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Maternal inheritance of P cytotype in *Drosophila melanogaster*: a "pre-P cytotype" is strictly extra-chromosomally transmitted

Stéphane Ronsseray, Bruno Lemaitre*, Dario Coen

Département "Dynamique du Génome et Evolution", Institut Jacques Monod, Tour 43, 2 place Jussieu, F-75251 Paris Cedex 05, France

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Abstract. In *Drosophila melanogaster*, transposition of the *P* element is under the control of a cellular state known as cytotype. The P cytotype represses *P* transposition whereas the M cytotype is permissive for transposition. In the long-term, the P cytotype is determined by chromosomal *P* elements but over a small number of generations it is maternally inherited. In order to analyse the nature of this maternal inheritance, we tested whether a maternal component can be transmitted without chromosomal *P* elements. We used a stable determinant of P cytotype, linked to the presence of two *P* elements at the tip of the *X* chromosome (1A site) in a genome devoid of other *P* elements. We measured *P* repression capacity using two different assays: gonadal dysgenic sterility (GD) and *P-lacZ* transgene repression. We show that zygotes derived from a P cytotype female (heterozygous for *P* (1A)/balancer devoid of *P* copies) and which inherit no chromosomal *P* elements from the mother, have, however, maternally received a P-type extra-chromosomal component: this component is insufficient to specify the P cytotype if the zygote formed does not carry chromosomal *P* elements but can promote P cytotype determination if regulatory *P* elements have been introduced paternally. We refer to this strictly extra-chromosomally inherited state as the "pre-P cytotype". In addition, we show that a zygote that has the pre-P cytotype but which has not inherited any chromosomal *P* elements, does not transmit the pre-P cytotype to the following generation. The nature of the molecular determinants of the pre-P cytotype is discussed.

Key words: *Drosophila melanogaster* – *P* element – Cytotype – Maternal inheritance – *P-lacZ* fusion gene

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* *Present address:* Université Louis Pasteur, Laboratoire de Biologie Générale, 12, rue de l'Université, F-67000 Strasbourg, France

Correspondence to: S. Ronsseray

Introduction

The hybrid dysgenesis syndrome in *Drosophila melanogaster* is produced by the family of *P* transposable elements. This syndrome includes chromosome rearrangements, male recombination, high mutability and temperature-sensitive agametic sterility (GD sterility, Kidwell et al. 1977; for a review see Engels 1989). Dysgenesis occurs in the germline of hybrids produced by crosses between M-type females (devoid of *P* elements) and P-type males (carrying 25–55 *P* elements scattered throughout the genome; Rubin et al. 1982; Bingham et al. 1982; Ronsseray et al. 1989). These genetic abnormalities are due to the mobilisation of *P* elements derived from the P strain. In the progeny of the reciprocal crosses as well as in the progeny of P strains, *P* elements show a very low transposition rate (Preston and Engels 1984); essentially no dysgenesis occurs. This indicates that the activity of the *P* family is regulated by a maternally transmitted cellular state referred to as P cytotype (Engels 1979). P cytotype, which is characteristic of P strains, represses transposition. Its absence (M cytotype), characteristic of M strains, permits it. Engels (1979) showed that cytotype determination involves both chromosomal and maternally inherited components and is governed by complex rules of inheritance (for a review see Engels 1989). Firstly it presents the maternal effect described above: the two types of G_1 females produced by the M female × P male and P female × M male crosses are genotypically identical but only the first type shows hybrid dysgenesis (Kidwell et al. 1977). Secondly, the transmission of cytotype over several generations shows, at least partially, maternal inheritance. The two types of G_1 females have, and transmit to their daughters, different cytotype properties. The female progeny produced by a P cytotype mother have and transmit greater *P* repression capacities than those produced by an M cytotype mother (Engels 1979; Kidwell 1981). The influence of this initial maternal inheritance can be detected for up to five generations, provided that chromosomal regulatory *P* elements are maintained in the genome at

each generation (Engels 1979). After this period, the level of equilibrium of *P* repression in the lineage depends mainly on the chromosomal *P* element complement (Engels 1979). The nature of the maternally inherited component is not completely understood. It is thought to result from the accumulation, in the oocytes of the P females, of *P* product(s) encoded by chromosomal *P* elements (Engels 1989; Rio 1990; Misra and Rio 1990).

Sved (1987) has shown that inheritance of the maternally transmitted component alone is not sufficient to maintain the P cytotype even for one generation: in the progeny of a P cytotype female that is heterozygous for regulatory *P* elements, females that did not inherit chromosomal *P* elements did not show any apparent remnant of P cytotype. He concluded that the maternally inherited component is not auto-replicative and that it must be replenished at each generation by chromosomal regulatory *P* elements. Although such females appear to have an M cytotype, it is still possible that they have in fact retained some regulatory properties, not detectable in the Sved experimental design, which are not sufficient to elicit the P cytotype in these females but which could stimulate the switch towards P in their progeny, provided that regulatory *P* elements were paternally reintroduced.

In this paper, we describe experiments carried out to investigate this possibility and the nature of P cytotype maternal inheritance. This was facilitated by the use of a line which contains, in a genome devoid of other *P* copies, two *P* elements inserted at the tip of the *X* chromosome (site 1A), which determine the P cytotype (Ronsseray et al. 1991). The use of *X*-linked markers enabled us to follow the segregation of the *P*(1A) regulatory elements. The *P* regulatory properties of tested females were analysed by measuring their ability to repress the occurrence of dysgenic sterility and to repress the activity of the *P* promoter of an enhancer-trap insertion (*P-lacZ* fusion gene; Lemaitre and Coen 1991) whose expression is restricted to the germline (J.L. Couderc and F. Laski, personal communication; Lemaitre et al. 1993).

We show the existence of a strictly extra-chromosomal component in P cytotype maternal inheritance. Zygotes produced by a P cytotype mother and that have not received maternal P elements do however show evidence of having received a maternal component which we call pre-P cytotype. This pre-P cytotype promotes the determination of the P cytotype, provided that regulatory *P* elements have been introduced by the paternal gamete. A zygote that has received such a pre-P cytotype, but which has not inherited chromosomal *P* elements, does not transmit the pre-P cytotype to the following generation. We discuss the nature of this extra-chromosomal component.

