Repression of Hybrid Dysgenesis in *Drosophila melanogaster* by Combinations of Telomeric *P*-Element Reporters and Naturally Occurring *P* Elements

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ABSTRACT

In *Drosophila melanogaster*, hybrid dysgenesis occurs in the germline of flies produced by crosses between females lacking *P* elements and males carrying 25–55 *P* elements. We have previously shown that a complete maternally inherited repression of *P* transposition in the germline (P cytotype) can be elicited by only two autonomous *P* elements located at the *X* chromosome telomere (cytological site 1A). We have tested whether *P* transgenes at 1A, unable to code for a *P*-repressor, may contribute to the repression of *P* elements. Females carrying a *P-lacZ* transgene at 1A ["*P-lacZ*(1A)"], crossed with P males, do not repress dysgenic sterility in their progeny. However, these *P-lacZ*(1A) insertions, maternally or paternally inherited, contribute to *P*-element repression when they are combined with other regulatory *P* elements. This combination effect is not seen when the *P-lacZ* transgene is located in pericentromeric heterochromatin or in euchromatin; however a *P-w,ry* transgene located at the *3*R chromosome telomere exhibits the combination effect. The combination effect with the *P-lacZ*(1A) transgene is impaired by a mutant *Su(var)205* allele known to impair the repression ability of the autonomous *P* elements at 1A. We hypothesized that the combination effect is due to modification of the chromatin structure or nuclear location of genomic *P* elements.

THE *P*-transposable element has invaded natural *Drosophila melanogaster* populations over the last five decades (Kidwell 1983). After a transient period of dysgenesis, various mechanisms of repression have developed that are, depending on the population, maternally inherited (P cytotype) (Engels 1979, 1989) or biparentally transmitted (*P* susceptibility) (Black *et al.* 1987; Simmons *et al.* 1990). Of the 30–60 *P* copies present in a *D. melanogaster* P strain genome, one third are complete *P* elements (Bingham *et al.* 1982; O'Hare and Rubin 1983; O'Hare *et al.* 1992). These elements can produce the transposase required for their mobility (Karess and Rubin 1984; Rio *et al.* 1986) and are therefore autonomous. The other two thirds are defective in transposase synthesis (O'Hare *et al.* 1992); however these nonautonomous elements can be mobilized in *trans* by complete *P* elements (Engels 1984, 1989).

The repression ability of a *P* element depends on its structure and its insertion site (Robertson and Engels 1989; Misra and Rio 1990; Rasmusson *et al.* 1993). We have previously sought to identify regulatory *P* elements in the chromosomes of natural populations (Ronsseray *et al.* 1989; Biémont *et al.* 1990) because in these populations selection has probably retained efficient regulatory copies, *i.e.*, the right structure at the right site. We have genetically isolated, in a genomic context devoid of other *P* elements, a pair of autonomous *P* elements inserted at the telomere of an *X* chromosome (cytological site 1A on the polytene chromosomes) from a Russian natural population (Ronsseray *et al.* 1991). In the germline, this line ["*Lk-P*(1A)"] can completely repress *P*-induced hybrid dysgenesis, *P* transposition and transcription from a *P-lacZ* transgene (Ronsseray *et al.* 1991, 1996; Lemaitre *et al.* 1993). The repression ability of the *Lk-P*(1A) line is maternally transmitted. The *P* elements in this line are inserted in *Telomeric Associated Sequences* (*TAS*) (Ronsseray *et al.* 1996) described by Karpen and Spradling (1992). These sequences have the properties of heterochromatin, including the ability to silence transgenes inserted within them. In addition, the repression ability of the *P*(1A) elements is sensitive to the dosage of HP1, a nonhistone heterochromatin protein (James and Elgin 1986; Wustmann *et al.* 1989), because heterozygosity for a null allele of the *Su(var)205* locus, which encodes HP1, strongly impairs this ability (Ronsseray *et al.* 1996, 1997). HP1 has been shown to bind to centromeres and telomeres (James and Elgin 1986; James *et al.* 1989). The repression ability of the *Lk-P*(1A) line probably requires the expression of the *P*(1A) elements because genetic analysis reveals a maternally transmitted component of repression termed the "P pre-cytotype" (Ronsseray *et al.* 1993). The *P*(1A) elements are expressed because transposase activity can be detected genetically (Ronsseray *et al.* 1996) and

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transcripts can be detected by RT-PCR (Roche *et al.* 1995).

However, the repression ability of the *Lk-P*(1A) line might not result exclusively from the expression of its *P* elements. A case of homology-dependent transgene silencing has been reported in tobacco (Vaucheret 1993). In this study, a plant number "*271*" carried a telomeric silencer locus composed of multiple copies of two chimeric transgenes: an antisense *nitrite reductase* cDNA (*RiN*) under the control of the *35S* promoter of cauliflower mosaic virus (*CaMV 35Spro*) and a *neomycin-phosphotransferase* gene under the control of the *CaMV 19S* promoter. This telomeric silencing locus is able to silence any euchromatic gene under the control of the *35S* promoter (Vaucheret 1993, 1994). The authors hypothesize that the silencing capacity of the telomeric *271* locus is due to the location of this locus. The telomeric position would allow a rapid scan of the entire genome and the silencer locus could recognize and inactivate a target at any genomic location.

Further, the paper by S. E. Roche and D. C. Rio (1998, this issue) provides evidence in favor of such a model in Drosophila. They found that a *P-lacZ* element located at the tip of the *X* chromosome can exert a *trans*-silencing effect: it represses the expression of a *P-vasa-lacZ* construct (described in Roche *et al.* 1995) located on an autosome. The silencing effect appears independent of the insertion site of *P-vasa-lacZ*. They call this effect "*trans*-silencing." We have observed a similar *trans*-silencing also using a *P-lacZ* at 1A but with a *P-lacZ* transgene as the euchromatic autosomal target instead of *P-vasa-lacZ* (S. Ronsseray and L. Marin, unpublished results). Roche and Rio (1998) propose that the *trans*-silencing occurs via a pairing between the *P-lacZ* located at 1A and the *P-vasa-lacZ* located on the autosome.

