Establishment and Maintenance of the P Cytotype Associated With Telomeric *P* Elements in *Drosophila melanogaster*

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ABSTRACT

P elements inserted near the left telomere of the *X* chromosome are associated with the P cytotype, a maternally transmitted condition that strongly regulates the activity of the *P* transposon family in some strains of Drosophila. The regulatory abilities of two such elements, *TP5* and *TP6*, are stable in homozygous stocks over many generations. However, these regulatory abilities are attenuated when the telomeric *P* elements are transmitted through heterozygous females, and they are utterly lost when the elements are transmitted through males. Paternally transmitted telomeric *P* elements reacquire regulatory ability when they pass through a female germ line. This reacquisition is enhanced if the females in which it occurs came from mothers who carried a telomeric *P* element. The enhancement has two components: (1) a strictly maternal effect that is transmitted to the females independently of the mother's telomeric *P* element ("presetting" or the "pre-P cytotype") and (2) a zygotic effect associated with inheritance of the mother's telomeric *P* element. One telomeric *P* element can enhance the reacquisition of another's regulatory ability. When *X* chromosomes that carry telomeric *P* elements are extracted through males and made homozygous by using a balancer chromosome, most of the resulting stocks develop strong regulatory abilities in a few generations. However, some of the stocks do not attain the regulatory ability of the original population.

THE *P* elements of *Drosophila melanogaster* have been extensively used as tools in genetic analysis (Engels 1989). These elements were discovered through their involvement in hybrid dysgenesis, a phenomenon observed in the offspring of crosses between different strains of Drosophila (Kidwell *et al.* 1977). *P* elements are found in P strains but not in M strains. Crosses between P males and M females produce offspring with a syndrome of abnormalities in the germ line, including a high frequency of sterility and elevated mutation rates. These traits are usually not seen in the offspring of crosses between P females and M males. The difference between reciprocal crosses therefore indicates that the phenomenon of hybrid dysgenesis is regulated by a maternally transmitted condition characteristic of P strains. This condition is called the P cytotype (Engels 1979a). M strains have a complementary condition called the M cytotype, which is permissive for hybrid dysgenesis. Genetic analyses have shown that the P cytotype depends on maternal transmission of the *P* elements themselves (Engels 1979a,b; Sved 1987).

For many years it was thought that the repression of hybrid dysgenesis by the P cytotype involved polypeptides encoded by the *P* elements. Complete *P* elements, 2.9 kb long, encode an 87-kD polypeptide, the P transposase, which catalyzes *P*-element excision and insertion (Karess and Rubin 1984). Incomplete *P* elements, <2.9 kb because some DNA sequences have been deleted, do not encode the P transposase. However, they can be excised and transposed if a complete *P* element that makes the P transposase is present in the genome (Engels 1984). The excision of particular incomplete *P* elements has been used to monitor transposase activity in genetic experiments (Engels 1989). In addition to the transposase, complete *P* elements encode a 66-kD repressor polypeptide (Laski *et al.* 1986; Rio 1990). This polypeptide is translated from an incompletely spliced *P*-element RNA. In the soma, only the 66-kD polypeptide is made. In the germ line, both the 66-kD repressor and the 87-kD transposase are produced.

These facts have led to the hypothesis that the P cytotype is a state in which the 66-kD polypeptide more or less completely represses the synthesis or activity of the P transposase (Roche *et al.* 1995). However, this hypothesis has been called into question by the discovery that incomplete *P* elements situated near the left telomere of the *X* chromosome are powerful regulators of the *P*-element family (Marin *et al.* 2000; Stuart *et al.* 2002). Because of their structure, these elements cannot produce the 66-kD polypeptide, although they may produce smaller repressor polypeptides like, for example, the *KP* element, which produces a polypeptide that binds to *P* elements and represses their transposition (Lee *et al.* 1998). However, unlike *KP*, particular telomeric *P* elements are not geographically widespread

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(Stuart *et al.* 2002)—a feature that would be expected if natural selection had favored them in Drosophila populations. Thus, repression by the telomeric *P* elements may not involve *P*-encoded polypeptides. Other mechanisms involving the organization of chromatin around the telomeric *P* elements have been proposed to explain their regulatory properties (Roche and Rio 1998; Ronsseray *et al.* 1998, 2001; Stuart *et al.* 2002).

One key feature of the regulation mediated by the telomeric *P* elements is that it shows a reciprocal-cross effect. Telomeric *P* elements repress hybrid dysgenesis only when they are transmitted maternally. When transmitted paternally, a telomeric *P*'s regulatory ability is lost (Stuart *et al.* 2002; Simmons *et al.* 2004, this issue). Regulation by the telomeric *P* elements therefore follows the same pattern as regulation by the P cytotype. This parallel is the primary reason for associating telomeric *P* elements with the P cytotype.

In this article we consider questions about how the P cytotype is established and maintained. Is the P cytotype stably maintained when a telomeric *P* element is transmitted maternally? Is it reestablished when a paternally transmitted telomeric *P* element is returned to the female germ line? What factors influence the process of reestablishment? We address these questions by studying the regulatory abilities of two incomplete *P* elements, *TP5* and *TP6*, inserted at the same site in the TAS repeats near the left telomere of the *X* chromosome. *TP5* is 1.8 kb long and *TP6* is 1.9 kb long. Neither of these elements encodes a known repressor polypeptide; however, they both have strong abilities to repress transposase activity in the germ line (Stuart *et al.* 2002; Simmons *et al.* 2004, this issue).