Materials and methods

Strains employed. Lerik-18P(1A) *sc* w^{sp} *m f*, designated *Lk-P*(1A): This line has two full-length *P* elements at the cytological site 1A in a chromosomal context devoid of other *P* elements (Ronsseray et al. 1991). We made this line by genetically recombining the tip of an *X* chromosome (between 1A and 1B) from a natural population (Lerik USSR 1983, kindly provided by C. Biémont; Biémont et al. 1990) onto the *X* chromosome of an M strain and introducing this recombinant into the background of autosomes from M laboratory stocks. The *Lk-P*(1A) line presents all the properties of a P cytotype strain as tested by germline regulation assays (Ronsseray et al. 1991). In particular, it fully represses the occurrence of dysgenic sterility when crossed to P strain males and its *P* repression capacity is maternally transmitted. This line manifests transposase activity in genetic tests (destabilisation of the sn^w-allele, Engels 1984), showing that at least one of the two *P* elements at 1A is autonomous. In fact the two *P* elements at 1A have been separated in vivo by an excision experiment. The two complementary lines thus formed, each of which carries a single *P* element, both show transposase activity. This shows that each of the two *P* copies of the *Lk-P*(1A) line is autonomous (S. Ronsseray, unpublished data). Control studies of the *Lk-P*(1A) lines show that this line is stable, probably because of its P cytotype.

Muller-5 (M) designated *M5*: an M line with the *Muller-5* balancer *X* chromosome (*Basc* chromosome marked with *Bar* w^a; Lindsley and Zimm 1992). *Bar* is semi-dominant.

Canton-*y*: an M line marked with a spontaneous allele of *yellow;* this strain is derived from the long established laboratory strain Canton-S (Kidwell et al. 1977).

Harwich-2: an inbred P line derived from two females collected in Harwich, Massachusetts (Kidwell et al. 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.

BC69: a line harbouring an insertion of *P(lacZ, ry*$^+$*)A* (referred to as the *P-lacZ* element) on the second chromosome, balanced by *CyO*. *P(lacZ, ry*$^+$*)A* contains an in-frame translational fusion of the *Escherichia coli* β-galactosidase gene *(lacZ)* to the second exon of the *P* transposase gene and the *rosy*$^+$ gene as a marker for transformation (O'Kane and Gehring 1987). This strain has no other *P* sequences and has an M cytotype. The *BC69 P-lacZ* insertion was isolated in a screen for female sterile mutations and expresses the *P-lacZ* transgene only in the germline tissues of ovaries and testes (J.L. Couderc and F. Laski, personal communication). We have previously used this strain to demonstrate P cytotype repression of the *P* promoter in the germline (Lemaitre et al. 1993).

C(1)DX, y f/w v l(1)44/Y, designated *C(1)DX*(M): an M stock with compound-*X* chromosomes that was used to collect virgin females. *l(1)44* is a thermosensitive *X*-linked lethal mutation (Busson et al. 1983).

P(1A); *BC69/Xa:* this designation refers to males bearing the *X* chromosome from the *Lk-P*(1A) line with the *BC69 P-lacZ* insertion on the second chromosome and an M-derived autosomal translocation $(T(2;3)ap^{Xa}$, see Lindsley and Zimm 1992). These males were derived from a cross between *P*(1A); *Xa* females and *BC69/CyO* males. This mating scheme was begun with P maternal

inheritance from *P*(1A) females which had been shown to prevent the mobilisation of *P* sequences (see Discussion).

P *repression assays*

1. Gonadal dysgenesis assays (A* assay). The *P* regulatory properties of females were determined by measuring their ability to repress the occurrence of dysgenic sterility (Kidwell et al. 1977). The A* test cross (females of tested strain × P strain males) was performed using Harwich-2 as the P reference strain. For each test cross, 10–20 pairs were mated en masse and immediately placed at 28.5° C. Approximately 2 days after the onset of eclosion, progeny from the test cross were collected and allowed to mature for 2 days at room temperature. From this progeny, 30–50 females were then taken at random for dissection. Dissected ovaries were scored as unilaterally dysgenic (S1 type) or bilaterally dysgenic (S0 type; Schaefer et al. 1979). The frequency of gonadal dysgenesis was calculated as % GD = % S0 + ½%S1. This percentage will be referred to as the percentage of GD A*. The M cytotype, which allows *P* elements to be active, results in a high percentage of GD A* (>95%), whereas the P cytotype, which represses *P* element activity, results in a low percentage of GD A* (<5%). Intermediate percentages indicate incomplete repression capacities.

2. Quantitative measurement of β-galactosidase activity. In a previous study, comparisons were made of the expression of several *P-lacZ* fusions in P or M cytotypes. The β-galactosidase activity of these *P-lacZ* fusion genes was strongly repressed in the P background but not in the M background, showing that *P* repressor(s) repress(es) in vivo the *P* promoter activity (Lemaitre and Coen 1991; Lemaitre et al. 1993). The regulatory properties of females were measured following the method of Lemaitre and Coen (1991), using the germinally expressed *P-lacZ* insertion, *BC69*. Measurements were performed directly on crude extracts of ovaries of the females tested. Three ovaries were homogenised in Z buffer (Miller 1972) and centrifuged for 10 min at 10000 rpm (4° C) to remove debris. β-Galactosidase activity was measured as described in Miller (1972). The protein concentration was determined in each sample by the Bradford protein assay (BioRad), using BSA as a standard. Results are given in nmol/min per mg of protein. *P-lacZ* activity and GD sterility repression assays produced results which are generally strongly correlated (Lemaitre 1992).

3. Southern blot analysis. Genomic DNA was digested with the restriction enzyme *Eco*RI which cleaves only once within the complete *P* element. Digests were electrophoresed through 1% agarose gels, transferred to a nitrocellulose filter by blotting and hybridised with a *P* element probe made by nick-translating the plasmid Pπ25.7BWC (O'Hare et al. 1992). This plasmid contains a *P* element that lacks 39 bp from the left (5′) end and 23 bp from the right (3′) end and has no flanking genomic DNA. Hybridisation conditions were 5 × Denhardt's solution, 6 × SSC and 0.5% SDS at 65° C. The filters were washed for 60 min in 2 × SSC, 0.1% SDS at 65° C.

