The Maternally Inherited Regulation of *P* Elements in *Drosophila melanogaster* Can Be Elicited by Two *P* Copies at Cytological Site 1A on the *X* Chromosome

Stéphane Ronsseray, Monique Lehmann and Dominique Anxolabéhère

Mécanismes Moléculaires de la Spéciation, Université Paris 6, 75005 Paris, France

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ABSTRACT

Two *P* elements, inserted at the cytological site 1A on an *X* chromosome from an *Drosophila melanogaster* natural population (Lerik, USSR), were isolated by genetic methods to determine if they are sufficient to cause the P cytotype, the cellular condition that regulates the *P* family of transposable element. The resulting "Lerik P(1A)" line (abbreviated "Lk-P(1A)") carries only one *P* element *in situ* hybridization site but genomic Southern analysis indicates that this site contains two, probably full length, *P* copies separated by at least one *Eco*RI cleavage site. Because the Lk-P(1A) line shows some transposase activity, at least one of these two *P* elements is autonomous. The Lk-P(1A) line fully represses germline *P* element activity as judged by the GD sterility and sn^w hypermutability assays; this result shows that the P cytotype can be elicited by only two *P* element copies. However, the Lk-P(1A) line does not fully repress Δ2-3(99B) transposase activity in the soma, although it fully represses Δ2-3(99B) transposase activity in the germline (Δ2-3(99B) is an *in vitro* modified *P* element that produces a high level of transposase activity in both the germline and the soma). The germline regulatory properties of the Lk-P(1A) line are maternally transmitted, even when the Δ2-3(99B) element is used as the source of transposase. By contrast, the partial regulation of Δ2-3(99B) somatic activity is chromosomally inherited. These results suggest that the regulatory *P* elements of the Lk-P(1A) line are inserted near a germline-specific enhancer.

THE *P* transposable element family in *Drosophila melanogaster* is responsible for the syndrome of hybrid dysgenesis which includes chromosome rearrangements, male recombination, high mutability and temperature-sensitive agametic sterility [called gonadal dysgenesis (GD) sterility] (KIDWELL, KIDWELL and SVED 1977) (for a review see ENGELS 1989). Dysgenesis occurs in the germline of hybrids produced by crosses between M type females which are devoid of *P* elements and P type males, which carry 25–55 *P* elements scattered throughout the genome (RUBIN, KIDWELL and BINGHAM 1982; BINGHAM, KIDWELL and RUBIN 1982). These genetic abnormalities are due to the mobilization of the *P* elements derived from the P strain. In the progeny of the reciprocal crosses as well as in the progeny of P strains themselves, the transposition rate of *P* elements is very low (PRESTON and ENGELS 1984) and essentially no dysgenesis occurs. This indicates that the activity of the *P* family is regulated by maternally transmitted factors. The members of the *P* element family are heterogeneous in size, consisting of both 2.9-kb complete elements and various smaller elements which are derived from the complete elements by internal deletions (O'HARE and RUBIN 1983). Complete *P* elements are autonomously transposable (SPRADLING and RUBIN 1982). The transposase has been shown to be an 87-kd protein encoded by a single cistron comprising four exons (KARESS and RUBIN 1984; LASKI, RIO and RUBIN 1986; RIO, LASKI and RUBIN 1986). In a P strain, about one third of the elements appear to be structurally complete and may therefore be autonomous (O'HARE and RUBIN 1983; K. O'HARE, personal communication). *P* element activity is controlled by a cellular state referred to as cytotype. The P cytotype, which is characteristic of P strains, represses transposition and the M cytotype, which is characteristic of M strains, permits it (ENGELS 1979a). In the short-term, the cytotype is maternally determined but in the long-term, it is chromosomally determined by the *P* elements themselves (ENGELS 1979a; KIDWELL 1981; ENGELS 1989). Expression of the P cytotype requires the presence of chromosomal *P* elements (SVED 1987).

The structure of the *P* elements that determine the P cytotype has not been fully elucidated. The study of the regulatory properties of lines harboring only complete *P* elements has suggested that the complete *P* elements do not establish the P cytotype (ANXOLABÉHÈRE *et al.* 1987; DANIELS *et al.* 1987; PRESTON and ENGELS 1989). Recent investigations have studied the properties of lines harboring single *P* elements that had been modified *in vitro* (ROBERTSON and ENGELS 1989; MISRA and RIO 1990). Although a few of these

in vitro-modified elements clearly have shown regulatory potential, none has been able to repress GD sterility nor has any exhibited the classical maternal inheritance of the P cytotype (ROBERTSON and ENGELS 1989; MISRA and RIO 1990; RIO 1990). These results have been attributed by the authors to an insufficient amount of the repressor in the stocks carrying these elements, either because the copy number was low or, because a strong germline specific enhancer was not present near the *P* element's insertion site; such as enhancer would be necessary to activate transcription of the regulatory *P* element in the germline where maternal inheritance would be determined.

Another way to identify elements that determine the P cytotype is to isolate them from the chromosomes of natural P cytotype strains. Indeed, it is possible that selection against dysgenesis could preferentially retain such elements at suitable genomic sites, *i.e.*, near germline-specific enhancers (see above). This approach was initially undertaken by NITASAKA, MUKAÏ and YAMAZAKI (1987) who suggested that repression of GD sterility was associated with a *P* element that could produce a truncated transposase. However, several other types of *P* element were present in the genome of the tested strain, complicating the interpretation of the results. In addition, the transmission of the regulatory ability in this strain was not investigated.

We have previously shown a correlation between the P cytotype and the occurrence of a *P* hybridization site in region 1A of the polytene *X* chromosome. This was observed both at intrapopulation (RONSSERAY and ANXOLABÉHÈRE 1986; IZAABEL 1988; BIÉMONT *et al.* 1990) and interpopulation levels (RONSSERAY, LEHMANN and ANXOLABÉHÈRE 1989). In the present study, we have constructed a line harboring only the 1A *P* elements (two copies were detected by molecular investigations) from an inbred line derived from a Russian wild population. The characteristics of these elements and the regulatory properties of the line carrying them are investigated.

MATERIALS AND METHODS

Drosophila stocks: The following stocks were used.

Lerik 1983: The Lerik laboratory strain was established from a sample of 50 flies collected in Lerik (Azerbaïdjan, USSR) at the end of 1983. This strain has the M cytotype and carries about 18 *P* elements as determined by *in situ* hybridization (BIÉMONT and GAUTIER 1988; RONSSERAY, LEHMANN and ANXOLABÉHÈRE 1989). It is therefore classified as an M′ strain.

Lerik-18 line: From the Lerik mass sample, 17 inbred lines were established in October 1984 and subsequently maintained by one sib pair each generation. In October 1988 (generation 62), three out of the 17 lines had the P cytotype as determined by GD sterility assays. Only these three lines had *P* elements at position 1A (Biémont *et al.* 1990). To determine if these elements caused the P cytotype, we isolated them from a line that had 16 *P* copies. This line is designated "Lerik No. 18."

Canton-*y*: an M strain marked with a spontaneous allele of *yellow*; this strain is derived from the long established laboratory strain "Canton-S" (KIDWELL, KIDWELL and SVED 1977).