MATERIALS AND METHODS

Drosophila stocks and mutability assay for P transposase activity: Genetic symbols for the Drosophila stocks are explained in Lindsley and Zimm (1992) or in other references cited in the text. Experimental cultures were maintained on a standard cornmeal-molasses-dried yeast medium at 25° unless stated otherwise. The *X*-linked telomeric *P* elements *TP5* and *TP6* were derived from natural populations by recombination with pure M strains (Stuart *et al.* 2002). Subsequently, these elements were combined on the same *X* chromosome with the double *P*-insertion mutation *singed weak* (sn^w), a hypomorphic allele of the *singed* bristle locus. In the presence of the P transposase, sn^w becomes hypermutable due to the excision of one or the other of the two incomplete *P* elements inserted in the 5′ region of the *singed* gene (Roiha *et al.* 1988). When one *P* element is excised, the sn^w allele changes to sn^e, an allele with an extreme mutant phenotype. When the other *P* element is excised, sn^w changes to sn^+, an allele that is phenotypically indistinguishable from wild type. To detect these changes, males in which they were occurring were mated individually to *C(1)DX, y f* females with attached-*X* chromosomes and their sons were scored for bristle phenotype. Sons were counted on day 13 or 14 after the test cultures were established and again on day 17. The combined frequency of sn^e and sn^+ flies among those scored within a culture was used to estimate the sn^w mutation rate.

Polymerase chain reactions: Crude DNA solutions were obtained from single flies by squashing the flies in 100 μl buffer with a sterile toothpick (Gloor and Engels 1992). Samples (2 μl) from these solutions were used to seed amplification reactions catalyzed by *Taq* DNA polymerase. Each reaction contained the four deoxyribonucleotides, polymerase, polymerase buffer, $MgCl_2$, and appropriate primers. The reactions were carried through 30 cycles of amplification, with each cycle consisting of 1 min at 92°, 2 min at 60°, and 3 min at 72°. During the first cycle, the time at 72° was extended by 4 min. Reaction products were analyzed on 0.7% agarose gels by electrophoresis. Two types of reactions were carried out: (1) amplification with a primer complementary to a segment of the *P* element's terminal inverted repeat and (2) amplification with an element-specific primer (either *TP5* or *TP6* specific) and a primer complementary to a segment near the 3′ end of the *P* element. The sequences and positions of these primers within the complete *P* element are given in Stuart *et al.* (2002).

RESULTS

Stocks with telomeric *P* elements repress transposase activity consistently over time: Previous analyses have demonstrated that stocks carrying either the *TP5* or the *TP6* telomeric *P* elements repress *P*-element excisions in the germ line. To investigate the consistency of this repression over time, we tested homozygous *TP5* and *TP6* stocks at 6-month intervals for repression of transposase-catalyzed excisions from sn^w, a double *P*-insertion allele of the *X*-linked *singed* bristle locus. In males, the sn^w allele causes a weak malformation of the bristles. When one or the other of the *P* elements inserted in the sn^w allele is excised by transposase action, alleles with different phenotypes—either extremely malformed bristles (sn^e) or essentially wild-type bristles (sn^+)—are created. The frequency of these phenotypes estimates the sn^w mutation rate, which can be used as an index of transposase-catalyzed *P*-excision activity in the father's germ line. Repression of this activity is indicated by a reduction in the sn^w mutation rate.

Females were sampled from homozygous *TP5 w* sn^w (*w, white* eyes), *TP6 w* sn^w, and control *w* sn^w stocks on three occasions separated by 6-month intervals. The *TP5 w* sn^w and *TP6 w* sn^w stocks were created 3 years prior to sampling by making a single *X* chromosome homozygous in each case (Stuart *et al.* 2002). During and before the sampling period, these stocks were maintained at 21° by mass transfers of adult flies to new cultures every generation. The only *P* elements present in these stocks were the two incomplete *P* elements inserted in the *singed* locus, an incomplete *P* element tightly linked to *singed* (Roiha *et al.* 1988), and a telomeric *P* element, which was also incomplete. Because no complete *P* elements were present, the stocks were not selected for repression of transposase activity during this time. The collected *(TP)* sn^w females were crossed *en masse* to males homozygous for *H(hsp/CP)2*, a *hobo* transgene on chromosome *2* that encodes the P transposase (Simmons *et al.* 2002a), and their *(TP)* sn^w*; H(hsp/*

TABLE 1

Reciprocal-cross analysis of repression of *sn*^{w} mutability by *TP5* and *TP6* stocks

	M control			*TP5*			*TP6*		
Sample	No. vials	No. flies	Mutation rate[a]	No. vials	No. flies	Mutation rate[a]	No. vials	No. flies	Mutation rate[a]
Maternal transmission									
1	50	1175	0.533 ± 0.018	47	1180	0.010 ± 0.004	50	1445	0.061 ± 0.013
2	33	599	0.506 ± 0.025	30	657	0.052 ± 0.013	29	601	0.094 ± 0.020
3	48	1320	0.500 ± 0.018	48	1529	0.007 ± 0.004	49	1623	0.063 ± 0.012
Paternal transmission									
2	29	725	0.571 ± 0.025	29	756	0.539 ± 0.019	29	704	0.588 ± 0.026

[a] Unweighted mean mutation rate ±SE.

CP)2/+ sons were assayed for *sn*^{w} mutability. Each son was crossed to 3 or 4 *C(1)DX, y f* (*y, yellow* body; *f, forked* bristles) females carrying attached-*X* chromosomes, and the male progeny were scored for the weak singed, extreme singed, and wild bristle phenotypes.