However, when we cross these *P-lacZ*(1A) females with P males (for example Harwich-2 males), the female offspring are almost completely sterile [>98% gonadal dysgenesis (GD); see Table 1], showing that *P-lacZ*(1A) lines are not equivalent to *P*(1A) lines carrying autonomous *P* elements at 1A, probably because they lack a *P*-encoded repressor. We have tested whether telomeric *P-reporter* insertions combined with other *P* elements can elicit significant *P*-repression capacities in crosses in which neither component alone can do so. We show in this article, that a *P reporter* at the *X* or *3*R telomere can contribute to *P* repression when placed in hybrid females with P chromosomes that carry regulatory *P* elements. Telomeric *P* sequences, unable to encode a repressor, can therefore play a role in *P*-element repression.

MATERIALS AND METHODS

Drosophila stocks: Canton^{y} is a typical M line (Kidwell *et al.* 1977) containing no *P* elements and marked with a spontaneous allele of *yellow*. Sevelin and Oregon are two M lines. *Muller5* is an M line with the *Basc* balancer chromosome, marked with *Bar* and w^{a} (Lindsley and Zimm 1992). *E24* is a line deriving from *Lk-P(1A)* that has only one of the two *P* elements at 1A (Ronsseray *et al.* 1996). *Exe24* is a line deriving from *E24* that has lost the *P*(1A) element: it is therefore a neo-M line. *Ch-P*(1A) is a line with a single autonomous *P* element at 1A from a French natural population (Ronsseray *et al.* 1996). *Saltillo-P*(1A) [abbreviated "*Salt-P*(1A)"] is a line with a defective *P* element at 1A deriving from a Mexican population (L. Marin and S. Ronsseray, unpublished results). Harwich-2 is a P line; the subline used here shows more than 80 *P* labels by *in situ* hybridization (11/12 larvae tested had no *P* label at 1A). It has an unidentified autosomal recessive marker (sepia-colored eye) that appeared in our Harwich stock. Tautavel 67 is a strong P strain collected in France (1967) that shows 40–50 *P* labels by *in situ* hybridization; no *P* element has been found at 1A (nine larvae tested; S. Ronsseray and M. Lehmann, unpublished results). *Texas 007/Cy* is a P line that has strong *P* repression ability linked to a wild derived second chromosome called "*Texas 007*" (Périquet and Anxolabéhère 1982); it shows 20–25 *P* labels including one at the 1A site (three larvae tested). The properties of these strains in regard to *P*-induced hybrid dysgenesis are given in Table 1. w^{m4}; *Su(var)2-5*^{04}*/Cy Roi*: this *Su(var)205* allele encodes a truncated nonfunctional HP1 protein (Eissenberg *et al.* 1992). It strongly impairs the repression ability of *Lk-P*(1A) (Ronsseray *et al.* 1996, 1997). A number of *P-reporter* insertions at various locations in the genome were used: the insertion sites are given in Tables 1–6. The structure of the transgene is indicated further in Tables 1–6 by the two first letters of the name and is as follows: *WG = P{ry[+t7.2] = 1ArB} = P-lacZ, ry, adh*, from Walter Gehring; *GR = P{w[+t11.7] ry[+t7.2] = wA} = P-w, ry*, from Gerry Rubin; *GR′ = P{w[+tAR] ry[+t7.2AR] = wA[R]} = P-w, ry*, from Gerry Rubin; *JLC = P[lac-ry+]A = P-lacZ, ry*, from Jean-Louis Couderc; *LW = P{hsp26-pt-T hsp70-w+}, P-barley, w*, from Lori Wallrath; and *DP = P{ry[+t7.2] = 23.1} = P-ry*, from Daniel Pauli.

Gonadal dysgenesis assays (%GD A*): 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 test cross, 3–10 pairs were mated *en masse* and immediately 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. Twenty-five 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, calculated as %GD = %S0 + 1/2 %S1, will be referred to as the 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.

The ability of lines to induce dysgenic sterility was measured by the "A assay" (Kidwell *et al.* 1977). This assay reflects the activity of autonomous *P* elements in the tested line. Males of the tested line were crossed with M females (Canton^{y}). For each test cross, 5–10 pairs were mated *en masse* and immediately placed at 29°. Parents were discarded after 3 days of egg laying. The percentage of GD sterility was measured as in the A* assay (see above).

Interactions between *P-reporters* and P genomes: *Maternal and zygotic contributions of the P-reporter:* Five females of the line tested (*P-reporter* or control M line) were crossed at 20° to five

males that carry regulatory *P* elements. Different males were used depending on the experiment [*Lk-P*(1A), Harwich-2, *Texas 007/Cy* and Tautavel 67]. Replicate sets of three to five G_1 females were then crossed with five Harwich-2 males at 29°. Parents were discarded after 3 days of egg laying and the percentage of GD sterility was estimated in their progeny (see the A* assay).

Zygotic contribution of the P-reporter: Five males of the line tested were crossed to five *E24* females at 20°. Replicate sets of three to five G_1 females were crossed to five Harwich-2 males at 29°. Parents were discarded after 3 days of egg laying and the percentage of GD sterility was estimated in their progeny (see the A* assay).

G_1 males from the "maternal and zygotic component" of the previous assays were also tested for their ability to induce GD sterility when crossed with M females (A assay). Five to 10 G_1 males were crossed with 10 Canton^y females at 29°. Parents were discarded after 3 days of egg laying and GD sterility was measured in their daughters after dissection, as in the A* assay.

The sensitivity of the combination effect to *Su(var)205* was also tested. The mating scheme is presented with the results in Figure 1.