Gruta: an old laboratory M strain (ANXOLABÉHÈRE *et al.* 1987).

Muller-5 (M): an M strain with the *Muller-5* balancer chromosome (marked with *Bar* w^a) (LINDSLEY and GRELL 1967).

Harwich-2: an inbred P strain derived from two females collected in Harwich, Massachusetts (KIDWELL, KIDWELL and SVED 1977). We used a subline with an unidentified autosomal recessive marker which spontaneously appeared in our Harwich stock. Its phenotype resembles that of the *sepia* mutation.

π_2: an inbred P strain derived from a natural population in Madison, Wisconsin (ENGELS and PRESTON 1979).

$M5/sn^w;\pi_2$: a P strain with the genetic background of π_2 (ENGELS 1985). It has both the *Muller-5* balancer chromosome and an *X* chromosome with two defective *P* elements inserted at the *singed* locus. This mutation called *singed-weak* (sn^w) causes a slight malformation of the bristles (ENGELS 1979b, 1981, 1984; ROIHA, RUBIN and O'HARE 1988). In the M cytotype, sn^w is unstable in the presence of transposase, mutating to sn^e (a more extreme allele) or to sn^+ (apparent wild type). In each case, the change of phenotype is due to the excision of one of the two *P* elements. Because the $M5/sn^w;\pi_2$ strain has the P cytotype, its sn^w allele is stable. The use of this strain allows the regulatory capacity of another strain to be tested by measuring the instability of sn^w in appropriate crosses (see below).

$y^{84}\ z^1\ w^{1118}\ sn^w\ P[w^{d1}\ 9.3](19DE)$, abbreviated "$sn^w$ *9.3*": this M cytotype strain carries the sn^w allele and a $P[w^{d1}\ 9.3](19DE)$ transgene (COEN 1990) which is highly instable under dysgenic conditions. The $P[w^{d1}\ 9.3](19DE)$ transgene is labeled with the *white* gene and gives a coloured eye phenotype. This strain has no other *P* sequences and cannot produce the transposase. Using appropriate crosses, the two hypermutable systems (sn^w and $P[w^{d1}\ 9.3](19DE)$) in this strain provide tests for the transposase and regulatory activities of other strains.

$w\ sn^3$: when made homozygous with the various derivatives of sn^w, sn^3 enhances their expression and makes scoring easier. The dominance relations of the singed alleles are $sn^+ > sn^w > sn^e = sn^3$ (SIMMONS 1987).

$ry^{506}\ P[ry^+\ \Delta 2\text{-}3](99B)$, abbreviated "Δ2-3(99B)": this M cytotype strain is a transformed derivative of a ry^{506} M strain. It carries an *in vitro*-modified *P* element called $P[ry^+\ \Delta 2\text{-}3]$ from which the last intron has been removed. The splicing of this intron is necessary for the production of transposase by the complete *P* element and is naturally restricted to the germline; this is responsible for the limitation of the complete *P* element activity to the germline (LASKI, RIO and RUBIN 1986). The $P[ry^+\ \Delta 2\text{-}3]$ element, therefore, produces transposase in both the somatic and germline cells. In the "Δ2-3(99B)" strain, the $P[ry^+\ \Delta 2\text{-}3]$ element has been inserted in cytological site 99B through germline transformation and is essentially immobile (ROBERTSON *et al.* 1988).

ry^{506} *Sb* $P[ry^+\ \Delta 2\text{-}3](99B)$, *Sb/TM6 Ubx*, abbreviated "Δ2-3(99B)*Sb*": This strain has the Δ2-3(99B) *P* element linked to the dominant marker *Sb*.

$ry^{506}\ P[ry^+\ \text{AluI}](86E)$ and $ry^{506}\ P[ry^+\ \text{SalI}](89D)$. These two stocks were generated by transformation of a ry^{506} M strain with *P* elements that had been mutated in exons 0 and 3 respectively (KARESS and RUBIN 1984). In each case, only a single copy of the *P* construct is present; the insertion

sites are indicated in parentheses. These stocks will be abbreviated as "*P*[AluI](86E)" and "*P*[SalI](89D)," respectively.

Muller-5 Birmingham: an inbred M′ strain derived from a *Muller-5* balancer stock from the University of Birmingham (BINGHAM, KIDWELL and RUBIN 1982). The line used here carries about 60 *P* elements; all of them are nonautonomous and devoid of regulatory properties (ENGELS *et al.* 1987). When combined with the Δ2-3(99B) transposase source, the Birmingham chromosomes cause a high level of pupal lethality, probably due to *P*-mediated chromosome breakages in the imaginal discs or histoblast cells. This pupal lethality occurs in the M cytotype and is suppressed by the P cytotype (ENGELS *et al.* 1987).

Birmingham *2/ CyO*: this strain has only the second chromosome of the *Muller 5* Birmingham strain balanced with the *CyO* balancer chromosome. The *X* and third chromosomes are devoid of *P* sequences. When combined with the Δ2-3(99B) transposase source, the Birmingham second chromosome produces some pupal lethality (ENGELS *et al.* 1987).

C(1)DX, y f/w v 1(1)44/Y, abbreviated "*C(1)DX(M)*": An M stock with compound-*X* chromosome that was used to collect virgin females. *1(1)44* is a thermosensitive *X*-linked lethal mutation (BUSSON *et al.* 1983).

C(1)DX, y w f (P)/w v 1(1)44(P)/Y; Harwich, abbreviated "*C(1)DX (P)*": a compound-*X* strain with the autosomes of the Harwich P strain. This strain therefore has the P cytotype (COEN 1990).

GD assays: For each test cross, 10–20 pairs were mated *en masse* and immediately placed at 28.5°. Approximately 2 days after the onset of eclosion, F_1 progeny were collected and allowed to mature for 2 days at room temperature. At least 50 F_1 females were then taken at random for dissection. Dissected ovaries were scored as unilaterally dysgenic (S1 type) or bilaterally dysgenic (S0 type) (SCHAEFFER, KIDWELL and FAUSTO-STERLING 1979). The frequency of GD was calculated as %GD = %S0 + ½ %S1. Cross A* (females of tested strain × P strain males) assesses the cytotypic properties of the tested strain. The M cytotype, allowing *P* elements to be active, results in a high or intermediate frequency of GD whereas the P cytotype, repressing *P* element activity, results in a low frequency of GD (<5%). Cross A (M strain females × males of tested strain) measures P activity potential. It reflects the ability of the tested strain to mobilize *P* elements in an unregulated state. Harwich-2 and π_2 were used as reference P strains and Canton-*y* and Gruta as reference M strains.

***sn*^{w} hypermutability assays:** The hypermutability of the *sn*^{w} allele can be used to test both the regulatory capacity and the transposase activity of a given strain.

For the regulation assay, 20–30 tested females were crossed *en masse* to *M5/sn*^{w};π_2 males at 20°. Twenty F_1 virgin females were crossed *en masse* to 20 *w sn*^{3} males at 25° and allowed to lay eggs in successive bottles for 10 days. Among the F_2 progeny, only *sn*$^+$ and *sn*^{w} individuals were scored and the mutation rate was calculated as follows: $u = (sn^+/(sn^+ + sn^w)) \times 100$.