Results

An extra-chromosomal P component can be transmitted with a chromosomal M complement

P cytotype females usually transmit to their progeny a cellular state which promotes determination of P cytotype (Engels 1979; 1989). The question addressed in Experiment 1 was the following: can a chromosomally M gamete, produced by a P cytotype female, promote the P cytotype state in a zygote into which chromosomal *P* elements have been introduced by the paternal gamete? In other words, is a strictly extra-chromosomal component involved in P cytotype determination? We compared cytotype properties of females that had maternally inherited a chromosomal M complement from P or from M cytotype mothers and which had received the regulatory *P*(1A) elements from males of the *Lk-P*(1A) line. Figure 1 presents both the mating schemes and the results of repression assays. The genotype of females was determined by using the semi-dominant marker on the *Basc* chromosome (*Bar*).

G_1 females *Basc*/*P*(1A) were constructed starting from a P (Fig. 1A) or an M (Fig. 1B) mother. Sets of G_1 females were crossed to Harwich-2 males in order to test their regulatory properties with the A* assay. As expected, the two kinds of G_1 females, although genotypically similar, differed strongly in their regulatory properties: daughters of *Lk-P*(1A) mothers showed a nearly complete P cytotype (Fig. 1A) whereas female progeny of an *M5* (M) mother clearly showed the M cytotype (Fig. 1B). We then measured the ability of gametes bearing the (*Basc*) M chromosome produced by these two kinds of G_1 females to promote the switch towards the P cytotype. We crossed G_1 females to *Lk-P*(1A) males and measured the cytotype of G_2 females *Basc*/*P*(1A) using the A* assay or the *P-lacZ* repression assay. These females had inherited the *Basc* M chromosome from their mothers. We also measured similarly the cytotype of *P*(1A)/*P*(1A) G_2 females.

The results of both GD and *P-lacZ* repression assays showed that the two kinds of G_2 *Basc*/*P*(1A) females differed strongly in their cytotypic properties (compare Fig. 1A and B). In the A* assay, the difference between 4.2% and 40.4% GD was highly significant by the non-parametric Wilcoxon test with replicate tests as independent units ($P<0.001$). This test was used because the standard deviation values were clearly different between the two groups compared. The G_2 *Basc*/*P*(1A) females deriving from a P cytotype grandmother had a nearly complete P cytotype (4.2% GD is close to the value obtained on testing the *Lk-P*(1A) line: 2.2%): indeed the P cytotype was not destabilised over the three generations. G_2 *Basc*/*P*(1A) females with an M grandmother showed a partial switch towards P but this switch was far from complete (40.4% GD as against 2.2%; Wilcoxon

