females were crossed with males of the tested lines at 20°. Replicate sets of 3–5 $G_1$ females [$Cy^+$], carrying both the *P-1152* transgene and the tested cluster, were crossed with 5 Harwich-2 males (A* assay) at 29° and the percentage of GD sterility was measured.

transmitted by the P females. Lines with a single *P-lacZ* do not significantly repress GD sterility (see *P-1152*, *P-605*, and *6-2*). Among lines carrying tandem arrays of *P-lac-w* transgenes, only one represses GD sterility. The exception is the *T-1* line, which partially but significantly represses the occurrence of dysgenic sterility (74.3% GD). The comparison of the *T-1* repression ability with that of the M control (Canton$^y$) is highly significant ($P < 0.01$) as judged by the Mann-Whitney test. In addition, for some of the GD assay replicate tests (16 out of 20) performed with *T-1*, the $G_1$ females bearing the cluster [$Cy^+$] and their siblings lacking the cluster [Cy] were dissected separately. Similar percentages of GD sterility were found for the two kinds of progeny: [$Cy^+$], $m = 77.2\%$, $s = 15.5$, $n = 16$; [Cy], $m = 79.3\%$, $s = 16.6$, $n = 16$. This shows that $G_1$ females that did not inherit the *T-1* cluster nonetheless inherited the capacity to repress GD.

*(b) Combination effect between tested lines and a P-lacZ telomeric insertion:* The combination of a telomeric transgene with regulatory *P* elements results in a combination effect, *i.e.*, an enhancement of the repression ability of the regulatory *P* elements (Ronsseray *et al.* 1998). We tested if the combination of such a telomeric transgene with a transgene cluster leads to enhancement of the ability of the cluster to repress GD sterility. We crossed *P-1152* females, which carry a *P-lacZ* element at the telomere of the *X* chromosome, with males of the lines under test and measured the repression abilities of the $G_1$ females by crossing sets of these females with P-strain males (Harwich-2) at 29° (Table 1, right). As expected, the cross of *P-1152* females with M males (Canton$^y$) produced $G_1$ females with no repression capacity (99.6% GD). As shown previously (Ronsseray *et al.* 1998), crossing these females with *Lk-P*(1A) males produces females with strong repression ability (33.1% GD). However, no significant enhancement of the repression ability of $G_1$ females bearing the *P-1152* telomeric transgene with a single *P-lac-w* (*P-605, 6-2*) or with two *P-lac-w* copies (*1A-6*) was observed. By contrast, all the other clusters with a higher number of transgenes showed enhanced repression of GD sterility. The maximum was obtained with *T-1* (21.0% GD).

Taken together, these results show that a cluster (*T-1*) can elicit a significant repression of dysgenic sterility by itself and that the combination of a cluster (four copies or more) with a telomeric *P-lacZ* leads to enhanced levels of repression. In addition, strong repression (21.0% GD) can be reached by combining *T-1* with *P-1152*.

*(c) Repression of a P-lacZ element in the germline:* The *BQ16 P-lacZ* insertion was used in this assay. In an M context, this insertion is strongly expressed in the nurse cells and in the oocyte (Canton$^y$, Figure 3A). $G_1$ females