For the transposase activity assay, 20–30 tested males were crossed *en masse* to *sn*^{w} *9.3* females at 20°. Twenty virgin F_1 females were crossed *en masse* to 20 *w sn*^{3} males at 25° and allowed to lay eggs in successive bottles for 10 days. Data were collected and analysed as in the regulation assay.

Test for the regulation of the Δ2-3(99B) somatic and germline activities: The mating scheme illustrated in Figure 1 was designed to compare the regulatory capacities of the Lk-P(1A) *P* insertions when inherited from either the grandmother or the grandfather. Because the regulatory *P* insertions in the Lk-P(1A) strain are located at 1A, they are tightly linked with the *yellow* locus. The strain Lk-P(1A) carries a *y*$^+$ allele, whereas the *sn*^{w} *9.3* strain carries *y*. Consequently, only the F_2 sons with wild type body color should carry the regulatory *P* elements located at 1A. Among the different F_2 males produced by recombination between the *sn*^{w} *9.3* and Lk-P(1A) *X* chromosomes, two kinds were chosen for the regulation assays: those with the Lk-P(1A) insertions (*y*$^+$ phenotype) along with the *P*[*w*^{d1} 9.3](19DE) transgene and (as negative controls) those with the *P*[*w*^{d1} 9.3](19DE) insertion but without the Lk-P(1A) insertions (*y* phenotype). All these F_2 males carried the Δ2-3(99B) transposase source.

Somatic regulation was assessed by the degree of eye color mosaicism resulting from the excision of the *P*[*w*^{d1} 9.3]-(19DE) transgene. Two levels of mosaicism were distinguished: the "severe" form in which the central part of the eye (half of the diameter) contains some ommatidia with little or no pigment (due to the excision of the *P*[*w*^{d1} 9.3]-(19DE) transgene and expression of the *w*^{1118} allele from the *sn*^{w} *9.3* line or the *w*^{sp} allele from the Lk-P(1A) line—see Figure 1) and the "nonsevere" form in which this part of the eye is fully pigmented (wild coloration when *P*[*w*^{d1} 9.3]-(19DE) has not been excised). Mosaicism was quantified by the percentage of eyes that showed the severe form in each class of the F_2 males that were examined (see above).

The germline regulation assay was performed on the same F_2 males by crossing them to *C(1)DX(P)* females and scoring their F_3 progeny for *w*^{1118} or *w*^{sp} sons. These types of sons result from excision of the *P*[*w*^{d1} 9.3](19DE) transgene in the germline of their fathers. No mosaicism occurs in the F_3 because the F_2 mothers have the P cytotype. Two parameters were used for quantification: (1) the frequency of F_2 males that produced at least one excision event among their progeny (only the F_2 males with at least 20 F_3 sons were considered); (2) the overall percentage of *P*[*w*^{d1} 9.3]-(19DE) excision, calculated by pooling the progeny of all the *y* or *y*$^+$ F_2 males. In this assay, all generations were reared at 25°, except the F_1 which was reared at 18°.

Pupal lethality rescue assay: Twenty females from the line under test were crossed at 18° to 20 Δ2-3(99B)*Sb* males (Figure 2). Twenty F_1 males, carrying the Δ2-3 element (*Sb* phenotype) were crossed to 20 *Muller-5* Birmingham females or to 20 Birmingham *2/CyO* females and the progeny were allowed to develop at 25°. In either cases, the *Sb*$^+$ and *Sb* phenotypes were scored among the F2 daughters that were not *Curly*. In the absence of regulation, the *Sb* individuals are expected to die.

***In situ* hybridization:** Hybridization to polytene chromosomes was performed using a tritium dCTP-labeled probe made from the plasmid Pπ25.1 (O'HARE and RUBIN 1983) which contains a complete *P* element plus genomic DNA from cytological region 17C. The 17C label was used as a positive control of hybridization.

Southern blot analysis: The number and structure of the *P* sequences in the Lk-P(1A) line were analyzed by genomic Southern blots. Genomic DNA from the Lk-P(1A) line was digested with the restriction enzymes *Eco*RI, *Acc*I and *Dde*I. Genomic DNA from the control lines *P*[AluI](86E) and *P*[SalI](89D) was digested with *Acc*I and *Dde*I. All digests were run on 0.9% agarose gels, transferred to a nitrocellulose filter by blotting and hybridized with a *P* element probe made by nick-translating the plasmid Pπ25.7BWC. This plasmid contains a *P* element that lacks 39 bp from its left (5′) end and 23 bp from its right (3′) end and has no flanking genomic DNA (K. O'HARE, personal communication). Hybridization conditions were 5 × Denhardt's solution, 6 × SSC and 0.5% SDS at 65°. The filters were washed for 30 min in 2 × SSC, 0.1% SDS at room temperature.