The results of these experiments are shown in the top of Table 1. The control *sn*^{w} mutation rates, which ranged from 0.500 to 0.533, are consistent with previous estimates for flies carrying the *H(hsp/CP)2* transposase source (Simmons *et al.* 2002a; Stuart *et al.* 2002). Both the *TP5* and the *TP6* stocks strongly repressed *sn*^{w} mutability. At all three sampling times, the mutation rates for the flies with either of the telomeric *P* elements were low compared to the controls. Thus, during the yearlong sampling period, the stocks with the telomeric *P* elements maintained a strong ability to repress transposase activity similar to that observed when they were first tested [2.5 years earlier; see Stuart *et al.* (2002, Table 4)].

The repression ability associated with a telomeric *P* element is lost by passage through the male germ line: Previous analyses have indicated that telomeric *P* elements lose their ability to repress transposase activity when they are transmitted through the male germ line (Stuart *et al.* 2002). To verify this result, males collected from the *TP5 w sn*^{w}, *TP6 w sn*^{w}, and control *w sn*^{w} stocks at the second time point in the study described above were crossed to *C(1)DX, y f ; H(hsp/CP)2* females and their *sn*^{w}*; H(hsp/CP)2/+* sons were assayed for *sn*^{w} mutability. The bottom of Table 1 presents the results of these experiments. The control mutation rate, 0.571, is somewhat higher than the corresponding rate from the experiment in which the *sn*^{w} allele was maternally derived. This greater value might be due to maternal transmission of the transposase activity encoded by the *H(hsp/CP)2* transgene (Simmons *et al.* 2002b). The mutation rates of the *TP5* and *TP6* stocks are indistinguishable from this higher control rate. Thus, the ability of a telomeric *P* element to repress *sn*^{w} mutability is lost when that element is transmitted from father to son.

Paternally transmitted telomeric *P* elements regain their ability to repress transposase activity when they are reestablished in homozygous stocks: To determine if paternally transmitted telomeric *P* elements can regain their regulatory abilities when they are returned to the female germ line, we "extracted" *TP w sn*^{w} (*TP* is *TP5* or *TP6*) *X* chromosomes from homozygous stocks by crossing individual males to *C(1)DX, y f* females from a pure M strain and then made the extracted *X* chromosomes homozygous using an *FM7* balancer *X* chromosome marked with the semidominant mutation *Bar* (*B*) eyes. This work was initiated at the same time as the analysis of sample 1 in Table 1. Sons from the cross involving the *C(1)DX, y f* females were mated to *FM7/sc*^{7} *l* (*sc, scute* bristles; *l*, recessive lethal) females from a pure M stock, and their *TP w sn*^{w}*/FM7* daughters were backcrossed to *TP w sn*^{w} males carrying the extracted chromosome to establish a homozygous *TP w sn*^{w} stock. Each of these stocks was maintained in small, mass-mated cultures without selection for repression of hybrid dysgenesis. At generations 2, 7, and 11, females from these stocks were mated to *H(hsp/CP)2* males to obtain *TP w sn*^{w}*; H(hsp/CP)2/+* sons, which were tested for germ-line *sn*^{w} mutability by crossing them individually to *C(1)DX, y f* females.

Table 2 summarizes the results of these tests. The M strain controls show that the *H(hsp/CP)2* transgene induced a high rate of *sn*^{w} mutability (0.532–0.577). All of the lines extracted from the *TP5* and *TP6* stocks were able to repress this mutability significantly. In the first test after they were made homozygous, most of the lines repressed *sn*^{w} mutability to a level below 0.10. However, a few lines (*e.g.*, TP5.5, TP6.1, TP6.3, TP6.8, and TP6.9) were less effective as repressors, with mutabilities ranging from 0.141 to 0.405. PCR with *TP5*- and *TP6*-specific primers was used to determine if the telomeric *P* elements were present in 10–20 test males from three of these lines (TP5.5, TP6.1, and TP6.3); all the males in these samples proved to carry the appropriate telomeric *P* element.

TABLE 2

Repression of sn^w mutability by homozygous lines established from the *TP5* and *TP6* stocks

	Generation 2			Generation 7			Generation 11		
Line	No. vials	No. flies	Mutation rate[a]	No. vials	No. flies	Mutation rate[a]	No. vials	No. flies	Mutation rate[a]
M control	50	1751	0.577 ± 0.016	50	1693	0.532 ± 0.014	47	1748	0.539 ± 0.013
TP5.1	48	1373	0.019 ± 0.008	30	815	0.000 ± 0.000	28	700	0.001 ± 0.001
TP5.2	46	1085	0.075 ± 0.021	29	744	0.007 ± 0.004	30	826	0.018 ± 0.011
TP5.3	48	1641	0.059 ± 0.016	29	429	0.003 ± 0.002	30	996	0.002 ± 0.000
TP5.4	NT			8	235	0.012 ± 0.008	30	967	0.000 ± 0.000
TP5.5	49	1663	0.177 ± 0.019	30	757	0.041 ± 0.013	30	1093	0.045 ± 0.011
TP5.6	27	702	0.045 ± 0.016	29	569	0.030 ± 0.018	29	964	0.006 ± 0.002
TP5.7	50	1714	0.039 ± 0.009	30	819	0.008 ± 0.004	28	941	0.043 ± 0.014
TP5.8	46	1235	0.072 ± 0.016	30	818	0.010 ± 0.004	25	870	0.007 ± 0.005
TP5.9	48	1593	0.095 ± 0.017	30	818	0.030 ± 0.014	30	793	0.027 ± 0.015
TP5.10	49	1712	0.016 ± 0.005	29	535	0.025 ± 0.016	29	1034	0.000 ± 0.000
TP6.1	47	904	0.379 ± 0.027	30	913	0.121 ± 0.021	30	947	0.282 ± 0.030
TP6.2	46	951	0.037 ± 0.013	30	650	0.008 ± 0.008	30	906	0.039 ± 0.011
TP6.3	48	1240	0.405 ± 0.029	30	874	0.203 ± 0.025	30	914	0.284 ± 0.026
TP6.4	47	979	0.049 ± 0.013	30	942	0.015 ± 0.007	30	893	0.030 ± 0.011
TP6.5	48	1047	0.059 ± 0.013	30	882	0.017 ± 0.007	30	790	0.013 ± 0.005
TP6.6	49	1610	0.103 ± 0.017	30	760	0.028 ± 0.011	30	852	0.020 ± 0.010
TP6.7	38	1342	0.062 ± 0.014	30	790	0.007 ± 0.003	29	918	0.056 ± 0.011
TP6.8	50	1757	0.239 ± 0.024	30	758	0.087 ± 0.023	30	1038	0.028 ± 0.015
TP6.9	48	1569	0.141 ± 0.021	30	791	0.030 ± 0.010	29	1033	0.021 ± 0.008