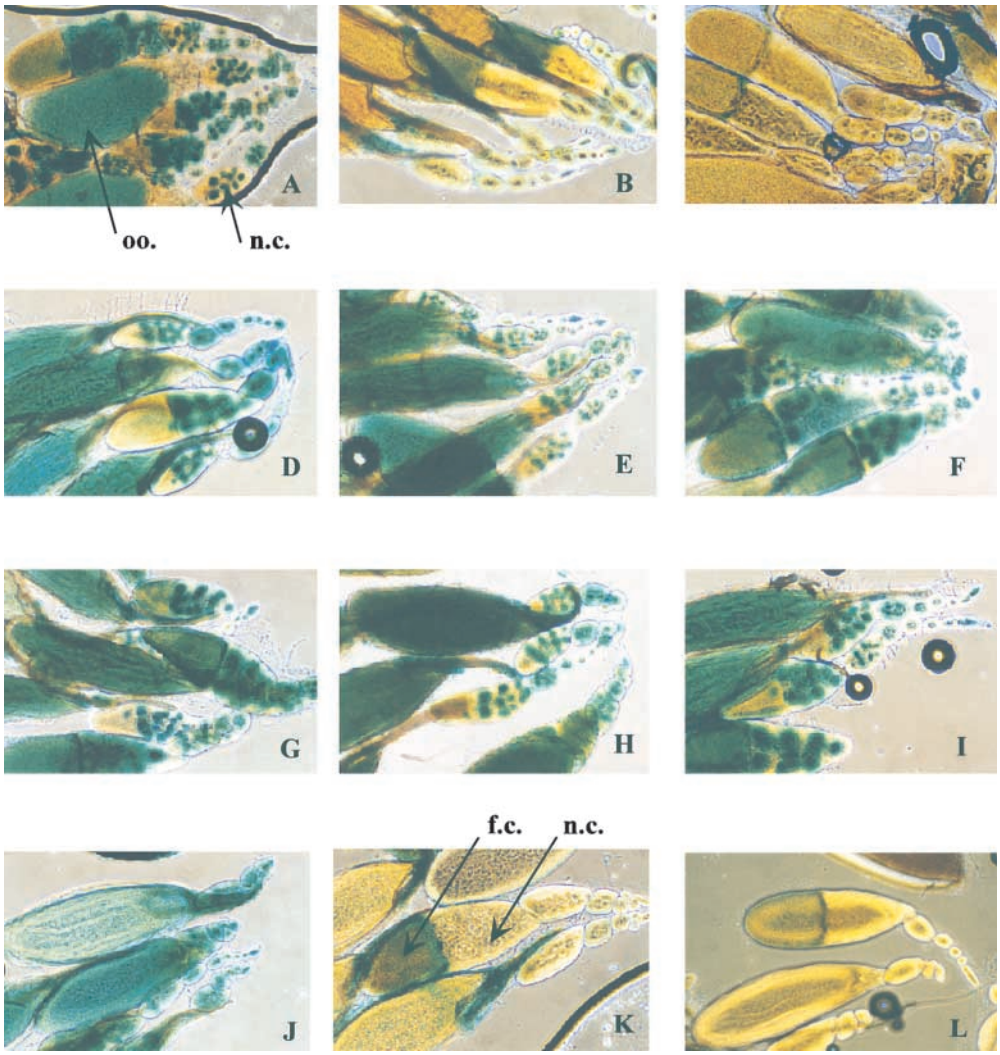



FIGURE 3.—(A–K) Repression assay for *P-lacZ* in *BQ16*. The *BQ16* insertion expresses β-galactosidase in the ovarian germline tissues (nurse cells and mature oocyte). Each picture shows the staining of ovaries of $G_1$ females from the cross of tested females with *BQ16* males, reared at 20°. Tested females: (A) Canton$^y$, (B) *Lk-P*(1A), (C) *P-1152*, (D) *P-605*, (E) *6-2*, (F) *1A-6*, (G) *6-4*, (H) *DX1*, (I) *BX2*, (J) *C-2*, (K) *T-1*. Staining reactions were performed overnight in the same experiment to allow a visual comparison. (L) Overnight staining of ovaries from the *T-1* line performed separately. n.c., nurse cells; oo., oocyte; f.c., follicle cells.

from the crosses between *Lk-P*(1A) females and *BQ16* males strongly repressed *lacZ* activity in the germline (Figure 3B). However, weak staining in follicle cells (due to *BQ16*) is still detected. Such a restriction of *Lk-P*(1A) repression ability to germline tissue has previously been shown (RONSSERAY *et al.* 1991; LEMAITRE *et al.* 1993). Females carrying the *P-1152* transgene also strongly repressed *BQ16* expression (Figure 3C). This result is consistent with that of ROCHE and RIO (1998) and MARIN *et al.* (2000) in which *P-1152* repressed a *P-lacZ* transgene in the germline. Among the other lines tested, *T-1* completely repressed *BQ16* expression in the germline (nurse cells; Figure 3K) whereas other clusters did not (Figure 3, D–J), except for slight repression with *C-2*. Therefore, like *P-1152*, *T-1* shows the ability to silence, *in trans*, a homologous transgene specifically in the germline.