Statistical analysis: The repression abilities of G_1 females from experiments described above were compared using the nonparametric Mann-Whitney *U*-test performed on A* assay replicates.

RESULTS

Combination effect of telomeric *P-reporters* with regulatory *P* elements: *Maternal and zygotic component:* We investigated the properties of two lines carrying an insertion of a *P-lacZ* fusion transgene at the 1A site. These lines (*WG-1103* and *WG-1152*) were chosen from among a collection of enhancer-traps established in Walter Gehring's laboratory (Wilson *et al.* 1989). Neither exhibited any *lacZ* staining in the ovaries even after an overnight reaction (S. Ronsseray and L. Marin, data not shown). Both *WG-1103* and *WG-1152* are flanked by *TAS* (Roche and Rio 1998).We crossed *WG-1103* and *WG-1152* females with Harwich-2 males at 29° and found 95–100% of GD sterility (Table 1), a level similar to that obtained when crossing M line females to Harwich-2 males under similar conditions. Therefore these *P-lacZ*(1A) insertions do not themselves repress dysgenic sterility. To test for an indirect effect of these *P-lacZ*(1A) insertions on *P* regulation, *WG-1103* or *WG-1152* females were crossed with *Lk-P*(1A) males at 20° and the repression ability of G_1 females was evaluated by the A* assay, *i.e.*, by crossing them with Harwich-2 males at 29° and scoring GD sterility in the G_2 females. As a control, the same experiment was carried out with M females instead of *P-lacZ*(1A) females in the G_0 and also with females from two different lines [E24 and *Ch-P*(1A)] that have autonomous regulatory *P* elements at 1A (Ronsseray *et al.* 1996).

Among the M lines used as a control, some have *X*-linked *TAS*, as tested by *in situ* hybridization, whereas others apparently lack such sequences (L. Tolar, F. Sheen and R. Levis, personal communication; L. Marin and S. Ronsseray, unpublished results). Starting the experiment with M females in the G_0, the G_1 females had no detectable repression ability (Table 2, first column). This result is expected since the *Lk-P*(1A) elements were paternally introduced in the G_0 and the repression ability of these elements is known to be maternally inherited (Ronsseray *et al.* 1991, 1996). Whether or not the M lines have *X*-linked *TAS*, there is a similar lack of repression ability in the G_1 females, indicating that the presence of the *X*-linked *TAS* does not affect the results of this assay. By contrast, using *WG-1103* or *WG-1152* females in the G_0 produced G_1 females with increased repression ability (Table 2, first column). Each comparison between the results of a given M line and those of *WG-1103* or *WG-1152* is significant ($P < 0.01$). "*P*(1A)" females [carrying autonomous *P* elements at 1A from the *E24* or *Ch-P*(1A) lines] produced G_1 females with nearly complete repression ability. This resulted because these lines repress the occurrence of GD sterility by themselves (Table 1). How-

TABLE 1

Properties of the strains in regard to the P-M system of hybrid dysgenesis

	Tested line females × Harwich-2 males			Canton^y females × Tested line males		
Tested line	*m*	*s*	*n*	*m*	*s*	*n*
M lines						
Canton^y	100	0.0	10	0.7	0.6	3
Muller5	99.5	1.0	4	1.3	1.5	4
Sevelin	100	0.0	2	1.0	1.4	2
Oregon	99.2	1.3	3	0.0	0.0	3
Exe24	100	0.0	10	0.0	0.0	7
P lines						
Harwich-2	0.3	0.6	5	100	0.0	10
Tautavel 67	0.0	0.0	2	86.0	1.2	5
Texas 007	0.0	0.0	5	98.6	1.2	3
"*P*(1A)" lines						
Lk-P(1A)	1.1	2.0	35	1.1	1.5	5
E24	19.0	8.7	6	1.0	1.4	5
Ch-P(1A)	19.2	14.4	5	4.2	10.1	11
Salt-P(1A)	100	0.0	7	0.6	1.6	6
Telomeric *P-reporter* lines						
WG-1103	100	0.0	9	0.4	0.9	5
WG-1152	98.8	1.3	10	0.0	0.0	4
GR-833	99.8	0.5	4	0.0	0.0	4

The ability of lines to repress *P*-induced gonadal dysgenesis was tested by crossing replicate sets of 3–10 females of these lines with five Harwich-2 males (A* assay). The ability of lines to induce *P*-induced gonadal dysgenesis was tested by crossing replicate sets of 5–10 males of these lines with 10 Canton^y females (A assay). In both cases, the G_1 flies were allowed to develop at 29°. For each replicate test cross, 60 to 100 ovaries were examined in the progeny. *m*, *s*, *n* are, respectively, the mean percentage of GD sterility, the standard deviation among replicates, and the number of replicates performed. "*P*(1A)" lines are lines with naturally occurring *P* element(s) at 1A.

TABLE 2

Combination effect with a maternally inherited *P-reporter* insertion at 1A

	G_0 males					
	Lk-P(1A)			Harwich-2		
G_0 females	*m*	*s*	*n*	*m*	*s*	*n*
M lines without *X*-linked *TAS*						
Canton^y	99.8	0.6	10	82.7	5.2	5
Sevelin	100	0.0	8	99.4	1.6	8
Muller5	99.8	0.4	5	100	0.0	10
M lines with *X*-linked *TAS*						
Oregon	97.8	0.7	9	87.3	14.0	7
Exe24	100	0.0	8	99.2	1.2	6
P-reporters at 1A						
WG-1103	80.4	12.6	11	13.2	9.2	9
WG-1152	25.3	12.5	10	4.9	8.2	8
"*P*(1A)" lines						
E24	3.0	2.7	9	1.0	1.4	8
Ch-P(1A)	5.8	6.9	10	0.3	0.6	3
Salt-P(1A)	33.2	24.4	9	0.2	0.4	6

The G_0 cross (five females × five males) was performed at 20°. The *P* repression ability of the G_1 was tested by crossing replicate sets of three to five G_1 females with five P strain males (Harwich-2) at 29°. For each replicate G_1 test cross, 50 to 100 ovaries were examined in the progeny. *m*, *s*, *n*, see legend to Table 1.