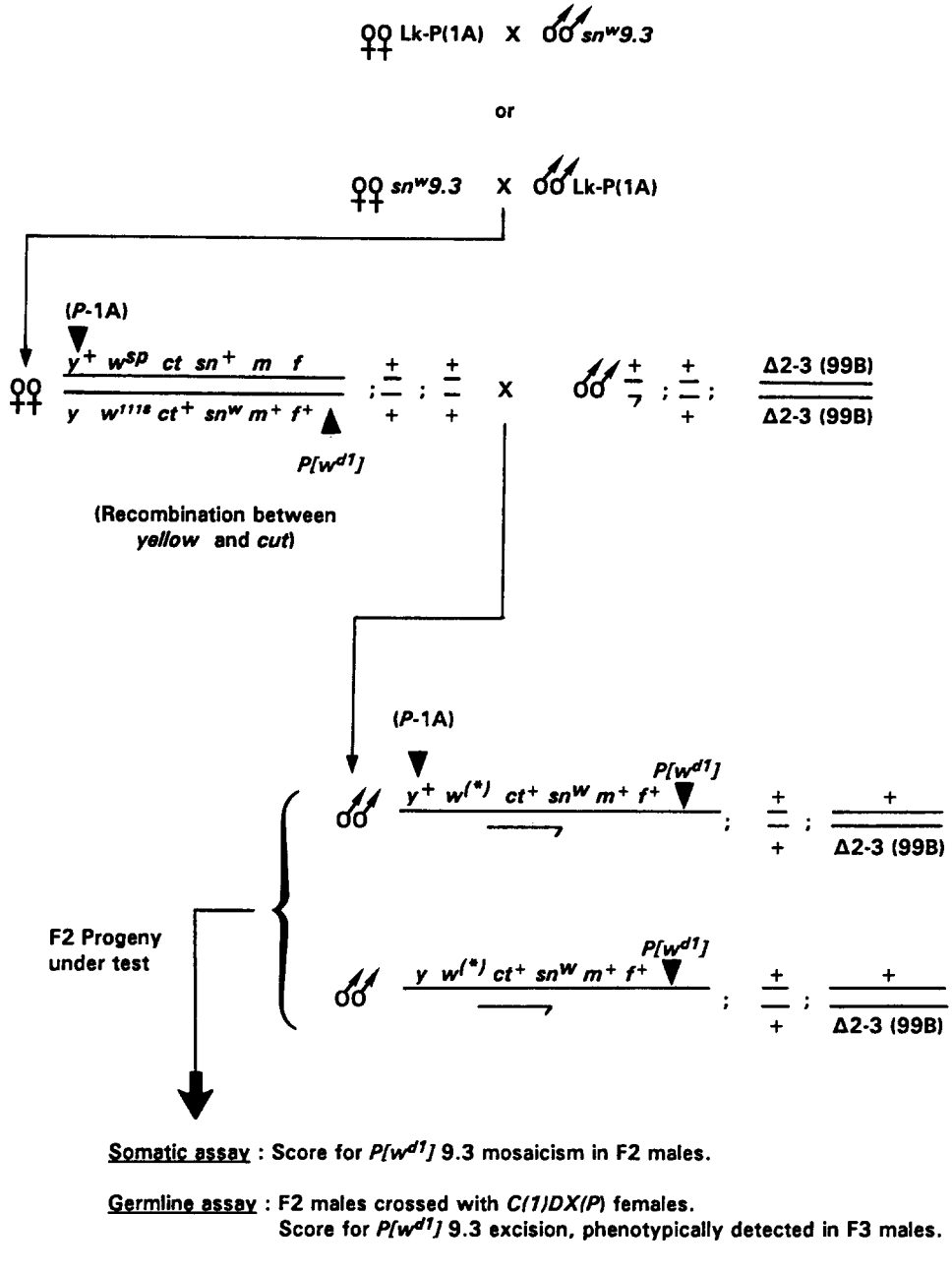

FIGURE 1.—Mating scheme to test for the regulation of Δ2-3(99B) somatic and germline activities. The symbol "$w^{(*)}$" means either w^{1118} or w^{sp} depending on where recombination occurred between *y* and *ct* in the F_1 female germline.

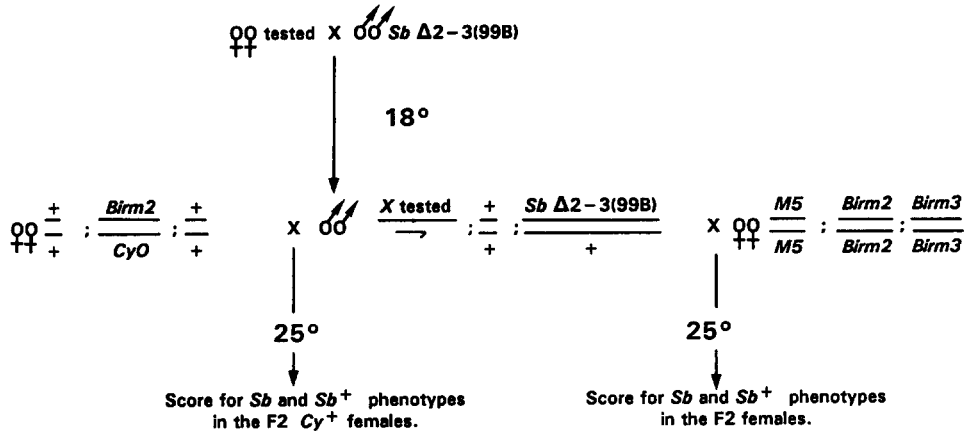

FIGURE 2.—Mating scheme of pupal lethality rescue assay.

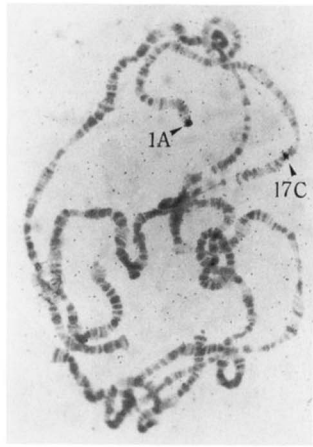

FIGURE 3.—*In situ* hybridization of the Lk-P(1A) polytene chromosomes using a tritium labelled *P* element clone as a probe. The Pπ25.1 clone used here contains the complete *P* element plus genomic DNA from the 17C region. The *P* insertion site at 1A and the genomic hybridization site (at 17C) are indicated.

RESULTS

The synthesis of a line with only one *P* hybridization site at 1A: A line carrying the tip of the *X* chromosome from the inbred "Lerik-18" line in an M type autosomal genetic background was synthesized. The first step of this synthesis was the substitution of the Lerik-18 autosomes with autosomes devoid of *P* elements. Males from the Lerik-18 line were initially crossed to *X*-attached M type females (*C(1)DX(M)*) and then six successive backcrosses between males and *X*-attached females were carried out at 18°. *In situ* hybridization was used to identify a subline from these crosses which carried *P* elements on the *X* chromosome, including site 1A, but not on the autosomes (data not shown). In the second step in the isolation of the 1A *P* elements, we removed the proximal *P* elements on the *X* chromosome by recombination between *yellow* (at 1A) and *scute* (at 1B), using a multiply marked *X* chromosome from an M strain. The crosses were carried out at 18°. A recombinant *X* chromosome (whose genotype was y^+ *sc* w^{sp} *m f*) was obtained and made homozygous using the *Muller-5* balancer *X* chromosome (LINDSLEY and GRELL 1967). The resulting y^+ *sc* w^{sp} *m f* line was checked by *in situ* hybridization; only one *P* element hybridization site was found and it was at 1A (Figure 3). This line is designated Lerik-18 P(1A) *sc* w^{sp} *m f* but it will be abbreviated as "Lk-P(1A)" hereafter. The stability of the P element insertion in this line was checked by performing *in situ* hybridization on five sublines (reared at 18°) over a period of more than ten generations: no changes occurred during this period, showing that the *P* elements in the Lk-P(1A) line were stable.

The Lk-P(1A) line represses GD and sn^w hypermutability: The ability of the Lk-P(1A) line to repress GD was investigated using the A* cross. P cytotype strains can be defined as those in which the frequency of GD sterility is less than 5% in the progeny of this cross. The result of the A* GD assay for the Lk-P(1A) line is given in Table 1 along with results for M and P cytotype controls. This assay was performed with two different P strains. For the Lk-P(1A) line, complete repression of sterility was observed, indicating that it was as strong a repressor as the reference P strain, Harwich-2.

TABLE 1

GD and germline sn^w hypermutability regulation

Tested strain	% GD sterility (1)	% GD sterility (2)	Mutation rate
Lk-P(1A)	0.1	0.0	0.1
	(402)	(207)	(1774)
Canton-*y*	100	100	6.9
	(100)	(100)	(1227)
Gruta	100	99.0	7.1
	(150)	(100)	(707)
Harwich-2	3.8		0.2
	(144)		(2095)

Gonadal sterility regulation: cross A* was performed using males from two different P strains: (1) = Harwich-2; (2) = π2. The percentage of atrophic ovaries is given with the number of females examined in parentheses.

Germline sn^w hypermutability regulation: hypermutability was scored among F_2 males and females produced by the classical regulation assay. The results with the two sexes were similar and are therefore pooled. The mutation rate is equal to $u = (sn^e/(sn^e + sn^w)) \times 100$. The numbers in parentheses correspond to the denominator of the fraction.