*(d) Repression of various P-lacZ insertions:* We tested whether germline silencing by the *T-1* line is restricted to the *BQ16* insertion or whether it can be extended to other transgenes. We tested four other *P-lacZ* insertions that are expressed in the germline. *ABOO*, *BC69*, and *P-1039* are located on the second chromosome and *R3-29* is located on the *X* chromosome. The expression pattern of each insertion was determined in the ovaries of female progeny from crosses between males from lines bearing these insertions and Canton$^y$ females (Figure 4, A, C, E, and G). In parallel, the same males were crossed with *T-1* females (Figure 4, B, D, F, and H). In each case, the *T-1* cluster completely repressed the activity of the *P-lacZ* in the nurse cells. *T-1* did not show any repression of *ABOO* and *P-1039* expression in somatic follicle cells (Figure 4, D and H).

Finally, we used a different construct as a target to test whether the proximity of the promoter driving LacZ expression and *P* sequences is a necessary condition for silencing to occur. The *P-Co-1* line contains a transgene named *P-Co* (see MATERIALS AND METHODS and Figure 5E) in which *lacZ* is not fused in frame with the 5′ *P* sequence but is driven by the *otu* promoter. In this construct, the *otu* promoter is located at a central position between *white* and *lacZ*, *i.e.*, >3 kb away from the *P*