TABLE 3

Combination effect with a paternally inherited *P-reporter* insertion at 1A

G_0 males	*m*	*s*	*n*
M lines without *X*-linked *TAS*			
Canton^y	94.0	5.8	9
Sevelin	97.3	4.3	9
M lines with *X*-linked *TAS*			
Oregon	100	0.0	8
Exe24	99.6	0.8	7
P-reporters at 1A			
WG-1103	61.2	21.6	8
WG-1152	48.8	20.0	8
"*P*(1A)" lines			
E24	19.0	8.7	6
Ch-P(1A)	4.5	2.1	2

The G_0 cross was performed at 20° (five tested line males × five *E24* females). The *P* repression ability of the G_1 was tested by crossing replicate sets of three to five G_1 females with five P strain males (Harwich-2) at 29°. For each replicate G_1 test cross, 50 to 100 ovaries were examined in the progeny. *m*, *s*, *n*, see legend to Table 1.

ever, the case of *Salt-P*(1A) is different. It has a naturally occurring defective *P* element at 1A and by itself does not repress GD sterility (Table 1). When *Salt-P*(1A) females are crossed to *Lk-P*(1A) males in the G_0, their G_1 daughters have significant but incomplete repression ability, similar to the G_1 daughters of by *P-lacZ*(1A) females crossed to *Lk-P*(1A) males.

These results show that: (1) a maternally inherited *P-lacZ*(1A) insertion alone is unable by itself to repress GD sterility; (2) paternally inherited regulatory *P* elements at 1A alone are also unable to repress GD sterility; (3) the combination of a maternally inherited *P-lacZ* insertion and paternally inherited regulatory *P* elements at 1A significantly represses GD sterility (there is therefore a "combination effect"); and (4) this regulatory effect can also occur when a naturally occurring defective *P* element at 1A is combined with paternally inherited regulatory *P* elements at 1A. It should be noted, however, that this combination effect is weaker than the effects obtained with maternally inherited complete *P* elements at 1A (as in the *E24* or *Ch-P*(1A) lines; Table 2).

We also tested whether these *P-lacZ*(1A) elements can present a combination effect with regulatory *P* elements that are not located at 1A, but that are scattered throughout the genome. The Harwich-2 line, as recently tested by *in situ* hybridization, carries approximately 80 *P* elements per haploid genome. A *P* label at 1A was found in only 1 slide out of 12 analyzed, but at least two autosomal telomeric labels were observed on each slide (S. Ronsseray and M. Lehmann, unpublished results). In the G_0, we crossed females from the *P-lacZ*(1A), M, or *P*(1A) lines with Harwich-2 males [instead of *Lk-P*(1A) males as in the previous assay] at 20°. There is no GD sterility in the progeny at this temperature of development. Then we tested the repression ability of the G_1 females as above. When M females were used in the G_0 crosses, their G_1 daughters had a negligible or, at best, weak ability to repress GD sterility (Table 2, second column). By contrast, when *WG-1103* or *WG-1152* females were used in the G_0 crosses, strong repression ability was seen in their G_1 daughters. This effect was also observed when *P*(1A) females were used in the G_0 crosses even when the G_0 females come from the *Salt-P*(1A) line. Each comparison between results from a given M line and those from *WG-1103*, *WG-1152*, or a *P*(1A) line is significant ($P < 0.01$). These results show that the combination of a maternally inherited *P-lacZ* insertion at 1A and paternally inherited *P* elements at locations other than 1A (including euchromatic and autosomal telomeric locations) represses GD significantly.

Zygotic component: When *Lk-P(1A)* females are crossed to M males in the G_0, their G_1 daughters usually have strong repression ability because of the maternal inheritance of the *Lk-P*(1A) regulatory factors (Ronsseray *et al.* 1991). *E24* has only one of the two *P* elements present in *Lk-P*(1A) and its repression ability is accordingly weaker than that of *Lk-P*(1A) (Table 1; see also Ronsseray *et al.* 1996). Crossing *E24* females with M males produces G_1 females that have negligible repression ability (Table 3). Because these hybrid females from *E24* mothers cannot repress GD sterility, we can investi-

gate the effect of combining a paternally inherited *P-lacZ*(1A) element with a maternally transmitted *P* element from the *E24* line. *P-lacZ*(1A) males were crossed with *E24* females and the repression ability of the G_1 females was measured by the A* assay as in the previous experiments. Using either *WG-1103* or *WG-1152* males in the G_0 cross, we find that their G_1 daughters have significant repression ability (Table 3). Each comparison between the results from a given M line and those from *WG-1103* or *WG-1152* is significant ($P < 0.01$). As in the first set of experiments (Table 2), these repression properties are weaker than those obtained with autonomous *P* elements at 1A [*E24* or *Ch-P*(1A)] instead of *P-lacZ*(1A) transgenes introduced in the G_0. These results show the contribution of the *P-lacZ*(1A) insertions to the combination effect can be at least partially transmitted through the male.

Effects of P-reporter constructs at other locations: The ability of *P-reporter* constructs inserted at other sites of the genome to induce a combination effect was investigated. None of these *P-reporter* lines had any significant *P* repression ability as tested by A* assay (data not shown). The capacity of these lines to influence the repression ability of *P* elements at 1A was investigated by crossing females carrying these *P-reporter* constructs to *Lk-P*(1A) males and testing their daughters by the A* assay. Among 15 euchromatic insertions tested, none resulted in a combination effect in the *P-reporter*/*P*(1A) hybrid females (Table 4). In addition, a "multi-lac" line that carries four *P-lacZ* insertions on the *X* chromosome also failed to produce a combination effect in the hybrid females. It must be pointed out that, among the euchromatic *P-reporter* insertions tested, some (*BC69, BQ16* and *ABOO*) show very strong *lacZ* staining in the nurse cells and in the mature oocytes (J. L. Couderc, personal communication), indicating that these *P-reporter* insertions are vigorously expressed in these tissues. By contrast, *WG-1103* and *WG-1152* are apparently not expressed in the nurse cells and in the oocyte (S. Ronsseray and L. Marin, unpublished results). Thus the *P-reporter*/*P* elements combination effect is not correlated with expression of the *P-reporter* insertion in the germline.