The ability of the Lk-P(1A) line to repress excision of the *P* elements inserted in the hypermutable sn^w allele was also investigated. Table 1 shows that the Lk-P(1A) line is an effective repressor of this aspect of P activity. The frequency of excision is similar to that seen with the P cytotype strain. Harwich-2, but very much less than the frequencies seen with the two M strains that were used as negative controls.

From the GD and sn^w assays, it is clear that the Lk-P(1A) line shows germline regulation of P activity similar to that of true P cytotype strains.

The inheritance of GD sterility repression is maternally inherited and thermosensitive: A peculiar characteristic of cytotype is its partial maternal inheritance. Indeed F_1 females produced by the two reciprocal crosses between M and P cytotype individuals show different regulatory properties when they are tested using the A* GD assay (ENGELS 1979a). Stronger repression is observed in the F_1 females whose mother had the P cytotype. We have investigated this phenomenon with the Lk-P(1A) line by crossing Lk-P(1A) and M cytotype individuals reciprocally and then testing their F_1 daughters using the A* GD assay. These investigations were carried out using two different M cytotype strains (Canton-*y* and sn^w *9.3*) to produce reciprocal hybrids with Lk-P(1A).

TABLE 2

Maternal inheritance and thermosensitivity of GD regulation

Initial cross	GD sterility assay on F_1 females: % of dysgenic ovaries among F_2 females		
	At emergence	AT18	AT28.5
♀♀ Canton-*y* × ♂♂ Lk-P(1A)	94.3 (4.3)	97.6 (2.9)	10.2 (5.5)
	9	7	6
♀♀ Lk-P(1A) × ♂♂ Canton-*y*	2.2 (2.9)	42.9 (9.4)	1.2 (2.1)
	9	8	5
♀♀ *sn*^{w} *9.3* × ♂♂ Lk-P(1A)	98.8 (1.6)		
	6		
♀♀ Lk-P(1A) × ♂♂ *sn*^{w} *9.3*	31.3 (5.7)		
	3		

F_1 was allowed to develop at 25°. Sets of 20 F_1 females were crossed to Harwich-2 males in order to perform the A* GD sterility assay after different thermal treatments on virgin F_1 females: at emergence = no treatment; AT18 = 15 days of aging treatment at 18°; AT2.85 = 10 days of aging treatment at 28.5°. For each replicate, a minimum of 50 F_2 females were dissected. The mean GD percentage calculated on the basis of all replicates is given with the standard deviation among replicates (in parentheses). The number of replicates is given under the standard deviation.

From the first column of Table 2, it is clear that the F_1 females with a Lk-P(1A) mother had much stronger repression ability (less GD sterility) than the F_1 females with a Lk-P(1A) father. Because the two types of females had similar genotypic constitutions, this shows that the regulation of P activity by the Lk-P(1A) line is maternally transmitted.

The determination of cytotype in F_1 females produced by crosses between M and P cytotype strains is strongly thermosensitive (RONSSERAY, ANXOLABÉHÈRE and PÉRIQUET 1984; RONSSERAY 1986). During imaginal life, an 18° treatment results in a switch toward the M cytotype, whereas a 25° (or higher) treatment results in a switch toward the P cytotype. These temperature-induced modifications can be reversed.

The thermosensitivity of cytotype determination in F_1 females resulting from crosses between Lk-P(1A) and Canton-*y* individuals was investigated by aging these females at different temperatures. Table 2 shows that the F_1 females from the cross Canton-*y* females × Lk-P(1A) males, which have the M cytotype at emergence (%GD = 94.3), remain M after aging at 18° (%GD = 97.6) but switch towards the P cytotype after aging at 28.5° (%GD = 10.2). F_1 females from the reciprocal cross, which have the P cytotype at emergence (%GD = 2.2), remain P after aging at 28.5° (%GD = 1.2) but switch to an incomplete repression level (%GD = 42.9) after aging at 18°. Thus, the F_1 females produced by crosses between Lk-P(1A) and M cytotype individuals show strong thermosensitivity in their cytotype determination.

Using these germline regulation assays, it is clear that the Lk-P(1A) line shows all the characteristics reported for true P cytotype strains.

TABLE 3

Regulation of the Δ2-3 somatic and germinal activities

Assay	Initial cross	
	♀♀ *sn*^{w} *9.3* × ♂♂ Lk-P(1A)	♀♀ Lk-P(1A) × ♂♂ *sn*^{w} *9.3*
(A)		
F_2 male *y*	90.9 (254)	93.5 (370)
F_2 male y^+	66.8 (388)	62.3 (268)
Harwich	2.9 (102)	3.6 (234)
(B)		
F_2 male *y*	10/10	12/12
F_2 male y^+	21/21	3/27
(C)		
F_2 male *y*	38.2 (14.4)	27.4 (8.0)
F_2 male y^+	22.5 (16.3)	0.7 (2.2)

See MATERIALS AND METHODS and Figure 1 for a description of the protocol. (A) Somatic regulation assay: percentage of males with "severe" mosaicism among F_2 males from the cross ♀♀F_1 × ♂♂ Δ2-3(99B). As a positive control, the somatic regulation assay was performed using a Harwich *white* line in the initial cross instead of the Lk-P(1A) strain. (B) Germline regulation assay: ratio of F_2 males with at least one germline excision event. The number of F_2 males that showed at least one P[w^{di}9.3](19DE) excision event among a minimum of 20 F_3 sons is given before the slash and the total number of F_2 males tested is given after the slash. (C) The mean excision rate calculated on the basis of all F_3 males examined is given with its standard deviation (in parentheses).

The Lk-P(1A) line differentially controls Δ2-3(99B) transposase activity in the germline and the soma: An experiment was designed to test the regulatory effects of the Lk-P(1A) *P* insertions on Δ2-3 transposase activity in the soma and in the germline. Individuals carrying both the Δ2-3(99B) transposase source and the *P*[w^{d1} 9.3](19DE) transgene in a w^{1118} background were produced either with or without the Lk-P(1A) insertions. These correspond to the F_2 male progeny described in the crossing scheme presented in Figure 1. As described in MATERIALS AND METHODS, the y^+ F_2 males carry the Lk-P(1A) insertions whereas the *y* F_2 males do not. To test for maternal inheritance of regulation by the Lk-P(1A) insertions, the experiment was started with reciprocal crosses between Lk-P(1A) and *sn*^{w} *9.3* individuals (Figure 1).

In the somatic assay, the percentage of severe mosaicism (see MATERIALS AND METHODS) was calculated for each kind of F_2 male. Table 3A shows that whether the grandmother was *sn*^{w} *9.3* or Lk-P(1A), partial repression of *P*[w^{d1} 9.3](19DE) mosaicism was observed in the y^+ males (66.8% and 62.3%). By contrast, the *y* males showed a higher level of mosaicism (90.9% and 93.5%). The control experiment, starting with the Harwich strain instead of Lk-P(1A), showed an almost complete repression of mosaicism (2.9% and 3.6%). It can be concluded that the Lk-P(1A) insertions cause incomplete repression of somatic mosaicism but that this repression does not show maternal inheritance (66.8% and 62.3% do not differ significantly, $\chi^2 = 1.37$, d.f. = 1, $P > 0.52$).