NT, not tested.
[a] Unweighted mean mutation rate ±SE.

The extracted lines were retested for repression of sn^w mutability in generations 7 and 11. All the lines except TP6.1 and TP6.3 repressed sn^w mutability to a level below 0.10, and most of them repressed it to a level below 0.05. The observed mutabilities for TP6.1 and TP6.3 ranged from 0.121 to 0.284. In generation 13, males from these two lines were examined by PCR for the presence of the *TP6* element. All the tested males (15 from TP6.1 and 14 from TP6.3) proved to carry this element.

These experiments demonstrate that telomeric *P* elements that have been passed through the male germ line can regain repression ability after being made homozygous. However, sometimes the original level of repression ability associated with a telomeric *P* element is not achieved.

Telomeric *P* elements maintain repression ability when they are transmitted maternally: We conducted a series of genetic experiments to investigate the role of the female germ line in the establishment and maintenance of repression by *TP5* and *TP6*. Each experiment was performed at three different times to assess the reproducibility of the results. The first and second of these replications coincided with the tests of samples 2 and 3 in Table 1.

The first type of experiment was designed to determine if the repression ability of the telomeric *P* element could persist through three generations of maternal transmission. During two of these generations, the element was heterozygous. Homozygous sn^w females were collected from the *TP5*, *TP6*, and control stocks and mated to *y* sn^w or *FM6, y dm B* males; the *y* locus is tightly linked to the left telomere of the *X* chromosome and *FM6* is a balancer *X* chromosome. The *(TP) w* sn^w*/y* sn^w or *(TP) w* sn^w*/FM6* heterozygous daughters from these crosses were mated to *y* sn^w males, and their *(TP) w* sn^w*/y* sn^w daughters were mated to homozygous *H(hsp/CP)2* males. The *y* sn^w (yellow body) and *(TP) w* sn^w (non-yellow body) sons from these last matings—both heterozygous for the *H(hsp/CP)2* transposase source—were then individually tested for sn^w mutability by crossing them to *C(1)DX, y f* females.

Table 3 summarizes the results of the three replications of this experiment. In the first two replications, only the y^+ flies from the last mating were tested for sn^w mutability. In the third replicate, both the y^+ and y flies from this mating were tested. The y flies, which did not carry a telomeric *P* element, provided an opportunity to see if repression ability could be transmitted through the egg cytoplasm independently of the telomeric *P* element itself.

The mutation rates for the control flies ranged from 0.471 to 0.554 and were similar to previous estimates of sn^w mutability obtained using the *H(hsp/CP)2* transposase source. The mutation rates for the *TP5* and *TP6* flies were significantly less than the control rates. When

TABLE 3

Repression of sn^w mutability by telomeric *P* elements transmitted through females for three generations

	M control			*TP5*			*TP6*		
Replicate[a]	No. vials	No. flies	Mutation rate[b]	No. vials	No. flies	Mutation rate[b]	No. vials	No. flies	Mutation rate[b]
Maternal + zygotic effect									
I (y^+)	49	1505	0.471 ± 0.018	49	1480	0.142 ± 0.021	52	1579	0.116 ± 0.021
II (y^+)	44	1556	0.539 ± 0.024	48	1981	0.177 ± 0.025	50	2020	0.307 ± 0.027
III (y^+)	50	1637	0.501 ± 0.014	48	1573	0.033 ± 0.009	49	1694	0.301 ± 0.024
Maternal effect only									
III (y)	49	597	0.554 ± 0.015	50	1748	0.549 ± 0.018	47	1491	0.580 ± 0.018

[a] In replicates I and II, the initial mating involved *y* sn^w males. In replicate III, it involved *FM6, y dm B* males.
[b] Unweighted mean mutation rate ±SE.

a maternally transmitted *TP5* was present in the tested flies, the rates ranged from 0.033 to 0.177, and when a maternally transmitted *TP6* was present, they ranged from 0.116 to 0.301. Thus, sn^w mutability was repressed by telomeric *P* elements that had been transmitted through females for three generations; in two of these generations, the elements were heterozygous. Furthermore, *TP5* appeared to be a stronger repressor than *TP6*.

The mutation rates from the y flies in replicate III were similar to the control mutation rates—if anything, slightly higher—even though in two cases, a telomeric *P* element was present in the mothers of the tested males. Thus, as Stuart *et al.* (2002) showed with a slightly different type of experiment, repression ability is not transmitted through the egg cytoplasm independently of the telomeric *P* elements themselves.