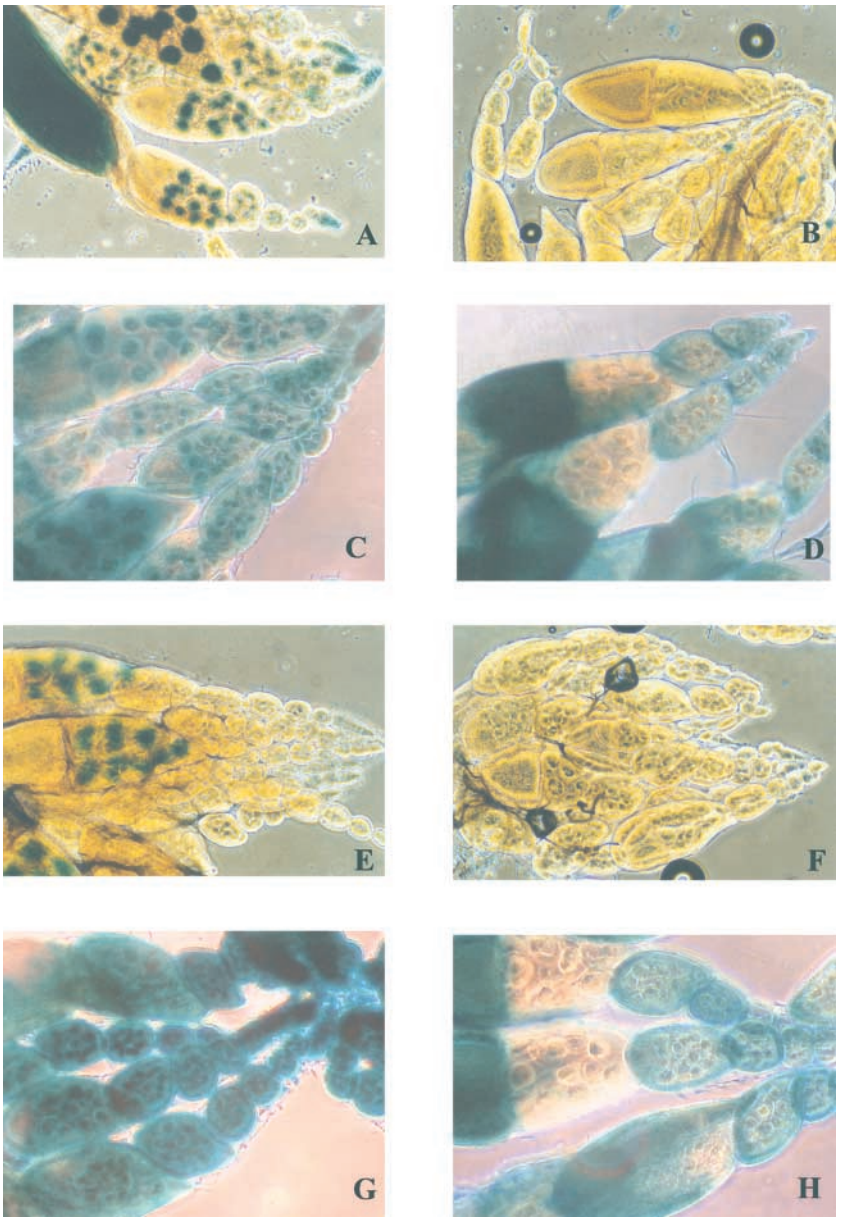



Figure 4.—Ability of *T-1* to repress several transgenes at different sites. Four different *P-lacZ* transgenes expressed in the female germline were tested as targets. (A–B) *BC69*, (C–D) *ABOO*, (E–F) *R3-29*, (G–H) *P-1039*. In each case, *P-lacZ* males were crossed with Canton$^{y}$ females at 20° (left) or *T-1* females (right). For each line tested, staining reactions were performed overnight in the same experiment to allow a visual comparison.

sequences. Because of the *otu* promoter, LacZ is strongly expressed in the germline (Figure 5A). The transgene is completely repressed by *P-1152* and *T-1* (Figure 5, B and C). However, *P-Co-1* is not repressed by the full-sized *P* elements of *Lk-P*(1A) (Figure 5D).

Taken together, these results show that the *T-1* cluster located at 50C on the second chromosome can repress germline expression of another *P-lacZ*, located either on the same chromosome at a different site (*BC69* in 35C) or on a different chromosome (*R3-29*, on the *X* chromosome; *P-Co-1* on chromosome *3*). Therefore, the *T-1* cluster causes homologous transgene silencing on a target located at a nonallelic position. In fact, silencing was found with all the targets tested, regardless of their insertion site (and therefore the neighboring enhancer) and regardless of their structure with respect to the position of the promoter driving LacZ expression. This last point suggests that this silencing can result not only from the *P*-element sequences shared by the silencer and the target, but also from the shared *lacZ* sequences. Such a mechanism is consistent with the lack of significant repression of *P-Co-1* by *Lk-P*(1A) *P* elements, which have no lacZ sequences.

**Maternal effect of the *trans*-silencing:** The regulatory properties of the naturally occurring autonomous *P* elements at 1A show a strong maternal effect for both the repression of dysgenic sterility (Ronsseray *et al.* 1991, 1993) and the repression of a *P-lacZ* element (Lemaitre *et al.* 1993). We tested whether the repression abilities of a telomeric *P-lacZ* insertion (*P-1152*) and of the *T-1* cluster also show a maternal effect. For these two lines, the two reciprocal crosses were performed with *BQ16*