Among four insertions in pericentromeric heterochromatin that were tested, no case of combination effect was detected (Table 4). This includes the pericentromeric *AS-CH(2)6* insertion which is flanked by *TAS-related* sequences (Zhang and Spradling 1995). Among five insertions at an autosomal telomere that were tested, one (*GR-833*) produced a combination effect (Table 4). The *GR-833* line carries a *P-w, ry* transgene located at 100F at the *3*R telomere (Hazelrigg *et al.* 1984). The combination effect with this insertion was also detected using Harwich-2 males in the G_0, *i.e.*, *GR-833* females × Harwich-2 males at 20°; less than 5% of GD sterility was found by the A* assay of the G_1 females from this cross ($m = 3.5$, $s = 5.1$, $n = 8$; compare with Table 2, second column). Because the *GR-833* transgene (*P-w, ry*) does not include the *lacZ* gene, the combination effect does not depend on *lacZ* presence or expression nor does it require that the *P-reporter* be inserted on the *X* chromosome. The *GR-833* insertion shows strongly variegated expression of its *white* transgene and it is flanked by *TAS*-related sequences (Levis *et al.* 1993). However, insertion in *TAS*-related sequences is not *per se* sufficient to produce a combination effect because the *LW-39C5* insertion (Wallrath and Elgin 1995) and the *WG-1155* insertion, neither of which produces a combination effect with *Lk-P*(1A), are also flanked by *TAS-related* sequences (L. Wallrath, personal communication; Roche and Rio 1998).

TABLE 4

Combination effect with *P-reporter* insertions at various genomic locations

G_0 males	*m*	*s*	*n*
Euchromatic *P-reporters*			
WG-1131 (1C)	100	0.0	6
WG-1164 (2D)	100	0.0	6
WG-1260 (3C1-7)	100	0.0	6
DP-1223 (20BC)	100	0.0	6
JLC-BC69 (35C)	100	0.0	6
GR′-832 (40A)	100	0.0	6
GR-798 (47A)	99.7	0.8	7
WG-1038 (50D)	100	0.0	6
GR-851 (59B)	100	0.0	4
WG-1064 (70F)	99.3	1.2	6
WG-1151 (91B)	100	0.0	6
GR-816 (91C)	100	0.0	7
R20A (100C-D)	100	0.0	6
JLC-BQ16 (nd)	99.9	0.4	7
JLC-ABOO (nd)	100	0.0	3
Multilac (*)	99.8	0.4	6
Pericentromeric heterochromatin *P-reporters*			
AS-CH(2)6 (*2*R, h42-44)	100	0.0	5
WG-993 (*3*R, 81F)	100	0.0	6
LW-118-E12 (*3*R)	99.5	1.4	8
LW-118E-10 (*4*)	100	0.0	8
Autosomal telomeric *P-reporters*			
LW-39C-5 (*2*L)	99.9	0.3	9
WG-1206 (*3*L, 61A)	100	0.0	6
*WG-1155 (3*R, 100F)	100	0.0	6
GR-833 (*3*R, 100F)	55.9	21.5	15
LW-118E15 (*4*)	100	0.0	5

The genomic location (chromosomal arm and/or cytological location) of the *P-reporter* tested is given in parentheses. The "multilac" line carries four *P-lacZ* insertions on the *X* chromosome. The G_0 cross (five tested line females × five *Lk-P*(1A) males) was performed at 20°. The *P* repression ability of the G_1 was tested by crossing replicate sets of three to five G_1 females with five P strain males (Harwich-2) at 29°. For each replicate G_1 test cross, 60 to 100 ovaries were examined in the progeny. *m, s, n,* see legend to Table 1; nd, not determined.

The combination effect is seen in different P strain genetic backgrounds: We tested for a combination effect between selected telomeric *P-reporter* insertions and the

TABLE 5

The combination effect works with various P strains

	G_0 males					
	Texas 007/Cy			Tautavel 67		
G_0 females	*m*	*s*	*n*	*m*	*s*	*n*
M lines						
Canton^y	51.9	14.6	8	78.9	23.6	8
Muller5	60.7	22.0	3	91.4	8.5	5
Oregon	87.7	6.7	7	87.0	21.2	10
Telomeric *P-reporters*						
WG-1103	4.4	3.1	9	5.7	9.3	7
WG-1152	1.6	1.6	8	0.0	0.0	8
GR-833	1.8	2.5	5	—	—	—
"*P*(1A)" line						
Lk-P(1A)	0	0	7	—	—	—

The G_0 cross (five females × five males) was performed at 20°. The *P* repression ability of the G_1 was tested by crossing replicate sets of three to five G_1 females (Cy^+ phenotype) with five P strain males (Harwich-2) at 29°. For each replicate G_1 test cross, 50 to 100 ovaries were examined in the progeny. *m*, *s*, *n*, see legend to Table 1.