Paternally transmitted telomeric *P* elements reacquire repression ability during one generation in a female: Having shown that a telomeric *P* element retains repression ability during transmission through heterozygous females, we next analyzed whether a paternally inherited telomeric *P* element could acquire repression ability during one generation in a female (Figure 1). In this experiment homozygous *w* sn^w females were crossed to *y* sn^w or *FM6, y dm B* males and their *w* sn^w/*y* sn^w or *w* sn^w/*FM6* daughters were crossed to *TP w* sn^w males. The two types of females that were produced by these F_1 crosses, (a) *TP w* sn^w/*w* sn^w females, which had white eyes, and (b) *TP w* sn^w/*y* sn^w or *TP w* sn^w/*FM6* females, which had red eyes, were mated to homozygous *H(hsp/CP)2* males, and the resulting F_3 males were tested for sn^w mutability. From the type a females, the males that lacked the telomeric *P* element were used as controls. These males were distinguished from their *TP* sn^w brothers by performing PCR with an element-specific primer after they had mated. From the type b females, only the sons that carried the telomeric *P* element were tested for sn^w mutability. These males could be distinguished from their non-*TP* brothers by body color (y^+ rather than y).

The results of three replica experiments are summarized in Table 4. The mutation rates for the control flies, which did not carry a telomeric *P* element, ranged from 0.522 to 0.636. The rates for the flies that carried telomeric *P* elements, derived from either type a or type b females, were consistently lower. For the flies carrying *TP5*, the rates ranged from 0.291 to 0.462, and the median value was 0.338. For the flies carrying *TP6*, the rates ranged from 0.394 to 0.458, and the median was 0.435. In a comparable experiment, Stuart *et al.* (2002) observed similar mutation rates—0.288 for *TP5* and 0.400 for *TP6*. These results therefore indicate that a telomeric *P* element that came from a male and passed through a female for one generation reacquires some ability to repress sn^w mutability. On average *TP5* seems to reacquire more repression ability than *TP6*. In six of seven comparisons based on the data in Table 4 and the data from Stuart *et al.* (2002), flies carrying *TP5* had lower mutation rates than flies carrying *TP6* ($P = 0.054$ by the sign test).

Reacquisition of repression ability is enhanced by the maternal effect of a telomeric *P* element: To determine if the reacquisition of repression ability by a paternally derived telomeric *P* element is influenced by maternally transmitted factors associated with a telomeric *P* element, we crossed TP_i *w* sn^w males to TP_j *w* sn^w/*y* sn^w or TP_j *w* sn^w/*FM6* F_1 females that were obtained by mating homozygous TP_j *w* sn^w females with *y* sn^w or *FM6* males (Figure 2). The F_2 TP_i *w* sn^w/*y* sn^w or TP_i *w* sn^w/*FM6* daughters from these crosses were then mated to males homozygous for the *H(hsp/CP)2* transgene, and their TP_i *w* sn^w*; H(hsp/CP)2*/+ sons, identified by having y^+ body color, were tested for sn^w mutability. The objective of these experiments was to see if TP_j, the telomeric *P* element in the F_1 females, enhanced the reacquisition of repression ability by the paternally derived element TP_i through a strictly maternal effect, *i.e.*, one transmitted to the F_2 females independently of TP_j itself. Ronsseray *et al.* (1993) called this effect the "pre-P cytotype."

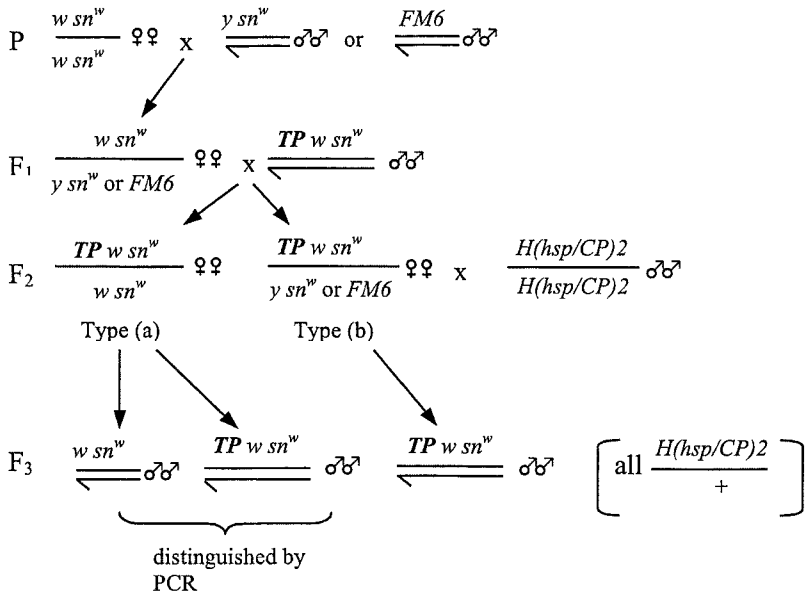

FIGURE 1.—Reacquisition of repression ability by a paternally inherited telomeric *P* element (*TP*).

We call it "presetting" and refer to TP_j as the "presetting" telomeric *P* element.