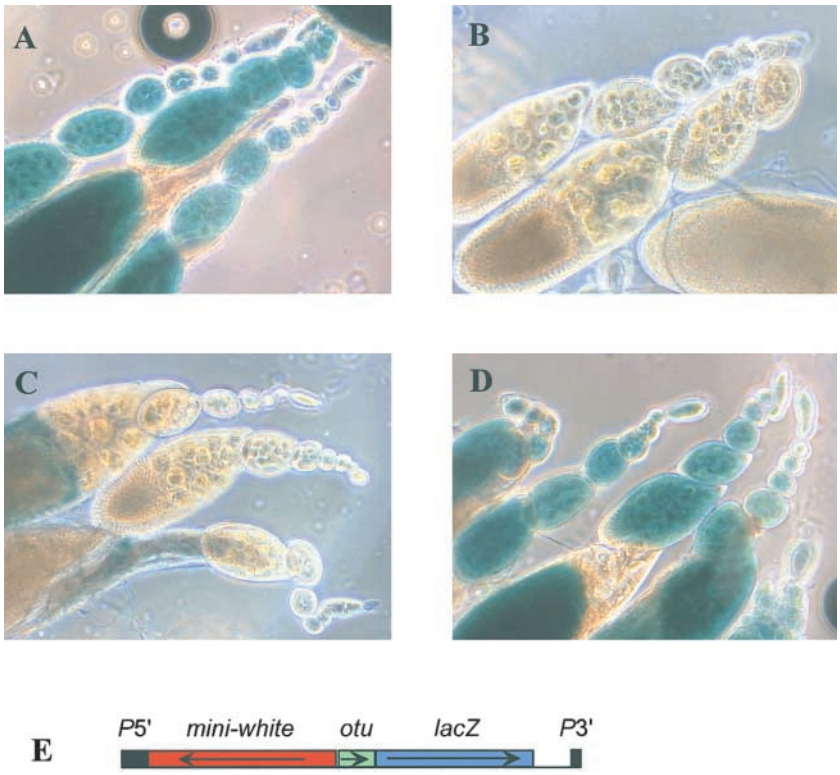



FIGURE 5.—Ability to repress a transgene driven by a heterologous promoter. The *P-Co-1* line containing the *P-Co* transgene, in which *lacZ* is under the control of the *otu* promoter, was tested as a target. Each picture shows the staining of ovaries of $G_1$ females from the cross of tested females with *P-Co-1* males, reared at 25°. (A) Canton$^y$, (B) *P-1152*, (C) *T-1*, (D) *Lk-P*(1A). Staining reactions were performed overnight in the same experiment. (E) Structure of the *P-Co* transgene.

at 20° and $G_1$ females were stained overnight for LacZ activity. Figure 6 shows that the repression ability of both *P-1152* and *T-1* involves a strong maternal effect. Repression is detected only when the *P-1152* or *T-1* transgenes are maternally inherited. The *trans*-silencing properties of *T-1* therefore present strong similarities with that of the telomeric *P*-reporter *P-1152*.

**Assay of the *Su(var)205* mutant allele effect on the *T-1* repressive properties:** We have tested whether the *trans*-silencing induced by *T-1* is affected by the dose of *Su(var)205* that encodes HP1. Indeed, it is known that the variegating phenotype of the clusters is suppressed by reducing the dose of HP1 (DORER and HENIKOFF 1994). In addition, HP1 was shown to bind the clusters in 50C with an intensity correlated to the number of transgenes (FANTI *et al.* 1998). Ovaries from females having the *P-Co-1* transgene and the *T-1* cluster and from sibling females having the same chromosomal complement but heterozygous *Su(var)205*$^5$/*Su(var)205*$^+$ were compared after overnight staining for lacZ. The two kinds of females showed a strong repression in *P-Co-1* expression, similar to that observed in Figure