TABLE 6

Telemeric *P-reporter* insertions do not affect the ability of males to induce hybrid dysgenesis

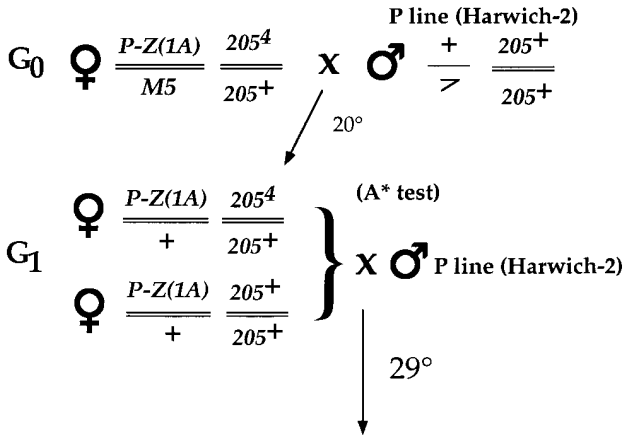
	G_0 males					
	Harwich-2			*Texas 007/Cy*		
G_0 females	*m*	*s*	*n*	*m*	*s*	*n*
M lines						
Canton^y	70.1	12.9	6	44.0	1.8	3
Muller5	72.7	7.7	4	33.1	6.6	4
Oregon	—	—	—	29.0	2.0	3
Telomeric *P-reporters*						
WG-1103	76.5	2.6	5	31.7	5.9	3
WG-1152	73.8	6.1	4	32.5	2.3	3
GR-833	75.0	8.5	4	34.6	15.0	3

The G_0 cross (5 females × 5 males) was performed at 20°. The ability of the G_1 to induce hybrid dysgenesis was tested by crossing replicate sets of 5–10 G_1 males (Cy^+ phenotype) with M strain females (Canton^y) at 29°. For each replicate G_1 test cross, 50 to 100 ovaries were examined in the progeny. *m*, *s*, *n*, see legend to Table 1.

P elements from two independent P strains (*Texas 007/Cy* and Tautavel 67). *In situ* hybridization with a *P* probe showed that these two lines have numerous *P* labels scattered through the genome (materials and methods). With Tautavel 67, there was no *P* label at 1A in the nine slides analyzed; however, 1–2 autosomal telomeric sites were labeled on each slide. With *Texas007/Cy*, a *P* label was seen at 1A and at the *3*R telomere (100F) in the three slides analyzed. In the G_0, females from the telomeric *P-reporter* lines were crossed with males from the P strains (*Texas 007/Cy* or Tautavel 67) and the repression ability of their G_1 daughters (Cy^+ phenotype) was measured by the A* assay. When M line females (Canton^y, *Muller5*, Oregon) were crossed in the G_0 with *Texas 007/Cy* or Tautavel 67 males, partial repression ability was observed in the G_1 daughters (Table 5). By contrast, when G_0 females with a telomeric *P-reporter* were crossed with either *Texas 007/Cy* or Tautavel 67 males, stronger repression ability was seen in the G_1 daughters, indicating a combination effect. The level of repression was close to that obtained with *Lk-P*(1A) G_0 females. These results therefore show that the combination effect does not depend on a particular P strain background.

Telomeric *P-reporter* insertions do not affect the ability of males to induce hybrid dysgenesis: Because telomeric *P-reporters* influence the repression ability of natural *P* elements in the genome, we tested whether they also affect the ability of these elements to induce GD sterility. P males were crossed at 20° to females with telomeric *P-reporter* insertions. Their G_1 sons were then crossed at 29° to M females (Canton^y) and the percentage of GD sterility in the G_2 daughters was estimated (A assay). These G_1 males have paternally inherited *P* elements and a maternally inherited telomeric *P-reporter*. As controls, the same experiment was carried out with M females instead of telomeric *P-reporter* females in the G_0. Table 6 shows that the presence of the insertions *WG-1103*, *WG-1152* or *GR-833* does not significantly affect the ability of the *P* elements inherited from Harwich-2 or *Texas 007/Cy* to induce GD sterility. Thus there is no combination effect on the ability to induce GD sterility.

The combination effect is impaired by a *Su(var)205* mutation: The repression ability of the *Lk-P*(1A) line is sensitive to *Su(var)205* mutations (Ronsseray *et al.* 1996, 1997). *Su(var)205* encodes HP1, a nonhistone heterochromatin protein (James and Elgin 1986; Wustmann *et al.* 1989) that binds the centromeres and the telomeres (James and Elgin 1986; James *et al.* 1989). The presence of a null allele of *Su(var)205* or of a truncated HP1 protein strongly impairs, in a dominant manner, the ability of the *Lk-P*(1A) elements to repress GD sterility. Neither the other *Modifiers of variegation* tested, nor *Y*-chromosome dosage, affects *Lk-P*(1A) repression ability (Ronsseray *et al.* 1996). The effect of a *Su(var)205* mutation on the combination effect was tested according to the mating scheme presented in Figure 1. The *Su(var)2-5^{04}/Cy Roi* line used in this experiment is devoid of *P* repression ability. The presence of the *Su(var)2-5^{04}* allele was previously shown to dramatically affect the repression ability of *Lk-P*(1A) (Ronsseray *et al.* 1996). G_1 females were constructed that carried the *WG-1103* insertion (maternally inherited) and *P* elements from Harwich-2 (paternally inherited); these females were either heterozygous *Su(var)205$^+$/Su(var)2-5^{04}* or homozygous *Su(var)205$^+$/Su(var)205$^+$*



Dissection of G_2 females: % of GD sterility

G_1 ($205^4/205^+$): GD G_2 = **94.8** ; s= 8.2 ; n=17

G_1 ($205^+/205^+$): GD G_2 = **54.8** ; s= 23.8 ; n=18

Figure 1.—Sensitivity of the combination effect to *Su (var)205* mutations. *Su(var)205* is located on the second chromosome. The chromosome with the *Su(var)205*$^+$ allele is marked with the *Cy* dominant mutation. The repression ability of the G_1 was tested by crossing replicate sets of three to five G_1 females with five P strain males (Harwich-2) at 29°. For each replicate test cross, 50 to 100 ovaries were examined in the progeny. *m, s, n*, see legend to Table 1; *M5*, *Muller5*; *P-Z*(1A), *P-lacZ*(1A) (*WG-1103 insertion*); *205*^{4}, *Su(var)2-5*^{04}.