The results of three replica experiments are presented in Table 5. To evaluate them, we must refer to the data showing the reacquisition of repression ability by a paternally derived telomeric *P* element when no presetting telomeric *P* element was present; these data are in the bottom third of Table 4. A comparison of the data in Tables 4 and 5 indicates that the reacquisition of repression ability by a paternally derived *TP5* or *TP6* was significantly enhanced by a strictly maternal effect of either element. In the absence of a presetting telomeric *P* element, the mutation rates for *TP5* ranged from 0.291 to 0.374 (bottom third of Table 4), whereas with *TP5* as the presetting element, they ranged from 0.069 to 0.094 (Table 5, top left), and with *TP6* as the presetting element, they ranged from 0.126 to 0.249 (Table 5, bottom left). In the absence of a presetting telomeric *P* element, the mutation rates for *TP6* ranged from 0.399 to 0.458 (bottom third of Table 4), whereas with *TP5* as the presetting element, they ranged from 0.154 to 0.300 (Table 5, top right), and with *TP6* as the presetting element, they ranged from 0.213 to 0.341 (Table 5, bottom right). For both of the paternally de-

TABLE 4

Repression of sn^w mutability by paternally inherited telomeric *P* elements that were transmitted through females for one generation

	TP5 paternally transmitted in F_1			*TP6* paternally transmitted in F_1		
Replicate[a]	No. vials	No. flies	Mutation rate[b]	No. vials	No. flies	Mutation rate[b]
			Control from type a females (no *TP* present)			
I	25	899	0.549 ± 0.031	26	840	0.522 ± 0.017
II	47	1273	0.636 ± 0.014	23	507	0.560 ± 0.023
III	47	1180	0.553 ± 0.020	40	1167	0.554 ± 0.013
			TP males from type a females[c]			
I	21	753	0.462 ± 0.033	32	1022	0.394 ± 0.026
II	52	1540	0.346 ± 0.019	74	1900	0.443 ± 0.018
III	50	1283	0.331 ± 0.023	55	1778	0.447 ± 0.018
			TP males from b females[c]			
I	29	786	0.291 ± 0.027	28	741	0.485 ± 0.032
II	50	1752	0.374 ± 0.021	50	1592	0.426 ± 0.020
III	48	1677	0.307 ± 0.023	49	1774	0.399 ± 0.023

[a] In replicates I and II, the initial mating involved *y* sn^w males. In replicate III, it involved *FM6, y dm B* males.
[b] Unweighted mean mutation rate ±SE.
[c] The *TP* present in the F_3 males that were tested was the same as the *TP* present in the F_1 males (see Figure 1).

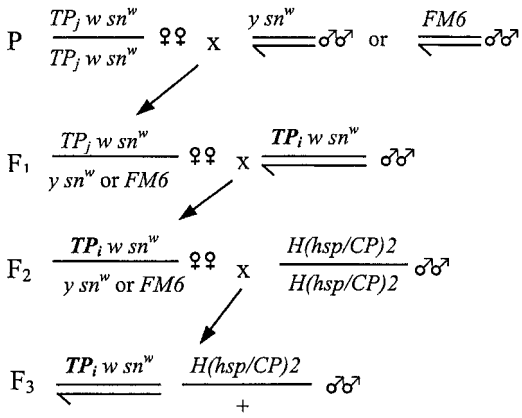

FIGURE 2.—Maternal effect of a telomeric *P* element (TP_j) on reacquisition of repression ability by a paternally inherited telomeric *P* element (TP_i).

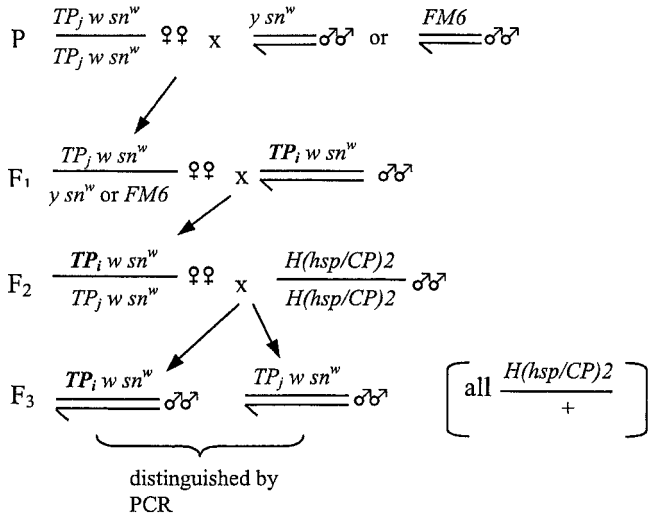

FIGURE 3.—Maternal and zygotic effects of a maternally inherited telomeric *P* element (TP_j) on reacquisition of repression ability by a paternally inherited telomeric *P* element (TP_i).

rived telomeric *P* elements, *z*-tests established that the mutation rates when presetting elements were present were significantly less than the corresponding rates when these elements were absent.

The data in Table 5 indicate that a strictly maternal effect of either *TP5* or *TP6* can enhance reacquisition of repression ability by either of these telomeric *P* elements—that is, the reacquisition of repression ability in a female is preset if her mother carried a telomeric *P* element even though that *P* element is not present in the female herself. In these experiments *TP5* reacquired greater repression ability than *TP6*, and *TP5* was the more effective presetting element. It is possible that presetting is due to the inheritance of one of the sn^w alleles that had coexisted with the telomeric *P* element in the F_1 (presetting) females. However, this hypothesis is not supported by the data from replicate III, in which the *FM6* balancer chromosome was used to preclude transmission of sn^w from the F_1 females to the F_2 females. Presetting therefore seems to involve a feature of the maternal cytoplasm that is transmitted to the F_2 females independently of either the telomeric *P* element or the sn^w allele.