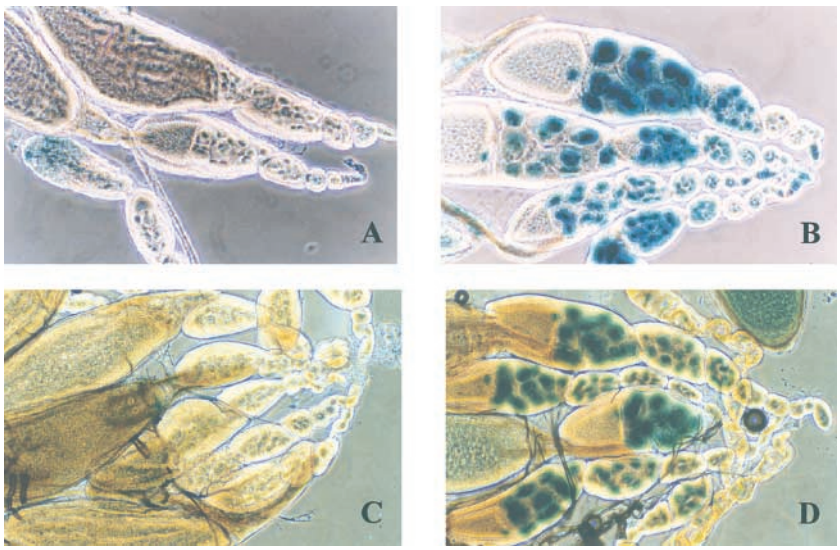



FIGURE 6.—Maternal effect assay on *trans*-silencing. Pictures show the overnight staining of ovaries of $G_1$ females reared at 20°. (A) *P-1152* females × *BQ16* males; (B) *BQ16* females × *P-1152* males; (C) *T-1* females × *BQ16* males; (D) *BQ16* females × *T-1* males. For each line tested (*P-1152* and *T-1*), staining reactions were performed overnight in the same experiment.

5C, whereas the control $G_1$ females having the *P-Co-1* transgene without the *T-1* cluster showed a strong staining similar to that observed in Figure 5A. Therefore, the presence of the *Su(var)205*$^5$ mutated allele did not impair the repression ability of *T-1*.

## DISCUSSION

**Repression of *P*-induced dysgenic sterility in the absence of a repressor-encoding *P* element:** The *P*-element family is able to establish the conditions for repression of its own activity. Transformation of an M genome with *P* elements can induce the development of *P* repression over generations (Anxolabéhère *et al.* 1987; Daniels *et al.* 1987; Preston and Engels 1989). The ability of a given *P* element to establish the P cytotype depends on its structure. Various studies have allowed the identification and characterization of some regulatory *P* elements. These include autonomous *P* elements (Ronsseray *et al.* 1991, 1996; O'Hare *et al.* 1992), copies lacking the last intron (Robertson and Engels 1989; Misra and Rio 1990; Gloor *et al.* 1993; Misra *et al.* 1993), and copies with a central deletion, termed *KP* elements (Black *et al.* 1987; Higuet *et al.* 1992; Rasmusson *et al.* 1993; Andrews and Gloor 1995). In each case, the *P* copy is able to encode a repressor polypeptide that is thought to repress *P*-element activity by transcriptional repression (Lemaitre and Coen 1991; Lemaitre *et al.* 1993) or by protein-protein interactions (Andrews and Gloor 1995).

It appears also that the genomic location of the *P* element is crucial with regard to its repression capacity. Two copies of autonomous *P* elements at the *X* chromosome telomere exhibit strong repression ability, as strong as that of a P strain carrying 50 *P* elements scattered throughout the genome (Ronsseray *et al.* 1996). In addition, when located at this site, a *P-lacZ* transgene, which almost certainly does not encode a repressor polypeptide, nonetheless has some repression ability (Roche and Rio 1998; Ronsseray *et al.* 1998): it is able to repress *in trans* a *P-lacZ* at another site in the genome, and it does this specifically in the germline. Only telomeric locations appear to confer such repression ability to *P*-reporter transgenes. At telomeric sites, *P* insertions are inserted in subtelomeric heterochromatin (Karpen and Spradling 1992; Ronsseray *et al.* 1996; Roche and Rio 1998; Cryderman *et al.* 1999) and no LacZ expression can be detected in the germline even after overnight staining (data not shown). The repression capacities of telomeric *P* insertions have thus been proposed to result from their ability to interact at the chromatin level with euchromatic targets (Roche and Rio 1998; Ronsseray *et al.* 1998). Alternatively, it can be proposed that they produce aberrant RNAs that lead to RNA interference. However, among the three telomeric transgenes that cause a *trans*-silencing effect, none can repress significantly, by themselves, the dysgenic sterility induced by a strong P strain (Ronsseray *et al.* 1998).