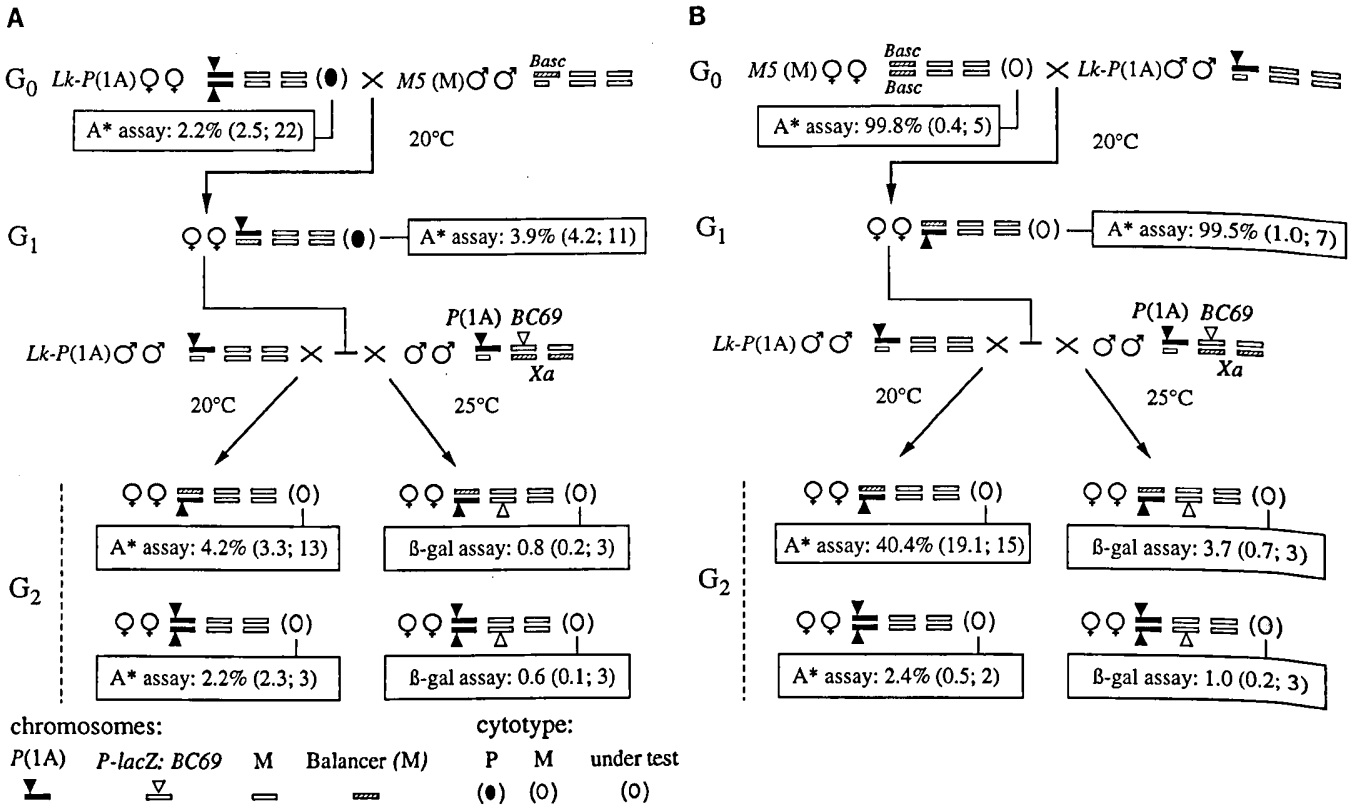

Fig. 1A, B. Crossing scheme for Experiment 1. A and B correspond, respectively, to a P and an M initial maternal inheritance. In each case, the G_2 *P*(1A)/*Basc* females have inherited the *Basc* chromosome maternally. They have thus received no *P* copies from their mother but are derived from a P or an M grandmother and mother (A vs B). The *P* regulatory properties of females were assayed by measuring their ability to repress the occurrence of dysgenic sterility (A* assay) or to repress the promoter of a *P-lacZ* transgene (β-galactosidase assay). A* assay: The A* test cross (Kidwell et al. 1977) was used to measure the repression of GD sterility. In each case, the females under assay were crossed to Harwich-2 males and GD sterility was measured in the female progeny of this test cross. For instance, to measure the regulatory properties of G_2 females, their *progeny* were dissected. For a given assay, several independent replica crosses were carried out. For each replicate, the percentage of atrophic ovaries was measured directly by dissection of 30–50 females. The mean percentage of GD sterility, calculated from all replicate tests, is given in the Figure with the standard deviation between replicates (SD) and with the number (n) of replicates performed: mean GD% (SD; n). In the progeny of the A* crosses involving heterozygous *P*(1A)/*Basc* females, females that had received a *Basc* or a *P*(1A) chromosome were assayed separately: in each case they produced similar percentages of GD sterility. The results of both genotypes were thus pooled. As examples, dissected females carrying a *P*(1A) or a *Basc X* chromosome give a score of 4.1% and 4.2%, respectively, in the A* assay of G_2 heterozygous females in A (4.2% when pooled); similarly in B, we obtained values of 41.0% and 40.1% (40.4% when pooled). β Galactosidase assay: *P-lacZ* activity repression was determined by measuring the β-galactosidase activity in crude extracts of ovaries of tested females. The mean activity (in nmol/min per mg of protein), calculated from all replicate tests, is given together with the standard deviation between replicates and with the number of replicates: mean activity (SD; n). As P and M controls, β-galactosidase activity was measured in the progeny of *Lk-P*(1A) female × *BC69* male and Canton-*y* female × *BC69* male crosses, respectively. The results were: *Lk-P*(1A) × *BC69*, 0.5 activity units (0.3; 3) and for Canton-*y* × *BC69*, 3.8 units (0.8; 5)

test, $P < 0.001$). Similarly in the *P-lacZ* repression assay, G_2 *Basc*/*P*(1A) females with a P grand-mother clearly differed from those with an M grand-mother: the difference between 0.8 and 3.7 activity units is highly significant (*t*-test $P < 0.01$). Females with a P grand-mother had a complete P cytotype: by a *t*-test, 0.8 does not differ from 0.5, the enzyme activity level measured in the progeny of the *Lk-P*(1A) female × *BC69* male cross (see the legend to Fig. 1). On the contrary, the level of repression observed for G_2 females from an M grandmother did not show even a partial switch towards P: 3.7 does not differ from 3.8, the level of β-galactosidase activity observed in the progeny of a Canton-*y* × BC69 male cross (see legend to Fig. 1; Canton-*y* is an M line). This last result suggests that GD sterility and *P-lacZ* repression assays can have different abilities to detect partial levels of *P* repression or do not measure exactly the same components of *P* regulation.

G_2 females *P*(1A)/*P*(1A) had a *P* element chromosomal complement similar to those of the *Lk-P*(1A) line. By both GD and *P-lacZ* repression assays, a complete switch towards P was observed even when the grandmother was M (Fig. 1B). This shows that the *Lk-P*(1A) *P* elements have very strong regulatory properties which require no more than two generations to switch the cytotype from M to P.

In order to test if the differences observed in Fig. 1 might be due to mobilisation of the *P*(1A) elements in the