TABLE 4

Pupal lethality rescue

	Birmingham autosomal complement			
	2 + 3		*2*	
Tested strain	*Sb*	*Sb*$^+$	*Sb*	*Sb*$^+$
Lk-P(1A)	0	170	11	185
Canton-*y*	0	346	0	242
Harwich-2	250	263	44	76

The *X* chromosome of the tested strain was combined with Birmingham autosomes in the presence (*Sb* phenotype) and in the absence (*Sb*$^+$ phenotype) of the Δ2-3(99B) transposase source. The number of individuals with these phenotypes is given.

Repression of the excision of the *P*[w^{d1} 9.3](19DE) transgene in the germline of the same F_2 males was also investigated. Excision events were detected by crossing these males with *C(1)DX(P)* females and by scoring for w^{1118} or w^{sp} F_3 sons. The results (Table 3B) show that all the F_2 males that did not carry the Lk-P(1A) insertions (yellow F_2 males) had at least one germline excision event. For the y^+ F_2 males carrying the Lk-P(1A) insertions, the situation differs depending on whether the grandmother was M or P. All F_2 males showed at least one excision event when the grandmother had the M cytotype whereas only 3 F_2 males out of 27 whose grandmother was Lk-P(1A) showed a germline excision event. This difference (21/21 and 3/27) is highly significant ($\chi^2 = 37.5$, d.f. $= 1$, $P < 0.001$). The same conclusion may be derived using the overall excision rate; strong repression was observed only in the y^+ F_2 males with Lk-P(1A) grandmothers (Table 3C). Thus it appears that the Lk-P(1A) line strongly represses Δ2-3(99B) activity in the germline and this repression ability is maternally inherited. This result contrasts with that for somatic regulation which shows that only a partial repression of Δ2-3(99B) somatic activity occurs, without any detectable maternal inheritance (Table 3A). Thus it is possible to differentiate between somatic and germinal regulation.

Pupal lethality rescue by the Lk-P(1A) line: The combination of defective *P* elements from the Birmingham M′ strain and the Δ2-3 transposase source leads to lethality at the pupal stage (ROBERTSON *et al.* 1988). The incidence of pupal lethality depends on both the number of defective *P* elements in the genome and the rearing temperature. It also depends on the regulatory state and can be fully repressed by the P cytotype. Rescue of pupal lethality therefore provides an assay for *P* element regulation (ROBERTSON *et al.* 1988). In order to test the ability of the Lk-P(1A) line to rescue pupal lethality, we have combined in the same female the Lk-P(1A) *X* chromosome with the Δ2-3(99B) transposase source and one or two Birmingham autosomes (Birmingham chromosomes *2* and *3* in one experiment and Birmingham chromosome *2* in another; see Figure 2). In these experiments, the Δ2-3 element was linked to the dominant marker *Sb*; therefore, in the absence of regulation, flies with this marker were expected to die, whereas their *Sb*$^+$ sibling were expected to live. In each experiment, the numbers of F_2 females with the *Sb*$^+$ and *Sb* phenotypes were scored; the results are given in Table 4. In the experiment performed with Birmingham chromosomes *2* and *3*, no *Sb* flies were observed in the crosses with the Lk-P(1A) and Canton-*y* strains. This shows that these strains were not able to repress the lethal effect of Δ2-3. In contrast, the positive control, Harwich-2, produced roughly equal numbers of *Sb* and *Sb*$^+$ flies, indicating that it possessed strong regulatory ability. In the experiment performed with the Birmingham second chromosome, the Lk-P(1A) strain showed some ability to rescue the *Sb* flies (11 *Sb* and 185 *Sb*$^+$), whereas, as expected, the Canton-*y* strain showed no such ability (0 *Sb* and 242 *Sb*$^+$). This difference is highly significant ($\chi^2 = 11.8$, d.f. $= 1$, $P < 0.001$). From these experiments it appears that the Lk-P(1A) line possesses only a weak ability to repress the Δ2-3-induced pupal lethality.

TABLE 5

Induction of GD sterility and *sn*^{w} hypermutability

	% GD sterility		
Tested strain	(1)	(2)	Mutation rate
Lk-P(1A)	1.0	0.4	0.85
	(100)	(135)	(23/2703)
Harwich-2	100	100	12.1
	(100)	(150)	(106/876)
sn^w *9.3*			0.0
			(0/2185)

Induction of GD sterility by the males: cross A was performed using females from two M strains: (1) = Canton-*y*; (2) = Gruta. The percentage of atrophic ovaries is given with the number of females examined in parentheses.

Tranposase activity: the transposase assay based on the hypermutable allele *sn*^{w} was performed. The scores of the two sexes were similar and are pooled. The mutation rate is equal to $u = (sn^r/(sn^r + sn^w)) \times 100$; the raw data are given in parentheses.

Induction of GD sterility and *sn*^{w} hypermutability: The ability of Lk-P(1A) males to induce GD sterility was tested by crossing them to two M strains. Table 5 shows that there was no significant sterility in the hybrid progeny of these crosses, indicating that the Lk-P(1A) males could not induce gonadal dysgenesis. In contrast, the strong P strain, Harwich-2, which was included as a positive control, induced complete sterility as expected.

The presence of transposase producing *P* elements in the Lk-P(1A) line was investigated using the *sn*^{w} hypermutability assay (see MATERIALS AND METHODS). Transposase producing elements were present because 23 *sn*^{e} individuals were observed among a total of 2703 flies (Table 5). There is therefore at least one

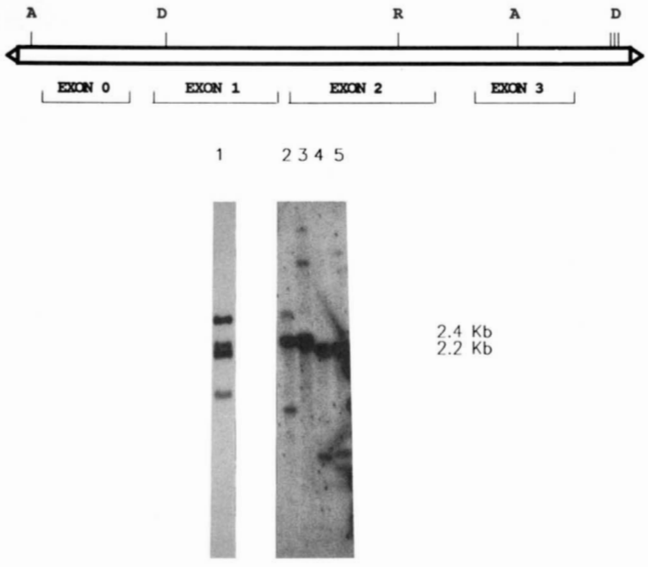

FIGURE 4.—Number and structure of the *P* elements in the Lk-P(1A) line as determined by Southern blotting. A, The structure of the complete *P* element showing the positions of the four exons. Restriction sites for *Eco*RI (R), *Acc*I (A) and *Dde*I (D) are indicated. B, Lk-P(1A) genomic DNA was digested with *Eco*RI (lane 1), *Acc*I (lane 3) and *Dde*I (lane 5). The genomic DNA of the line *P*[AluI]-(86E) and of the line *P*[SalI](89D) (Karess and Rubin 1984) were digested with *Acc*I (lane 2) and with *Dde*I (lane 4) respectively. The two small fragments in lane 5 do not comigrate with the 0.42-kb fragment produced by the digest of a *KP* element with *Dde*I (data not shown).