The clusters of *P-lac-w* transgenes analyzed here were suspected to share some properties with telomeric transgenes because they are also heterochromatinized due to their repeated structure (Dorer and Henikoff 1994, 1997; Fanti *et al.* 1998). The present study shows that one of them, *T-1*, has a significant ability to repress the GD sterility induced by a strong P strain (25% of the ovaries develop in the GD A* assay). Furthermore, when combined with a telomeric *P-lacZ* (*P-1152*), clusters with four copies or more have significant GD repression abilities. In the case of *T-1*, the repression ability is strongly enhanced (79% of developed ovaries). One case of *P*-induced GD sterility repression, without the production of any *P* repressor, was described previously (Rasmusson *et al.* 1993). A 0.5-kb *P* element called *SP*, which encodes only the first 14 amino acids of the transposase, was found to have the ability to repress the GD sterility induced by a weak P strain. Our results extend this result and show that the capacity to repress the dysgenic sterility induced by a strong P strain can be established in the absence of a *P*-encoded polypeptide repressor by, for example, combining a *P* transgene in subtelomeric heterochromatin and the *T-1* cluster.

**Comparison between *BX2* and *T-1*:** Both the *T-1* and *BX2* clusters have repressive properties in the occurrence of dysgenic sterility when combined with the telomeric *P-lacZ* transgene in the *P-1152* line. However, *T-1* has stronger abilities than *BX2*. In particular, *T-1* induces strong *trans*-silencing of a *P-lacZ* transgene in the germline, whereas *BX2* does not. The *T-1* line was derived upon X-ray treatment of the *BX2* line (Dorer and Henikoff 1997). What accounts for the different properties of these two lines? Southern blot analysis showed that the cluster itself is not rearranged in the *T-1* line when compared to the *BX2* line (Figure 2). We also carried out *in situ* hybridizations on polytene chromosomes of the *T-1* line with a *P*-element probe. Chromosomal rearrangements, including translocations, led to uninterpretable cytology, but only one *P*-hybridizing site was found (data not shown). This indicates that the cluster is still the only *P* sequence in the genome; it has not been divided into pieces and scattered throughout the genome. In addition, the site appears to be located in a median section of a chromosome segment: it is not at a neotelomeric position or close to a centromere.

If we compare the variegating eye color phenotype of the *T-1* and *BX2* lines in flies, the *T-1* cluster appears to be much more heterochromatinized than the *BX2* cluster. Although the *in situ* hybridization label indicates that the *T-1* cluster is not close to a centromere, it remains possible that the X-ray mutagenesis performed on *BX2* flies resulted in the translocation of a heterochromatic block in the vicinity of the cluster of transgenes. Precedence for this exists in the case of a translo-

cation resembling that which occurred at the *brown*$^{D}$ allele, which was generated upon insertion of a large block (1 Mb) of centric heterochromatin inside the *brown* gene on the second chromosome (Slatis 1955). In the *T-1* line, a translocation of heterochromatin near the cluster could mimic the situation that occurs when a transgene is in subtelomeric heterochromatin, thereby conferring an increased *P* repression ability.

**What is the mechanism of *P* repression by transgene clusters?** Does the repression ability of *T-1* involve a diffusible product of the cluster, an altered structure of the chromatin, or both? Under the diffusible product hypothesis, this product is probably RNA rather than protein since the *T-1* cluster has no detectable expression in the nurse cells (Figure 3L). RNA interference may occur in Drosophila (Kennerdell and Carthew 1998; Aravin *et al.* 2001) and has already been proposed to play a role in transposable element regulation (Jensen *et al.* 1999a,b; Aravin *et al.* 2001). Regarding *P* repression, Simmons *et al.* (1996) have shown that expression of antisense RNAs can lead to partial *P* repression. In addition, cosuppression has also been shown to exist in Drosophila both at the transcriptional and post-transcriptional levels (Pal-Bhadra *et al.* 1997, 1999; U. Bhadra, M. Pal-Bhadra and J. Birchler, personal communication). In the experiments performed to assay the ability of *T-1* to repress GD sterility, the $G_1$ females bearing the cluster and their sisters that carry the *Cy* balancer chromosome (and thus no *P* transgene) produced similar percentages of GD sterility, suggesting that a diffusible product is deposited in the oocyte and that this product is responsible for the observed repression of *P* activity in the offspring. This product could also explain the maternal effect of the *trans*-silencing induced by *T-1*.