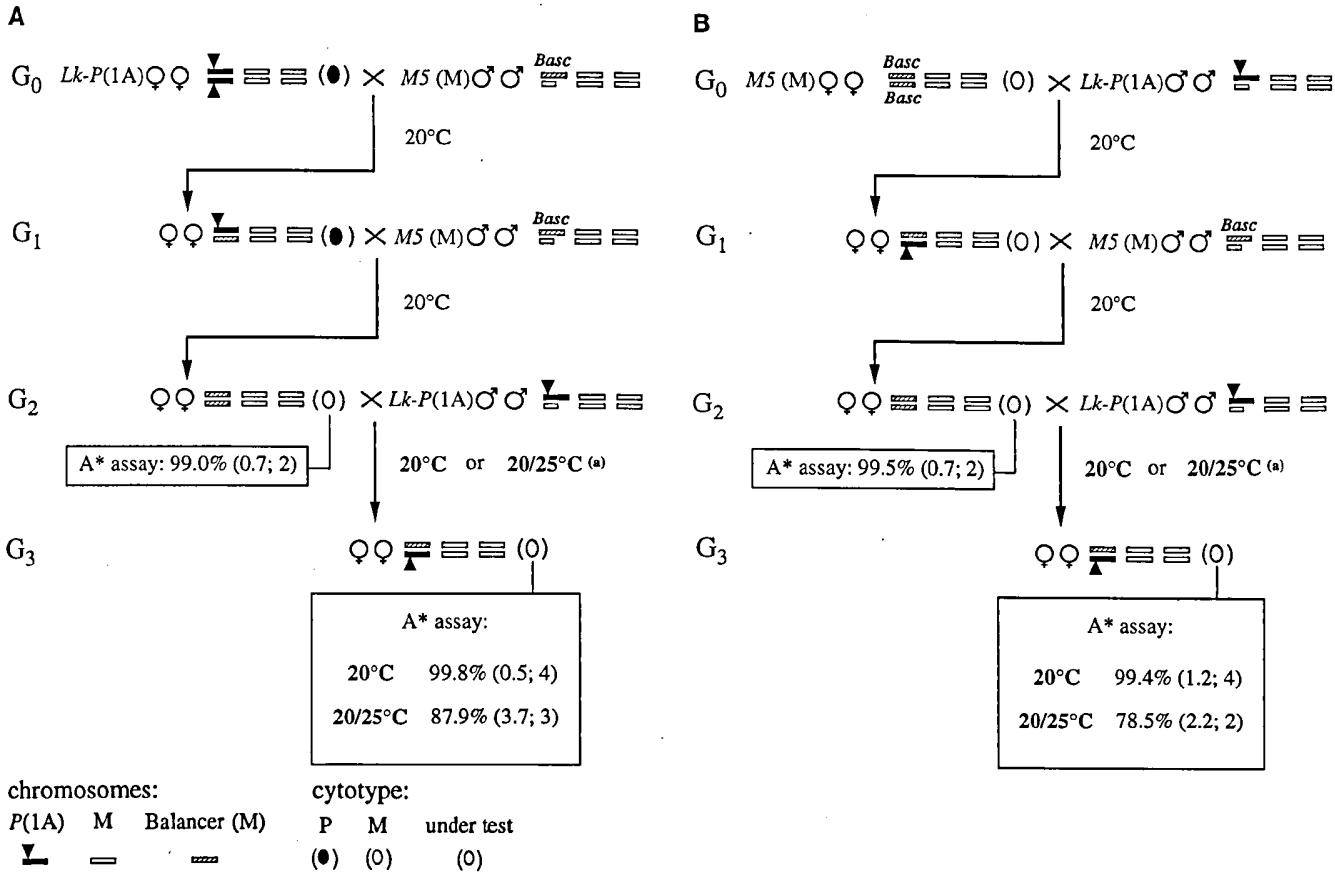

Fig. 2A, B. Crossing scheme for Experiment 2. Synthesis of chromosomal M type G_2 females descended from P cytotype (A) or M cytotype (B) G_1 females. A* assay: the assays were performed as described in the legend to Fig. 1 and results were calculated and are presented similarly. Results of A* assays for G_0 and G_1 females are given in Fig. 1. Different developmental temperature programme of G_3 females were performed. 20° C: all G_3 development was at 20° C and cytotype was tested at emergence; (a) 20/25° C: to induce a switch towards the P cytotype with more strength, embryonic and larval life was at 20° C and pupal life at 25° C; cytotype was then tested after 4 days of ageing at 28.5° C

germline of the progeny of the dysgenic cross (Fig. 1B), G_1 females produced by this cross were mated with *M5* males. Ten *P*(1A) G_2 males produced by different females were then crossed to *C(1)DX* (M) females to produce a number of G_3 males that had inherited the *X* chromosome from their fathers. Genomic DNA from G_3 males was analysed by Southern blotting after digestion with *Eco*RI. Hybridisation was performed with a *P* probe. DNA from the *Lk-P*(1A) line, digested with this enzyme and hybridised with a *P* probe, is known to produce four bands (Ronsseray et al. 1991). Any change in the location or the structure of the *P*(1A) elements should modify at least one of these bands. In the ten G_2 males tested (data not shown), four bands were detected which co-migrated with the four bands detected in the *Lk-P*(1A) line used as a control. In addition, no new bands were detected even after a long exposure. This shows that the ten G_2 males analysed had retained the two *P* elements at 1A with no apparent modification of their structure and with no transposition. Therefore, the differences between the G_2 regulatory properties of Fig. 1A and 1B cannot be interpreted as a consequence of *P*(1A) element excisions and/or transpositions.

From this first set of experiments, we concluded that, although devoid of *P* copies, the *Basc* gamete produced by the P cytotype *Basc/P*(1A) mother (Fig. 1A) can transmit, to the zygote, a state that we call pre-P cytotype. This extra-chromosomal state strongly promotes determination of P cytotype in zygotes into which regulatory *P* elements have been introduced by the paternal gamete.

A chromosomal M zygote which inherits the pre-P cytotype does not transmit it to the following generation

Experiment 2 was designed to test if a chromosomally M female *(Basc/Basc)*, which has inherited the pre-P cytotype state described above, can transmit it to its progeny. In this experiment, G_1 females (similar to those described in Fig. 1) were crossed to *M5* males in order to generate G_2 females *Basc/Basc*. Both P and M cytotype grandmothers were used (Fig. 2). The ability of these G_2 females to promote the switch towards P in G_3 females, which had received *P*(1A) elements from their father, was tested with the A* assay. The results are shown in

Fig. 2. The regulatory properties of G_0 and G_1 females were the same as in Fig. 1 and were discussed above.

Whatever the initial maternal inheritance for the lineage, no repression capacity was detected in *Basc/Basc* G_2 females. The regulatory properties of these females apparently do not differ from those of females from an M line. This result is not surprising and has been previously reported (Sved 1987). Clearly, the pre-P cytotype state, transmitted by G_1 females to their progeny (as shown in Experiment 1), is not sufficient by itself to elicit the P cytotype in *Basc/Basc* G_2 females (Fig. 2A) because of the absence of regulatory *P* elements in the genome of these females.

Comparison of the regulatory properties of *Basc/P*(1A) G_3 females (Fig. 2) tests the ability of *Basc/Basc* G_2 females to stimulate the switch towards P in their progeny, depending on whether these females received a P or M extra-chromosomal component. The results show that there is no remnant in G_3 females of such an extra-chromosomal component since the two kinds of G_3 females have similar properties. After developing at 20° C, they did not show any detectable regulatory properties (99.8% GD sterility vs 99.4%): they showed the properties normally observed for females produced by a M female × P male cross and developed at low temperature (Ronsseray et al. 1984; Ronsseray 1986). Replicate G_3 progeny, allowed to develop at a higher temperature, were assayed for regulatory properties after 4 days of imaginal life at 28.5° C. Such temperature conditions are known to enhance the switch towards P in G_1 females produced by an M female × P male cross (Ronsseray et al. 1984). The results showed here hat the degree of enhancement of the switch did not differ between the two types of G_3 females (no significant difference by a *t*-test was observed; 87.9% GD sterility vs 78.5%). The pre-P cytotype state, transmitted from G_1 to G_2 females, does not appear to be transmissible to G_3 by the chromosomally M G_2 females. This suggests that the determinants of the pre-P cytotype state cannot persist for even one generation in an individual devoid of chromosomal regulatory *P* elements and therefore are not auto-replicative.

Discussion

The pre-P cytotype: a strictly extra-chromosomally transmitted component of the P cytotype

Engels (1979) showed that cytotype determination of an individual involves both chromosomal *P* elements and maternally inherited factors. The transmission of cytotype over several generations has complex rules of inheritance. The cytotype is inherited at least in part maternally: G_1 females from M female × P male and P female × M male crosses have, and transmit to their daughters, different cytotype properties (Engels 1979; Kidwell 1981). The influence of this initial maternal inheritance can be detected for up to five generations. We have shown in Experiment 1 that a zygote that receives from a P mother a chromosomal complement devoid of *P* copies nevertheless receives an extra-chromosomal P component. This component will promote P cytotype determination provided that chromosomal regulatory *P* elements are introduced by the paternal gamete. This shows that the maternally inherited component of the P cytotype described by Engels (1979) can be transmitted strictly extra-chromosomally. We refer to this component as the pre-P cytotype. Determination of the P cytotype by an individual therefore requires the inheritance of both chromosomal regulatory *P* elements (maternally or paternally) and of the pre-P cytotype (maternally). To investigate the nature of the pre-P cytotype, we shall consider its properties in relation to the various models previously proposed to explain the determination of P cytotype.