autonomous *P* element at the 1A site. However, transposase activity is lower for the Lk-P(1A) line than for the Harwich-2 P strain which was included as a positive control. This difference is not surprising because the Harwich-2 strain carries 50 *P* elements, about one third of which are thought to be complete (O'HARE and RUBIN 1983). The absence of GD activity by the A test cross with the Lk-P(1A) line can be interpreted as a consequence of both the low copy number of complete *P* elements in this line and the low copy number of all *P* elements; indeed the induction of GD sterility probably requires both a minimum level of transposase synthesis and also a minimum number of *P* element targets (SIMMONS *et al.* 1987, RASMUSSON *et al.* 1990).

Two *P* elements are inserted in the 1A site: The transposase activity detected by the sn^w hypermutability assay indicates that the Lk-P(1A) line carries at least one complete *P* element. We investigated the number of *P* elements inserted at 1A by Southern blotting analysis. In Figure 4B (lane 1), DNA from the line Lk-P(1A) was digested with *Eco*RI and probed with complete *P* element DNA. Since one *Eco*RI site is present in the complete *P* element (Figure 4A), we would expect two *Eco*RI fragments to hybridize with *P* in the Lk-P(1A) DNA if this line contains only one complete *P* element. The size of these fragments would depend on the position of the *Eco*RI sites in the region adjacent to the insertion and therefore cannot be predicted. However, we found that four *Eco*RI restriction fragments hybridize with the probe indicating that more than one *P* element was present at the 1A site. Two of the four bands can be attributed to the complete *P* element detected genetically. The other two bands can either be due to another *P* element that contains the *Eco*RI site or to two defective *P* elements that have lost this site. In either case, all the *P* elements in the 1A site are separated by at least one *Eco*RI restriction site.

To investigate the structure of the *P* elements located at 1A, we have looked for the presence of internal restriction fragments of the complete *P* element in the Lk-P(1A) genomic DNA. DNAs from a line that contains only a modified *P* element *P*[AluI]-(86E) (Figure 4, lane 2) and from the Lk-P(1A) line (Figure 4, lane 3) were digested with *Acc*I and probed with the complete *P* element. This digest produces a 2.4-kb internal fragment in the case of a complete *P* element (Figure 4A) and in the case of the *P*[AluI](86E) element (KARESS and RUBIN 1984) which was used as a control. The Lk-P(1A) DNA (lane 3) contains a 2.4-kb *Acc*I fragment which hybridizes strongly with the probe and which comigrates with the *Acc*I fragment from the *P*[AluI](86E) element (lane 2). The intensity of the 2.4-kb fragment is stronger in the Lk-P(1A) line than in the *P*[AluI](86E) line suggesting the presence of two copies of this fragment in the former. Two other weak bands are also detected in the Lk-P(1A) line DNA; these bands could correspond to *Acc*I fragments that contains the 3′ end of a *P* element (Figure 4A) plus some adjacent sequence. One *P* element insertion in 1A would result in only one such band. (In the case of *P*[AluI](86E) (lane 2), both weak bands are observed but they are expected from the *P* construct (KARESS and RUBIN 1984).) This result strongly suggests that two full-length (or nearly full-length) *P* elements are inserted in 1A. A similar conclusion was reached when DNAs from the Lk-P(1A) line and from the *P*[SalI](89D) line (used as a control) were digested with *Dde*I (lanes 4 and 5). The complete *P* element, as well as the *P*[SalI](89D) element, produces a 2.2-kb internal *Dde*I fragment, which is found in the Lk-P(1A)DNA (lane 5). The strong intensity of this 2.2-kb fragment in the Lk-P(1A) DNA could correspond to the presence of two copies of this fragment in the Lk-P(1A) line. Two weak *Dde*I bands are also detected in the Lk-P(1A) DNA. These bands again suggest the presence of two *P* element insertions in the Lk-P(1A) line since each band probably contains the 5′ end of an element plus some adjacent DNA.

From these results, we conclude that two *P* elements are present at 1A, that they are separated by at least one *Eco*RI restriction site and that at least one of the elements (and possibly both) is complete.

DISCUSSION

In the Lk-P(1A) line, the P cytotype, including maternal inheritance, is elicited by only two *P* element copies: The P cytotype is the cellular state that represses *P* element transposition and hybrid dysgenesis. It is maternally transmitted in the short-term but in the long-term it is determined by the chromosomal *P* elements themselves (ENGELS 1979a, 1989; KIDWELL 1981). The nature of the *P* elements that are necessary to establish the P cytotype is not completely elucidated. No single element specifying all the features of the P cytotype, including maternal inheritance, has yet been described (ENGELS 1989; RIO 1990). Moreover, the regulatory properties of the *P* elements seem to depend, not only on their structure but also on their genomic position (ROBERTSON and ENGELS 1989; MISRA and RIO 1990; SIMMONS *et al.* 1990).

We have isolated the tip of a wild *X* chromosome with two *P* elements at cytological site 1A that establish the P cytotype, as judged by different germline regulation assays. The assays include GD sterility, sn^w hypermutability and Δ2-3(99B)-induced germline *P* element activity. The results show that the properties of the Lk-P(1A) strain with these *P* elements are identical to those of classical P cytotype strains such as Harwich or π_2. These similarities include the maternal inheritance of cytotype and the thermosensitivity of its determination in (M × P) F_1 hybrid females.

We do not have a complete picture of the exact structure of the *P* element(s) responsible for the P cytotype in the Lk-P(1A) strain, because there are apparently two *P* elements present at the 1A site. At least one of these is autonomous as some transposase activity has been detected. In fact, both elements appear to be structurally complete. It must be pointed out that the Lk-P(1A) line does not show the presence of a *KP* element. The *KP* element is a particular deleted *P* element (between positions 808 and 2560) which is abundant in the original Lerik strain (BIÉMONT *et al.* 1990) and which is suspected to play a role in *P* regulation (BLACK *et al.* 1987; JACKSON, BLACK and DOVER 1988; SIMMONS *et al.* 1990). The presence of a *KP* element was not detected by our Southern blot analysis (Figure 4B). Finally, it will of course be necessary to separate the two *P* elements of Lk-P(1A) strain, to determine their precise structure and to assay their regulatory abilities independently to identify what kind of elements are responsible for the P cytotype in this strain. It is also possible that the two P copies at 1A are necessary to cause the P cytotype if their functions are complementary.