(as a control). The repression ability of the two kinds of G_1 females was tested by the A* GD assay. G_1 females with two *Su(var)205* wild-type alleles had significant repression ability whereas their sisters carrying the *Su(var)2-5*^{04} allele did not. The difference between the two genotypes is highly significant ($P < 0.01$). Thus *Su(var)205* strongly affects the ability of the combination of a telomeric *P-reporter* and natural *P* elements to repress GD sterility.

DISCUSSION

The repression ability of *P*(1A) elements involves a combination of effects: The repression ability of a *P* element depends on both its structure and its genomic position (Anxolabéhère *et al.* 1987; Black *et al.* 1987; Daniels *et al.* 1987; Preston and Engels 1989; Robertson and Engels 1989; Misra and Rio 1990; Simmons *et al.* 1990; Ronsseray *et al.* 1991; Higuet *et al.* 1992; Gloor *et al.* 1993; Misra *et al.* 1993; Rasmusson *et al.* 1993; Andrews and Gloor 1995; Corish *et al.* 1996; Simmons *et al.* 1996). A strong position effect exists at 1A since two copies of autonomous *P* elements are sufficient to elicit the complete P cytotype whereas M lines transformed by the autonomous *P* element may reach around 15 copies without having a strong repression ability (Anxolabéhère *et al.* 1987; Daniels *et al.* 1987). The repression ability of autonomous *P* elements at 1A probably depends on an encoded product since *Lk-P*(1A) females transmit a strong maternally-transmitted component (pre-P cytotype) that promotes nearly complete *P* repression in progeny that inherit other regulatory *P* elements from their father (Ronsseray *et al.* 1993). The *Lk-P*(1A) elements are known to be expressed because each of the two elements, when separated, shows transposase activity (Ronsseray *et al.* 1996). In addition, Roche *et al.* (1995) have used RT-PCR to detect transcripts from these elements, including an incompletely spliced RNA which could lead to the production of a truncated transposase protein (*P*-66kD) in the germline; this protein has previously been shown to be a repressor of *P* activity (Robertson and Engels 1989; Misra and Rio 1990). However, the expression of the *Lk-P*(1A) elements cannot alone explain their strong repression ability because the expression is undetectable as judged by western analysis with an antibody against *P* proteins (S. E. Roche, S. Misra and D. C. Rio, personal communication). It is therefore likely that a peculiar property because of the telomeric position is also involved in repression by the *Lk-P*(1A) elements. The *trans*-silencing described in tobacco by Vaucheret (1993, 1994), Matzke *et al.* (1994) and Park *et al.* (1996) suggests that a chromatin-chromatin interaction may take place between a telomeric transgene insertion and a euchromatic insertion. The article from Roche and Rio (1998, this issue) provides data suggesting such an interaction: they have observed a *trans*-silencing effect (TSE) by telomeric *P*-transgenes, unable to encode a *P* repressor, on other *P-transgenes* in the genome.

TSE and the regulatory combination effect appear to be closely linked: The TSE described by Roche and Rio (1998, this issue) and the combination effect described here, present several similarities. They are both induced by *P-reporter*s but only at telomeric positions. There is a correlation between the TSE and the combination effect: for example, *GR-833* shows both phenomena whereas *WG-1155* shows neither. In fact, out of five insertions tested for both assays, there is no case of an insertion showing one but not the other phenomenon. Among lines that induce these effects, there is also a correlation for the strength of the effects: *WG-1152* and *GR-833* are stronger for both TSE and the combination effect than *WG-1103.* The TSE is suppressed by *Enhancer of zeste (E(z))* mutants that also impair the repression ability of *Lk-P*(1A) (Roche and Rio 1998, this issue). The combination effect is suppressed by a *Su(var)205* mutation (Figure 1) that also impairs repression by the *Lk-P*(1A) elements (Ronsseray *et al.* 1996). Both *Su(var)205* and *E(z)* are known to affect the structure of the chromatin. It will be interesting to test the effect of *E(z)* mutations on the combination effect and of

Su(var)205 mutations on TSE. However, differences exist between the two sensitivities, since *Su(var)205* mutations have an effect on regulation by *Lk-P*(1A) when paternally inherited whereas *E(z)* mutations have an effect only when maternally inherited.

An attractive model is that the combination effect induced by telomeric *P-reporter*s on the *P* elements of P strains [Harwich, Tautavel 67, *Texas 007/Cy* or *Lk-P*(1A)] is a consequence of a TSE on these *P* elements. Under this model, it can be hypothesized that a reduced expression of these *P* elements (because of the TSE) leads them to produce mainly repressor instead of transposase. Such a model, supported by genetic data, has been proposed by Lemaitre *et al.* (1993; see also Coen *et al.* 1994) and reinforced at the molecular level by Roche *et al.* (1995). Nevertheless a striking result remains unexplained: why does this trans-silencing not change the ability of males to induce dysgenesis? One explanation is that the *trans*-effects and the induction of GD sterility occur at different stages. TSE and the combination effect described here are observed in the female adult germline in which the maternal contribution of the *P* repression is elicited. By contrast, the crucial period for the induction of GD sterility by males takes place in the progeny at an earlier stage (embryo and first instar larvae; Engels 1989).