Reacquisition of repression ability is enhanced by a maternally inherited telomeric *P* element: The reacquisition of repression ability by a paternally derived telomeric *P* element is enhanced by the strictly maternal effect of a presetting *P* element. What happens if this strictly maternal effect is combined with the zygotic effect of the presetting *P* element? To answer this question, we crossed $TP_i\ w\ sn^w$ males with $TP_j\ w\ sn^w/y\ sn^w$ or $TP_j\ w\ sn^w/FM6$ F_1 females to obtain $TP_i\ w\ sn^w/TP_j\ w\ sn^w$ F_2 females, which were mated to homozygous *H(hsp/CP)2* males (Figure 3). The two types of sons, $TP_i\ w\ sn^w$; *H(hsp/CP)2*/+ and $TP_j\ w\ sn^w$; *H(hsp/CP)2*/+, were then tested for sn^w mutability. Males carrying different telo-

TABLE 5

Effect of maternal presetting on repression of sn^w mutability by paternally inherited telomeric *P* elements that were transmitted through females for one generation

	TP5 paternally inherited			*TP6* paternally inherited		
Replicate[a]	No. vials	No. flies	Mutation rate[b]	No. vials	No. flies	Mutation rate[b]
			Presetting by *TP5*			
I	27	737	0.094 ± 0.017	29	821	0.154 ± 0.027
II	49	1691	0.069 ± 0.013	50	1714	0.300 ± 0.029
III	50	1913	0.091 ± 0.016	49	1741	0.200 ± 0.028
			Presetting by *TP6*			
I	29	973	0.161 ± 0.028	30	683	0.213 ± 0.024
II	50	1728	0.249 ± 0.028	50	1691	0.341 ± 0.024
III	50	1895	0.126 ± 0.020	50	1693	0.228 ± 0.024

[a] In replicates I and II, the initial mating involved $y\ sn^w$ males. In replicate III, it involved *FM6, y dm B* males.
[b] Unweighted mean mutation rate ±SE.

TABLE 6

Repression of sn^w mutability by a paternally inherited telomeric *P* element that had been paired with a maternally inherited telomeric *P* element in a female for one generation

	TP5 sn^w males tested			*TP6* sn^w males tested		
Replicate[a]	No. vials	No. flies	Mutation rate[b]	No. vials	No. flies	Mutation rate[b]
			Females *TP5* homozygoes			
I	28	824	0.040 ± 0.015			
II	50	1976	0.058 ± 0.013			
III	50	1771	0.021 ± 0.007			
			Females *TP6* homozygotes			
I				30	1188	0.082 ± 0.012
II				47	1711	0.157 ± 0.024
III				50	1935	0.066 ± 0.011
			Females *TP5/TP6* heterozygotes (*TP5* was maternally inherited)			
I	24	1028	0.067 ± 0.018	36	1319	0.039 ± 0.012
II	51	1808	0.117 ± 0.018	47	1644	0.090 ± 0.013
III	47	1609	0.027 ± 0.008	52	1798	0.047 ± 0.015
			Females *TP6/TP5* heterozygotes (*TP6* was maternally inherited)			
I	22	816	0.097 ± 0.028	36	1292	0.150 ± 0.030
II	55	2839	0.166 ± 0.024	45	2309	0.206 ± 0.032
III	61	1572	0.040 ± 0.009	37	923	0.070 ± 0.013

[a] In replicates I and II, the initial mating involved *y* sn^w males. In replicate III, it involved *FM6, y dm B* males.
[b] Unweighted mean mutation rate ±SE.

meric *P* elements were distinguished by PCR with element-specific primers after the males had mated.

The results of three replica experiments are shown in Table 6. First, we consider the cases in which TP_i and TP_j were identical in the F_2 females (top half of Table 6). These cases are similar to the situation in the *TP5* and *TP6* stocks because the F_2 females that were crossed to *H(hsp/CP)2* males were homozygous for one or the other of the telomeric *P* elements. In the case where $TP_i = TP_j = TP5$, the observed sn^w mutation rates from the tested males were low, similar to values obtained by sampling a homozygous *TP5* stock (Table 1). Likewise, in the case where $TP_i = TP_j = TP6$, the mutation rates were similar to those obtained by sampling a homozygous *TP6* stock. Thus, under these conditions, repression ability is much like that in the original *TP5* and *TP6* stocks.

Next, we consider the cases in which TP_i and TP_j were different in the F_2 females (bottom half of Table 6). In either case (*TP5* paternally derived, *TP6* maternally derived, or vice versa), the paternally derived telomeric *P* element invariably showed more repression ability, *i.e.*, a lower sn^w mutation rate, than it did in the simple test for reacquisition of repression ability (*cf.* Table 4; $P = 0.016$ by the sign test) or in the test for reacquisition of repression ability with a presetting effect (*cf.* Table 5; $P = 0.016$ by the sign test). Furthermore, in five of six comparisons, the maternally derived telomeric *P* element showed more repression ability than it did in the test for simple maternal transmission of repression ability (*cf.* Table 3; $P = 0.094$ by the sign test). Thus, these results suggest that, in the F_2 females, the maternally and paternally derived telomeric *P* elements mutually facilitate the establishment of repression ability and that the maternally derived *P* element has both a presetting and a zygotic effect on the acquisition of repression ability by the paternally derived *P* element.

DISCUSSION

Stocks homozygous for the telomeric *P* elements *TP5* or *TP6* have maintained a strong ability to repress hybrid dysgenesis from the time they were first tested to the time of the experiments reported here—a period of 3.5 years. The regulatory abilities of these elements are therefore stable in stocks over many generations. However, genetic analysis demonstrates that both *TP5* and *TP6* lose their regulatory abilities when they are transmitted through the male germ line. If this loss were irreversible, the repression abilities of the *TP5* and *TP6* stocks would be expected to dissipate over time since an increasing fraction of the *X* chromosomes in these stocks would have passed through males. The fact that homozygous *TP5* and *TP6* stocks maintain strong repression ability over many generations indicates that paternally inherited telomeric *P* elements are restored to full

repression ability when they pass through the female germ line. In every generation the regulatory abilities of these elements must therefore be reestablished.