Cytotype switch can occur without excisions or transpositions of regulatory P *elements*

Rio (1991) proposed that the differences in cytotype properties between the progeny of M female × P male and P female × M male crosses could be mainly due to excision and/or transposition of regulatory *P* elements occurring in the first, but not in the reciprocal cross. Therefore it might be suspected that the *P*(1A) elements (which are autonomous) induce a detectable rate of self-excision in the germline of G_1 females produced by this cross (Fig. 1B), or in the germline of G_2 females, since the G_1 females still have an M cytotype. In this case, it is possible that the occurrence of these excisions in progeny of the M female × *Lk-P*(1A) male cross but not in those of the non-dysgenic reciprocal cross (Fig. 1A), could be responsible for the differences observed between the regulatory properties of the G_2 females (1A vs 1B).

We have confirmed that the *P*(1A) elements are not frequently mobilised in the germline of progeny from the *M5* (M) female × *Lk-P*(1A) male cross that were allowed to develop at the experimental temperature used here (20° C). We have analysed the *P* element complement of ten G_2 males produced by ten independent G_1 females issuing from the *M5* female × *Lk-P*(1A) male cross. Among the ten males tested, we did not detect any change in structure or location of the *P* elements. This indicates that, if excisions or transposition occur in the crosses performed, they are not frequent and cannot explain the differences described in the experiments reported here. In fact the stability of the *P*(1A) elements during the course of the experiment is not surprising, because although the *Lk-P*(1A) line shows transposase activity in dysgenic crosses, this activity is very weak – between 1% and 2% as measured with the hypermutable sn^w allele assay (in the same assay the value for the P line Harwich-2 is around 12%; Ronsseray et al. 1991).

In the *Lk-P*(1A) female × *M5* (M) male cross, stability of the *P*(1A) elements is expected to be nearly complete due to the initial P maternal inheritance and to the persistence of the P cytotype at each generation. Indeed, we have several indicators that the *P*(1A) elements are very stable in the P cytotype. On assaying many sublines of the *Lk-P*(1A) line, reared at 20° C over more than 20

generations, we detected no modifications in the *P*(1A) complement or in the regulatory properties of these lines. In addition, we have constructed several lines carrying the *P*(1A) elements in different genetic backgrounds. We began the mating schemes with a *Lk-P*(1A) female × M male cross: in no cases were the *P*(1A) elements modified (S. Ronsseray, unpublished).

We can thus exclude the possibility that the cytotype modifications described in our experiments are due to excisions or transpositions of the regulatory *P*(1A) elements. This shows that cytotype switch can occur without the mobilisation of regulatory *P* elements.

The pre-P cytotype state must be replenished each generation in the zygote by chromosomal P *elements*

Simmons and Bucholz (1985) proposed a model in which P cytotype could be determined by numerous extra-chromosomal *P* copies which could titrate the transposase and thus inhibit the transposition of the chromosomal *P* elements. To explain the maternal inheritance, such extra-chromosomal *P* elements were supposed to be autoreplicative. However, Sved (1987) has shown that inheritance of the P type extra-chromosomal contribution alone is not sufficient to specify the P cytotype. In the progeny of a P cytotype mother, heterozygous for its regulatory *P* elements, females having an M genotype did not show any apparent P cytotype remnant. Sved concluded that the extra-chromosomal component is not auto-replicative and must be replenished at each generation by chromosomal *P* elements. Nevertheless, we shown here that the oocytes from which this type of females issue have received, from their P cytotype mother, a cytoplasmic component capable of promoting the switch towards P if chromosomal regulatory *P* elements are present in the zygote. It was therefore possible that this pre-P cytotype could be maintained and transmitted to the progeny of these females. We have shown that this is not the case. Like the P cytotype, the pre-P cytotype cannot persist in a *Basc/Basc* zygote that has no chromosomal *P* copies. Like the P cytotype determinants, the pre-P cytotype determinants are not auto-replicative and their transmission to the following generation requires the presence of chromosomal *P* elements. This result reinforces the conclusions of Sved (1987).

Chromosomal imprinting cannot account for the maternal inheritance of P cytotype

Another model of the maternal inheritance could be proposed which supposes that a peculiar state of the chromatin is maternally but not paternally inherited, recalling the suggestions of Engels (1981a) and Sved (1976). The stable binding of *P* repressor to chromosomal *P* elements could induce such a modification of the chromatin. This modification would maintain the repressed state of these *P* elements. According to this model, it would be expected that the P cytotype can be elicited in a zygote only if it has received *P* elements which have been "imprinted" by the P cytotype of its mother. On the contrary, if an oocyte produced by a P cytotype female does not contain any *P* element(s), the zygote will not be of the P cytotype. Our results clearly rule out this type of model as the pre-P cytotype state can be inherited in a strictly extra-chromosomal manner.

The pre-P cytotype state probably results from the accumulation of a repressor in the oocyte