One of the models proposed to explain *P* element regulation is the "transposase titration" (SIMMONS and BUCHOLZ 1985). In this model, the regulation of *P* transposition is due to the presence of a large number of defective *P* elements. These elements titrate, by means of their DNA sequences, the transposase produced by the complete *P* elements. According to this model, a high *P* copy number is necessary to establish *P* transposition repression and a portion of these copies must be supposed to be extrachromosomal to explain maternally inherited *P* regulation. Such a situation is not likely for the Lk-P(1A) line which has only two *P* copies; this would imply that one, or both, elements at 1A generate many extrachromosomal *P* copies. Note, however, that such extrachromosomal *P* copies have never been detected.

A second model proposes that a subset of the *P* family is able to produce proteins that act as repressors of transposition. This model is supported by the work of ROBERTSON and ENGELS (1989) and MISRA and RIO (1990). These investigations have shown that lines containing single defective *P* elements that can only produce a truncated transposase corresponding to the first three exons exhibit some of the properties of the P cytotype. The *P*[SalI](89D) strain (ROBERTSON and ENGELS 1989) mimics the P cytotype when tested by the expression of different cytotype sensitive alleles. All of these alleles result from *P* insertions [*singed*, ROBERTSON and ENGELS (1989); *vestigial*, WILLIAMS, PAPPU and BELL (1988); $P[w^{d1}$ 9.3](19DE), COEN (1990)]. The *P*[SalI](89D) strain also represses Δ2-3(99B) activity in the soma and, to some extent, in the germline. Other strains containing single *P* elements that produce a truncated 66 kD protein were shown to repress sn^w hypermutability in the germline (MISRA and RIO 1990). However, none of the lines studied by ROBERTSON and ENGELS (1989) and by MISRA and RIO (1990) exhibit the maternal inheritance that is characteristic of the P cytotype. Moreover, the *P*[SalI]-(89D) strain of ROBERTSON and ENGELS (1989) failed to repress the occurrence of GD sterility when a classical P strain was used as the transposase source (MISRA and RIO (1990) did not perform this assay for their lines). To explain the lack of maternal inheritance the authors proposed two alternative explanations. (1) Many repressor-making elements must be present to cause maternal inheritance of cytotype and to repress the occurrence of GD sterility. (2) The repressor elements must be inserted at genomic locations that result in high levels of expression, especially during oogenesis. Our findings with the Lk-P(1A) strain argue against the first explanation because this strain has only 2 *P* elements. However, the second explanation is consistent with the regulatory abilities of the Lk-P(1A) strain.

The repression abilities of Lk-P(1A) are weaker in the soma than in the germline: Although *P* element transposition is naturally restricted to the germline, *P* regulation can be assessed in somatic tissues by using the Δ2-3(99B) transposase source or cytotype

dependent alleles. However, this approach to the study of cytotype is indirect and conclusions drawn from it may not apply to the situation in the germline. In fact, previous studies have suggested that *P* elements with strong regulatory ability in the soma but little or no ability in the germline are more frequently found that *P* elements with the opposite properties (ROBERTSON and ENGELS 1989; W. R. ENGELS, personal communication). The Lk-P(1A) strain shows a partial regulation in the somatic mosaicism assay and weak regulation in the pupal lethality assay. Further we have assayed the expression of the *vestigial*$^{21.3}$ allele; this allele is due to a *P* element insertion in the *vestigial* locus. Its phenotype is vg^{extreme} in M cytotype flies while it is almost wild type in P cytotype flies (WILLIAMS, PAPPU and BELL 1988). We have genetically obtained males which carry the Lk-P(1A) *X* chromosome and are homozygous for the *vestigial*$^{21.3}$ allele; the phenotype of these males is not rescued and is similar to the phenotype of the *vestigial*$^{21.3}$ control line which has an M cytotype (data not shown). Altogether, the results of the germline and somatic assays of regulation by the Lk-P(1A) strain contrast with those previously reported (ROBERTSON and ENGELS 1989; W. R. ENGELS, personal communication). The Lk-P(1A) situation can be explained if we suppose that the *P* elements responsible for the P cytotype in this line are more intensively expressed in the germline than in the soma, as they might be if they were under the control of a germline specific genomic enhancer located at 1A. Such an enhancer would stimulate the production and the accumulation of *P* products during oogenesis (see RIO 1990). Indeed such accumulation is probably necessary for the maternal inheritance of *P* repression as MISRA and RIO (1990) detected a large amount of the 66-kD truncated protein in the oocytes of the π_2 strain (which exhibits maternal inheritance) but not in the oocytes of their *P*66-kD transformed lines which do not exhibit maternal inheritance. To explain the fact that the classical P cytotype strains (Harwich or π_2) exhibit strong regulatory properties in both the soma and the germline, it can be proposed that several regulatory *P* elements are present in the genome of these strains. These elements are inserted at various genomic locations and the combination of the regulatory effects of all of them results in *P* regulation in both somatic and germinal tissues.

Maternal inheritance is detected only in the germline: The ability of the Lk-P(1A) insertions to regulate Δ2-3(99B) activity was tested in both the soma and the germline and tests were carried out to investigate the possibility of maternal inheritance. The results have clearly shown that the partial repression of *P*[w^{d1} 9.3](19DE) mosaicism in the eyes was biparentally inherited while the complete repression of germline Δ2-3(99B) activity was strongly maternally inherited (Table 3). Two genotypically identical males, carrying the Lk-P(1A) insertions, can show similar regulatory properties in the soma but dramatically different ones in the germline, depending on the cytotype of their grandmother. Results consistent with these were also observed in our laboratory by B. LEMAITRE and D. COEN using *P-LacZ* (enhancer-trap) insertions. They found that the *P* promoter is repressed by *P* regulatory products (LEMAITRE and COEN 1991) and that the maternal effect of this repression is restricted to the germline (B. LEMAITRE and D. COEN, personal communication). All of these results are consistent and indicate that only the germline, but not the soma, can exhibit the maternal inheritance of *P* regulation. On this point, it is important to recognize that germline assays are essential for the study of *P* regulation and for the identification of regulatory *P* elements.

The high frequency of *P* elements at 1A in natural P cytotype populations probably results from a selection process: A high frequency of *P* element insertions at 1A has been observed in natural populations (AJIOKA and EANES 1989; RONSSERAY, LEHMANN and ANXOLABÉHÈRE 1989). Further, these insertions are associated with the P cytotype (RONSSERAY, LEHMANN and ANXOLABÉHÈRE 1989). KIDWELL (1981) also found that the determinants of P cytotype in a strain mapped to the distal part of the *X* chromosome. Other studies have shown that the 1A site is not a hot-spot for *P* element *de novo* insertion (AJIOKA and EANES 1989). Moreover, *P* elements inserted at 1A have not lost their ability to excise because we succeeded in provoking excision of the Lk-P(1A) insertions using hybrid dysgenesis conditions (data not shown). So the simplest explanation for the high frequency of *P* insertions at 1A in P cytotype populations is that a selective process is operating.

We suggest that there is a genomic sequence near the *P* elements inserted in 1A that enhances *P* element expression in the germline at the stage when the immunity to the deleterious effects of dysgenesis is determined. Thus, whenever a potentially regulatory *P* element inserts in 1A, it evokes the P cytotype and prevents hybrid dysgenesis. The frequency of this insertion is expected to increase until the population acquires the P cytotype. Further analysis will be required to test this hypothesis.

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