Before attempting to dissect the reestablishment process, we monitored the repression abilities of *TP5* and *TP6* transmitted maternally through three generations—from homozygous females to heterozygous females to heterozygous females and then to hemizygous males in which repression ability was measured. Under these conditions, both *TP5* and *TP6* maintained repression ability, although this ability was less than that seen in males derived directly from homozygous *TP* females. Transmission through heterozygous females therefore attenuates the repression ability of a telomeric *P* element; furthermore, the attenuation is greater for *TP6*, which is the weaker of the two repressing elements.

Paternally transmitted telomeric *P* elements reacquire repression ability by passing through the female germ line. If the female does not carry another telomeric *P* element and if her mother did not carry such an element, the extent of this reacquisition is rather limited—and it is more limited for *TP6* than for *TP5*. Nevertheless, the repression ability of telomeric *P* elements in males derived from females in which reacquisition takes place is significant.

Reacquisition of repression ability is enhanced if the females in which the reacquisition occurs came from mothers who carried a telomeric *P* element. This enhancement has two components: (1) a strictly maternal effect that is transmitted to the females independently of the mother's telomeric *P* element and (2) a zygotic effect associated with inheritance of the mother's telomeric *P* element.

Ronsseray *et al.* (1993) referred to the first component as the pre-P cytotype; we call it presetting. This component suggests that either a product of the mother's telomeric *P* element or an effect of this element on some aspect of chromatic organization is transmitted through the egg cytoplasm to the females in which the paternally derived telomeric *P* element is partially restored to regulatory function. However, previous analyses with *TP5* and *TP6* have shown that repression ability itself is not transmitted through the egg cytoplasm independently of the telomeric *P* element (Stuart *et al.* 2002). Furthermore, the presetting effect does not seem to be mediated by an sn^w allele transmitted from the females that carry the presetting telomeric *P* element, and the presetting effect is not element specific. *TP5* can preset the reacquisition of repression ability by a paternally derived *TP6* or vice versa. However, *TP5* is more receptive to the presetting effect—*i.e.*, it reacquires more repression ability than *TP6*—and *TP5* is also the better presetting element—*i.e.*, it more strongly enhances the reacquisition of repression ability by a paternally derived *TP5* or *TP6*. Ronsseray *et al.* (1993) also obtained evidence that the pre-P cytotype is not element specific.

The strictly maternal effect of a presetting telomeric *P* element is further enhanced by a zygotic effect of that element. Maximal restoration of regulatory function is seen when a paternally inherited telomeric *P* element passes through a female who also inherited a telomeric *P* element from her mother. Under these circumstances, the repression ability of the paternally derived element is almost as strong as that seen in the homozygous *TP5* and *TP6* stocks. The repression ability of the maternally derived element is also boosted. Thus, maternally and paternally derived elements mutually facilitate the establishment of the P cytotype in the female germ line.

When *X* chromosomes that carry telomeric *P* elements are extracted through males and made homozygous by using a balancer chromosome, most of the resulting stocks develop a strong ability to repress hybrid dysgenesis. In a sample of 10 *TP5* and 9 *TP6* stocks, only two *TP6* lines, TP6.1 and TP6.3, failed to reach the repression ability characteristic of the original *TP6* stock. PCR experiments indicated that the *TP6* element was present in each of these lines; thus, their diminished repression ability cannot be attributed to loss of the telomeric *P* element. It might, however, be due to a genetic factor—possibly a feature of the telomere—that impairs repression by the *P* element. This factor might have been segregating in the *TP6* stock, and when lines were extracted from it, the factor might have been retained in some of them. A few test cultures in samples from the original *TP6* stock yielded sn^w mutation rates as high as or higher than the average rates seen in the TP6.1 and TP6.3 lines. Thus, the moderate repression ability of these lines could reflect variation that was already present within the *TP6* stock. Alternately, it could reflect a change that occurred when the lines were created.

The P cytotype is the paramount system for regulating *P* elements in the germ line. Genetic analysis has shown that this system is associated with *P* elements inserted near the left telomere of the *X* chromosome. It is not clear to what extent *P* elements inserted at other genomic locations may contribute to the P cytotype; however, some evidence suggests that they do (Ronsseray *et al.* 1998, 2001). Regulation by the P cytotype is established by the combined maternal and zygotic effects of telomeric *P* elements and, once established, is maintained stably over time. The mechanistic basis of this regulation is not known. One possibility is that it involves repression by some aspect of the chromatin organization of telomeres—a type of telomere position effect. However, the pre-P cytotype, or presetting effect, raises the possibility that a transmissible product of telomeric *P* elements, either a polypeptide or an RNA, is involved. It is not known if either *TP5* or *TP6* encodes a repressor polypeptide such as the one produced by the *KP* element. However, unlike *KP*, neither *TP5* nor *TP6* is widespread in natural populations of Drosophila (Stuart *et al.* 2002). A broad geographic distribution of these ele-

ments would be expected if they had been spread by natural selection based on the production of repressor polypeptides. The repression abilities of *TP5* and *TP6* might therefore not be related to their polypeptide-coding capacities. It is also not known if either *TP5* or *TP6* produces an RNA that could function as a regulatory factor, perhaps by interfering with the expression of transposase-encoding RNAs. Further work will be needed to determine if the P cytotype involves a product of telomeric *P* elements.

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