In O'Hare and Rubin's model (1983), P cytotype is the consequence of the presence of a repressor, encoded by *P* elements, which inhibits *P* transposition. In order to explain the maternal inheritance, the authors proposed that the repressor exerts a positive feedback on its own synthesis. Individuals produced by a P mother will synthesise more repressor than those that have a similar chromosomal *P* element complement, but which are descended from an M mother. Engels (1989) and Rio (1990) proposed that this could be due to the accumulation of repressor in the oocytes produced by a P mother. By Western blots, Misra and Rio (1990) have detected an accumulation of the 66 kDa protein (assumed to be a repressor) in the oocytes of the P strain π2. The concentration of the maternally transmitted repressor could differ in the two lineages over several generations until the repressor concentration increases and reaches a threshold in the lineage derived from an initial M female. Taking into account the fact that the P cytotype represses the *P* element promoter (Lemaitre and Coen 1991; Lemaitre et al. 1993), a model has been proposed to explain the positive feedback of the repressor on its own production. This model supposes that the splicing of the IVS3 from *P* pre-mRNA depends on the concentration of this pre-mRNA (O'Hare et al. 1992; Lemaitre et al. 1993). Unspliced mRNA will be translated as repressor while spliced mRNA will code for transposase (Rio et al. 1986; Robertson and Engels 1989; Misra and Rio 1990). This will ultimately determine the level of accumulation of repressor in the oocyte, its effect on *P* transcription, and therefore the level of positive feedback and of repression. We propose that the pre-P cytotype state, described here, is due to this accumulation of *P* repressor in the oocyte. The peculiar spatial distribution of *P-lacZ* staining activity in lines bearing germinally expressed *P-lacZ* insertions supports this hypothesis. For all *P-lacZ* insertions studied, β-galactosidase activity is mainly restricted to the nucleus (Bellen et al. 1989; Grossniklaus et al. 1989). This is presumably due to the presence in the *P-lacZ* fusion gene of the first exon and the 5′ end of the second exon of *P*. This region is thus thought to contain the sequence(s) responsible for the nuclear importation of the transposase. The only exception to this rule is the oocyte after stage 10, in which a strong staining can be observed in the cytoplasm (Grossniklaus et al. 1989; Dorn et al. 1993; Lemaitre et al. 1993; J.L. Couderc personal communication; our unpublished results). This cytoplasmic staining can be observed only in the late stages, after the nurse cells have begun to donate their cytoplasm to the oocyte (Mahowald and Kambysellis

1980). This accumulation in the oocyte probably results from the transport of products synthesised in the nurse cells. We suggest that this distribution reflects the spatial distribution of the *P*-encoded products since, as discussed above, the *P-lacZ* element contains sequences responsible for the spatial localisation of the *P* proteins. The *P* repressor could therefore have a similar localisation.

It should be mentioned that the oocyte is arrested in prophase 1 of meiosis until fertilisation (Mahowald and Kambysellis 1980). The two kinds of *X* chromosome [*Basc* and *P*(1A)] are therefore present in the oocyte until fertilisation, after which the first polar globule is ejected. The oocyte, which will transmit to the next generation a *Basc* (M) chromosomal complement, in fact contains the regulatory *P*(1A) elements until fertilisation. This does not affect the conclusions drawn from our experiments because transcription is inactive in the oocyte, except perhaps for a short period during late stage 9 and early stage 10 (Mahowald and Kambysellis 1980). The *P*(1A) complement is therefore silent at least after stage 10 and the pre-P cytotype can still be interpreted as a consequence of repressor accumulation in the oocyte, due to transport of the *P* products from the nurse cells to the oocyte.

The presence of repressor in the embryo and its persistence until the onset of zygotic transcription, would then protect the germline of the zygote from dysgenesis by immediate repression of zygotic transcription of the *P* elements. To explain the fact that the P cytotype and the pre-P cytotype state must be replenished each generation by the chromosomal *P* elements (Sved 1987; our results), we suggest that the repressor deposited in the oocyte is diluted and/or degraded during development. Repressor concentration must be maintained in the developing organism by de novo synthesis from chromosomal *P* elements.

When testing the regulatory properties of heterozygous *P*(1A)/*Basc* females, we observed that the two categories of dissected progeny females, issuing from the A* cross, showed similar frequences of GD regardless of whether they have inherited the *P*(1A) or the *Basc* chromosome from their tested mothers (see legend to Fig. 1). This shows that repression of GD sterility in these dissected females does not require the presence of the *P*(1A) regulatory element and depends only on their pre-cytotype.

Conversely, we obtained a different result on testing the regulatory properties of the progeny of females heterozygous for the *P*(1A) regulatory elements using a germline transposase assay (mobilisation of a $P[w^+]$ by *P*[Δ2–3](99B), Ronsseray et al. 1991; for information on *P*[Δ2–3](99B), see Robertson et al. 1988). In this case there was a strong difference in germline mosaicism between G_1 individuals that had or had not inherited the *P*(1A) elements. The pre-P cytotype alone was not able to repress *P* transposition. Strong repression was observed only in the presence of both pre-P cytotype and the *P*(1A) regulatory elements (see Fig. 1 and Table 3 of Ronsseray et al. 1991).

Two non-exclusive hypotheses can account for the discrepancy between these observations:

1. GD sterility is determined at early stages of development; in order to prevent sterility, repressor is necessary only at these stages. Indeed, at these stages, the pre-P cytotype repressor may not yet be diluted (or degraded) enough and thus be sufficient to prevent GD. Such a hypothesis is consistent with results of Engels and Preston (1979), who showed that temperature sensitivity for GD sterility is at its maximum just before and during the first larval instar, strongly decreases during the second instar, and is undetectable during and after the third instar (see also Engels 1981a). In contrast, *P*[Δ2–3](99B) produces transposase at both early and late stages (Robertson et al. 1988). Engels (1981b) already interpreted discrepancies between results of GD sterility and sn^w hypermutability assays in terms of differences in the period of sensitivity.

2. In the GD A* test cross, the P paternal gamete brings not only *P* elements able to induce GD, but also *P* elements with repression capacities. The repressor present in the embryo at the onset of zygotic transcription could be sufficient to exert a positive feedback on these regulatory *P* elements. They will therefore relay the pre-P cytotype and maintain the repressed state continuously during the whole life of the individual, thus preventing GD sterility. Conversely, *P*[Δ2–3](99B), which codes only for transposase (Laski et al. 1986), is devoid of regulatory capacities (Lemaitre and Coen 1991; Lemaitre et al. 1993). It is therefore unable to relay the pre-P cytotype repression which, alone, is unable to repress P transposition in the germline throughout the whole life of an individual.

It would be of great interest to determine to what extent and until which stage the pre-P cytotype is able to exert its repressive effect in the absence of chromosomal regulatory *P* elements, and which kinds of regulatory *P* elements can specify the pre-P cytotype.