The capacity of a telomeric *P*-insertion to repress GD sterility depends on its coding capacity: The structure of the *P* insertion at 1A still appears to be essential in regard to its ability to elicit, in the absence of other *P* elements, strong repression of GD sterility. *Lk-P*(1A), with two autonomous *P* elements, completely represses GD sterility; *E24*, *E60* and *Ch-P*(1A) each with one element partially repress it (Ronsseray *et al.* 1996). By contrast, the lines *WG-1103*, *WG-1152* and *GR-833* show no repression of GD sterility. In addition, *Salt-P*(1A), which carries a naturally occurring defective *P* element at 1A (L. Marin and S. Ronsseray, unpublished results) that is apparently unable to encode the *P* repressors described so far, *i.e.*, the *P*-66-kD truncated protein (Robertson and Engels 1989; Misra and Rio 1990; Gloor *et al.* 1993) and the *KP* protein (Black *et al.* 1987; Andrews and Gloor 1995; Lee *et al.* 1996), induces a combination effect but does not elicit significant repression of GD sterility by itself. Thus it behaves like a *P-lacZ*(1A) insertion. Furthermore, in the genetic experiments described in this article, autonomous *P* elements at 1A showed stronger levels of repression in the combination effect assays than telomeric *P-reporter* insertions or the *Salt-P*(1A) element. Clearly, *P-LacZ* insertions or defective *P* elements at 1A are not equivalent to autonomous *P* elements at this site. These differences are probably the consequence of the capacity of autonomous *P* elements at 1A to make a repressor.

The combination effect does not depend on telomeric *P-transgene* coding capacity: The results of this article indicate that the ability of *P-lacZ*(1A) insertions to induce a combination effect is not correlated to expression of the transgene in the germline. In fact, the combination effect and TSE appear to be independent of the coding capacity of the telomeric *P* insertions and in particuliar do not depend on the presence of *lacZ*. Indeed the *WG-1103* and *WG-1152* insertions have a structure differing from that of *GR-833*. The *GR-833* line carries a *P-w, ry* transgene and not *lacZ*. This shows that 587 bp of 5′ *P* sequence and 233 bp of 3′ *P* sequence are sufficient to induce the effect. It is possible that the *P* sequences flanking the transgenes are reponsible for the postulated interactions between telomeric and euchromatic *P* insertions. It would be interesting to test whether removing the *P*-sequences flanking one side of the transgene would abolish the combination effect and TSE.

The *TAS* adjacent sequences are perhaps necessary but not sufficient for the induction of the combination effect: The combination effect (Table 4) and TSE can be induced by a *P-reporter* at an autosomal telomere (*3*R), showing that these properties are not restricted at the *X* chromosome telomere. However, the *GR-833* insertion in 100F is also flanked by *TAS*-related sequences (Levis *et al.* 1993) and thus three out of three telomeric transgenes, which can induce the combination effect and TSE, are in *TAS* or *TAS-related* sequences. In addition three out of three naturally occurring *P* elements at 1A, which have the capacity to repress dysgenic sterility, are inserted in *TAS*. This suggests that *TAS* are necessary for the combination effect and consequently for *P* repression to occur. However, the *WG-1155* insertion (which has the same *P-reporter* structure as *WG-1103* and *WG-1152*) and the *LW-39C5* insertions are also inserted in *TAS-related* sequences (Roche and Rio 1998; L. Wallrath, personal communication) but they do not induce the combination effect or TSE. This shows that *TAS-related* adjacent sequences are not *per se* sufficient to induce these effects. One explanation may be that the size of the *TAS*-cluster differs between the insertions tested and is not sufficient in lines that fail to induce the combination effect and TSE (*WG-1155*, *LW-39C-5*). Another explanation is that the position of the *P*-transgene inside the *TAS-related*-repeats cluster differs in lines able or unable to induce the combination effect and TSE. Finally, it is possible that *TAS* have no functional role in the repression ability of telomeric *P*-insertions and that the apparent correlation is a consequence of the fact that *TAS* are hot spots for *P* insertions (Karpen and Spradling 1992).

The combination effect: a chromatin structure effect or a nuclear location effect: It appears that at least part of the ability of the *P* elements at 1A to repress hybrid dysgenesis and to cause TSE involves some coding-independent capacity to interact with other *P* elements. To explain TSE, Roche and Rio (1998), propose that a pairing occurs between *P-reporter* insertions at 1A and other *P-reporter* insertions in the genome. To explain

the combination effect, we propose a similar interaction between telomeric *P reporters* and natural *P* elements scattered through the genome. The combination effect would be a *trans*-stimulation by the telomeric insertions of the *P* repression elicited by the *P* elements of P strains. The latter *P* elements could be copies at 1A (*Lk-P*(1A)). They could also be euchromatic copies or copies located at autosomal telomeres since Harwich-2 and Tautavel 67 have both types of *P* elements (but no copies at 1A). *Texas 007/Cy* has *P* copies at 1A, at 100F and in euchromatin: each of them could play a role in the combination effect. The pairing would be facilitated by a scanning of the genome by the telomeric insertions as has been proposed to occur in plants. This pairing could be promoter-dependent as in tobacco (Vaucheret 1993). Because of this pairing, the other *P* elements or reporters in the genome would be repressed as suggested by TSE. This repression could be the consequence of a *trans*-heterochromatinization of the target *P* elements, since *P*-insertions at the telomeres are inserted in heterochromatic *TAS* sequences. An alternative possibility is that the telomeres are located in a special compartment of the nucleus. The relocalization of the other *P* elements or reporters in the genome would lead to their repression since the nuclear compartment may be important in regard to gene expression (Wakimoto and Hearn 1990). It will be interesting to analyze the *P* insertions at 1A and the other *P* insertions in the genome for the chromatin structure by accessibility to restriction enzyme digestion (Wallrath and Elgin 1995) and for their location in the nucleus by FISH. Similarities between animal and plant transposons have recently been revealed by the discovery of a "cosuppression-like" phenomenon in Drosophila (Pal-Badhra *et al.* 1997); the correlated combination and trans-silencing effects by *P* insertions at Drosophila telomeres mimic (at least partially) the telomeric trans-silencing in tobacco (Vaucheret 1993, 1994; Matzke *et al.* 1994; Park *et al.* 1996) and suggest that transposable elements have essential similarities in animals and plants.

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