However, it remains possible that a change in the chromatin at the level of the cluster contributes to the system. In such a model, telomeric sequences and the cluster could pair with homologous sequences in the genome and inactivate them *in trans*, either by *trans*-heterochromatinization or by relocalization of the sequences to another compartment of the nucleus. Alternatively, altered chromatin structure could be responsible for the production of aberrant RNAs with the ability to induce RNA interference (Voinnet *et al.* 1998). A good candidate for the induction of a change in the state of the chromatin could be HP1 since it has been shown to bind *P-lac-w* clusters at 50C (Fanti *et al.* 1998). Surprisingly, we show here that the ability of *T-1* to induce *trans*-silencing is not affected by the dose of HP1, suggesting that the normal dose of HP1 is not a necessary condition. Furthermore, HP1 binding on the clusters of *P-lac-w* transgenes is not a sufficient condition because clusters with two to seven copies, but with no rearrangements, are strong binding sites for HP1 on polytene chromosomes (Fanti *et al.* 1998), although they do not induce *trans*-silencing. An alternative explanation is that an effect of a *Su(var)205* mutated allele on *trans*-silencing requires maternal transmission of the mutated allele. Here, the *Su(var)205*$^{5}$ allele was introduced paternally because it is not possible to introduce it maternally since this would require recombination between the rearranged *T-1* chromosome and a nonrearranged chromosome *2*, carrying the *Su(var)205* mutated allele.

***Trans*-silencing effect: toward a general homology-dependent phenomenon:** We have previously shown that the ability of a telomeric *P* insertion to repress the expression of a *P* transgene in the germline requires some length of homology between the two *P* sequences (Marin *et al.* 2000). Indeed, a naturally occurring, defective *P* element at 1A [namely, *NA-P*(1A)], deleted for the first 871 bp at the 5′ side, is unable to repress a classical *P-lacZ* construct. In that case, only 233 bp are present both in the deleted *P* element and the *P* transgene. However, *NA-P*(1A) is able to repress a particular *P-lacZ* construct (namely, *PLH3*). In this case, 1.8 kb are present both in the deleted *P* element and the *P* transgene. In the present work, the telomeric full-sized *P* elements of the *Lk-P*(1A) line do not repress *P-Co-1* (Figure 5). Thus, the 800 bp of the *P* sequence (587 bp at the 5′ side and 233 bp at the 3′ side), present both in *Lk-P*(1A) and *P-Co-1*, are not sufficient to induce silencing. However, the *T-1* cluster and the *P-1152* telomeric *P-lacZ* insertion strongly repress the *P-Co-1* insertion. These results strongly suggest that the capacity of *T-1* or *P-1152* lines to suppress *P-Co-1* expression results not only from the 800 bp of *P*-element sequences shared by the silencer and the target, but also from the shared *lacZ* sequence (~3 kb), suggesting that this kind of suppression can be induced by a non-*P*-element sequence. We are currently investigating whether a *hobo-lacZ* transgene, expressed in the germline, can be repressed by a telomeric *P-lacZ*, due simply to the *lacZ* sequence homology. *Trans*-silencing induced by telomeric sequences could in fact represent a general phenomenon involved in some cellular functions of the germline. Such a suppression might have been adopted by the *P*-element family after its recent invasion of natural *D. melanogaster* populations, allowing its own regulation.

We thank Doug Dorer, Manika Pal-Bhadra, Utpal Bhadra, and James Birchler for personal communications. We thank J. L. Couderc for providing *P-lacZ* lines. We thank A. M. Pret and S. Charlat for their help in the preparation of the manuscript. We thank the communicating editor M. J. Simmons and an anonymous reviewer for their valuable comments and suggestions on the manuscript. S.R. thanks Jocelyne Besombe for helpful suggestions. We thank the Bloomington and Umea Stock centers for providing stocks and FlyBase for helpful informations. This work was supported by the Centre National de la Recherche Scientifique (UMR 7592), by the program Génome, by the Association pour le Recherche sur le Cancer, and by the Universités Paris 6-Pierre et Marie Curie and Paris 7-Denis Diderot (Institut Jacques Monod-UMR7592, Dynamique du Génome et Evolution). This work was carried out in compliance with the current laws governing genetic experimentation in France and in the United States.

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Communicating editor: M. J. Simmons