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References

Bellen HJ, O'Kane CH, Wilson C, Grossniklaus U, Pearson RK, Gehring WJ (1989) *P*-element-mediated enhancer detection: a versatile method to study development in *Drosophila*. Genes Dev 3:1288–1300

Biémont C, Ronsseray S, Anxolabéhère D, Izaabel H, Gautier C (1990) Localization of *P* elements, copy number regulation and cytotype determination in *Drosophila melanogaster*. Genet Res 56:3–14

Bingham PM, Kidwell MG, Rubin GM (1982) The molecular basis of P-M hybrid dysgenesis: the role of the *P* element, a P strain-specific transposon family. Cell 29:995–1004

Busson DM, Gans M, Komotopoulou K, Masson M (1983) Genetic analysis of three dominant female sterile mutations located on the *X* chromosome of *Drosophila melanogaster*. Genetics 105:309–325

Dorn R, Szidonya J, Korge G, Sehnert M, Taubert H, Archoukieh E, Tschiersch B, Morawietz H, Wustmann G, Hoffmann G, Reuter G (1993) *P* transposon-induced dominant enhancer mutations of position effect variegation in *Drosophila melanogaster*. Genetics 133: 279–290

Engels WR (1979) Hybrid dysgenesis in *Drosophila melanogaster:* rules of inheritance of female sterility. Genet Res 33:219–236

Engels WR (1981a) Hybrid dysgenesis in *Drosophila* and the stochastic loss hypothesis. Cold Spring Harbor Symp Quant Biol 45:561–565

Engels WR (1981b) Germline hypermutability in *Drosophila* and its relation to hybrid dysgenesis and cytotype. Genetics 98: 565–587

Engels WR (1984) A trans-acting product needed for *P* factor transposition in *Drosophila*. Science 226:1194–1196

Engels WR (1989) *P* elements in *Drosophila*. In: Berg DE, Howe MM (eds) Mobile DNA. American Society for Microbiology, Washington, D.C., pp 437–484

Engels WR, Preston CR (1979) Hybrid dysgenesis in *Drosophila melanogaster:* the biology of female and male sterility. Genetics 92:161–174

Grossniklaus U, Bellen HJ, Wilson C, Gehring WJ (1989) *P*-element-mediated enhancer detection applied to the study of oogenesis in *Drosophila*. Development 107:189–200

Kidwell MG (1981) Hybrid dysgenesis in *Drosophila melanogaster:* the genetics of cytotype determination in a neutral strain. Genetics 98:275–290

Kidwell MG, Kidwell JF, Sved JA (1977) Hybrid dysgenesis in *Drosophila melanogaster:* a syndrome of aberrant traits including mutation, sterility, and male recombination. Genetics 86:813–833

Laski FA, Rio DC, Rubin GM (1986) Tissue specificity of *Drosophila P* element transposition is regulated at the level of mRNA splicing. Cell 44:7–19

Lemaitre B (1992) L'élément *P* de *Drosophila melanogaster:* Mécanismes contrôlant la transposition. Thèse de l'Université Pierre et Marie Curie, Paris 6, France

Lemaitre B, Coen D (1991) *P* regulatory products repress *in vivo* the *P* promoter activity in *P-lacZ* fusion genes. Proc Natl Acad Sci USA 88:4419–4423

Lemaitre B, Ronsseray S, Coen D (1993) Maternal repression of the *P* element promoter in the germline of *Drosophila melanogaster:* a model for the P cytotype. Genetics: in press

Lindsley DL, Zimm GG (1992) The genome of *Drosophila melanogaster*. Academic Press, San Diego, California

Mahowald AP, Kambyselis MP (1980) Oogenesis. In: Ashburner M, Wright TRF (eds) The genetics and biology of *Drosophila*, vol 2d. Academic Press, pp 141–224

Miller JH (1972) Experiments in Molecular Genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York pp 352–355

Misra S, Rio DC (1990) Cytotype control of *Drosophila P* element transcription: the 66kd is a repressor of transposase activity. Cell 62:269–284

O'Hare K, Rubin GM (1983) Structure of *P* transposable elements and their sites of insertion and excision in the *Drosophila melanogaster* genome. Cell 34:25–35

O'Hare K, Driver A, McGrath S, Johnson-Schlitz DM (1992) Distribution and structure of *P* elements from the *Drosophila melanogaster* P strain π2. Genet Res 60:33–41

O'Kane CJ, Gehring WJ (1987) Detection *in situ* of genomic regulatory elements in *Drosophila*. Proc Natl Acad Sci USA 84:9123–9127

Preston CR, Engels WR (1984) Movement of *P* elements within a P strain. Dros Inf Serv 60:169–170

Rio DC (1990) Molecular mechanisms regulating *Drosophila P* element transposition. Annu Rev Genet 24:543–578

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

Rio DC, Laski FA, Rubin GM (1986) Identification and immunochemical analysis of biologically active *Drosophila P* element transposase. Cell 44:32–32

Robertson HM, Engels WR (1989) Modified *P* elements that mimic the P cytotype in *Drosophila melanogaster*. Genetics 123:815–824

Robertson HM, Preston CR, Phillis RW, Johnson-Schlitz D, Benz WK, Engels WR (1988) A stable genomic source of *P* element transposase in *Drosophila melanogaster*. Genetics 118:461–470

Ronsseray S (1986) P-M system of hybrid dysgenesis in *Drosophila melanogaster:* thermic modifications of the cytotype can be detected for several generations. Mol Gen Genet 205:23–27

Ronsseray S, Anxolabéhère D, Periquet G (1984) Hybrid dysgenesis in *Drosophila melanogaster:* influence of temperature on cytotype determination in the P-M system. Mol Gen Genet 196:17–23

Ronsseray S, Lehmann M, Anxolabéhère D (1989) Distribution of *P* and *I* mobile element copy number in *Drosophila melanogaster* populations. Chromosoma 98:207–214

Ronsseray S, Lehmann M, Anxolabéhère D (1991) 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. Genetics 129:501–512

Rubin GM, Kidwell MG, Bingham PM (1982) The molecular basis of P-M hybrid dysgenesis: the nature of induced mutations. Cell 29:987–994

Schaefer RE, Kidwell MG, Fausto-Sterling A (1979) Hybrid dysgenesis in *Drosophila melanogaster:* morphological and cytological studies of ovarian dysgenesis. Genetics 92:1141–1152

Simmons MJ, Bucholz LM (1985) Transposase titration in *Drosophila melanogaster:* a model of cytotype in the P-M system of hybrid dysgenesis. Proc Natl Acad Sci USA 82:8119–8123

Sved JA (1976) Hybrid dysgenesis in *Drosophila melanogaster:* a possible explanation in terms of spatial organization of chromosomes. Aust J Biol Sci 29:375–388

Sved JA (1987) Hybrid dysgenesis in *Drosophila melanogaster:* evidence from sterility and Southern hybridization tests that P cytotype is not maintained in the absence of chromosomal *P* factors. Genetics 115:121–127