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Drosophila *P* element: transposition, regulation and evolution

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Abbreviations: kb: = kilobase; kDa = kiloDalton

Introduction

The *P* transposable element family in *Drosophila melanogaster* is responsible for the P-M system of hybrid dysgenesis, a syndrome of abnormal germ-line traits which includes chromosomal rearrangements, male recombination, high mutability and temperature sensitive agametic sterility (called GD sterility for gonadal dysgenesis – Kidwell, Kidwell & Sved, 1977; for a review see Engels, 1989). *P* transposition and associated dysgenesic phenomena occur exclusively in the germ-line of hybrids produced by crosses between M type females which are devoid of *P* elements and *P* type males, which carry numerous *P* elements scattered throughout the genome (Rubin, Kidwell & Bingham, 1982; Bingham, Kidwell & Rubin, 1982; see Fig. 1). These genetic abnormalities are due to the mobilization of the *P* elements. In the progeny of the reciprocal crosses as well as in the progeny of *P* strains themselves, the rate of *P* element transposition is almost null and no dysgenesis occurs (Fig. 1).

P elements are heterogeneous and can be classified into two broad types: autonomous (full-length) elements of 2.9 kb (Fig. 2), and non-autonomous elements that usually result from internal deletions of various sizes (O'Hare & Rubin, 1983). The transposase is a 87 kDa protein encoded by a single cistron comprising four exons (Karess & Rubin, 1984; Laski, Rio & Rubin, 1986; Rio, Laski & Rubin, 1986). The germ-line limitation of transposition is achieved by an alternative splicing: the third intron (IVS3) is spliced exclusively in the germ-line of both sexes, restricting the synthesis of transposase to this tissue (Laski, Rio & Rubin, 1986; see further and Fig. 2).

Populations and strains may be characterized on the basis of two properties related to the phenotypic effect of their *P* elements. The 'P activity potential' refers to the capacity of a strain to mobilize *P* elements in a permissive background (or 'susceptible' state). The potential of a strain to regulate or suppress *P* element activity is referred to as the 'P susceptibility' (see further). It comprises the joint action of all mechanisms affecting *P* element regulation (maternally transmitted P cytotype and zygotic repression, see further). Based on these properties, strains may be classified into four broad types according to their phenotypic characteristics in diagnostic test crosses and according to the number of *P* elements they possess. P strains have low to high levels of P activity potential; they also present a low level of P susceptibility. Q strains have extremely low levels of both P activity potential and P susceptibility. Individuals of the P type or of the Q type carry generally between 25 to 60 copies of *P* sequences (complete or deleted) per haploid genome (Bingham, Kidwell & Rubin, 1982; Rubin, Kidwell & Bingham, 1982; Ronsseray, Lehmann & Anxolabéhère, 1989).

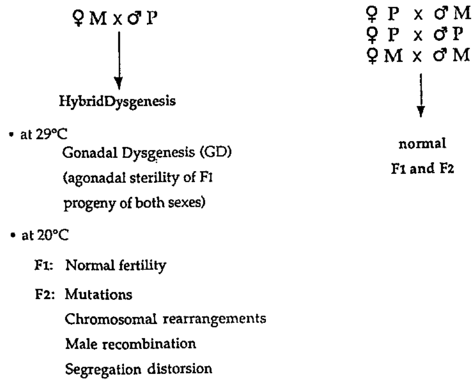

Fig. 1. The P–M system of hybrid dysgenesis.

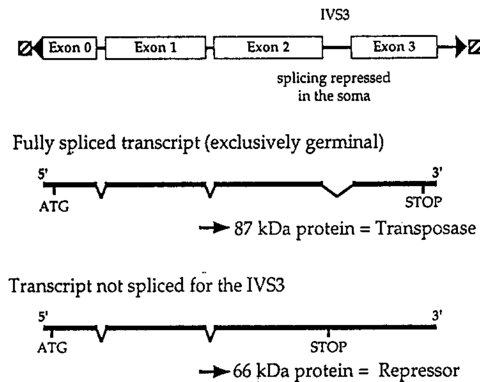

Fig. 2. Structure of the complete autonomous *P* element (2907 bp) and of its main transcripts. Hatched boxes: 8bp target duplication. Arrowheads: 31 bp terminal inverted repeats. Open boxes or thick lines: exons. Thin lines: introns or untranscribed.

M and M′ strains rarely have any significant level of P activity potential. M strains are devoid of any *P* elements and have an extremely high level of P susceptibility. M′ strains carry *P* elements from a few up to 50 copies per haploid genome. Most, if not all of these elements are defective (Bingham, Kidwell & Rubin, 1982; Black *et al.*, 1987; Izaabel, Ronsseray & Anxolabéhère, 1987). M′ strains vary for P susceptibility from extremely high to moderately low (Anxolabéhère *et al.*, 1985).

This review does not intend to be extensive but we will present data on *P* element evolution, transposition and regulation mainly based on the contribution of our laboratory to this field.

Population and evolutionary studies

Temporal and geographical distributions of P *elements*

The existence of *Drosophila melanogaster* strains without any *P* sequences in their genome has raised very interesting questions about the intraspecific distribution of the *P* element and its phylogenetic origin.

A study concerning more than two hundred *Drosophila melanogaster* stocks collected on all continents between 1920 and 1982 has clearly established a strong correlation between the date of sampling of a strain and its type in the P-M system of hybrid dysgenesis (Kidwell, 1983). The oldest laboratory strains are exclusively of the M type while the proportions of P and Q strains increase when the dates of sampling are more recent. These results have been confirmed at the molecular level (Anxolabéhère *et al.*, 1985). Only the strains isolated in laboratories many years ago are devoid of *P* sequences. Conversely, all the strains recently derived from natural populations bear *P* sequences on their chromosomes. Their characteristic in the P-M system (P, Q or M′ type) is dependent on the number and properties of their *P* elements.

The first appearance of *P* elements is detected in strains collected during the 1950s and early 1960s in the Americas. *P* sequences are detected only at the end of the 1960s on other continents. In America, the latest strains completely devoid of *P* sequences were collected in the mid-1960s. Conversely, such strains have been found as late as 1969 in France, and 1974 in the former USSR. Analysis performed during the 1980s revealed that, at this date, all natural populations of *Drosophila melanogaster* carried *P* elements. Clear quantitative and qualitative differences in *P* element distribution between geographical regions can be observed, probably as a result of genetic drift, founder effects, migration, natural selection and development of mechanisms regulating *P* element transposition (Anxolabéhère *et al.*, 1985, 1990; see further).

Since the P-M hybrid dysgenesis system is a consequence of the recent introduction of *P* elements in *Drosophila melanogaster* populations, it seems unlikely that the geographical differentiation presently observed reflects a worldwide stable equilibrium. We will only be able to understand the evolution of current distributions with a working knowledge of the dynamics of *P* element invasion. This evolution has to be tested in nature over time.

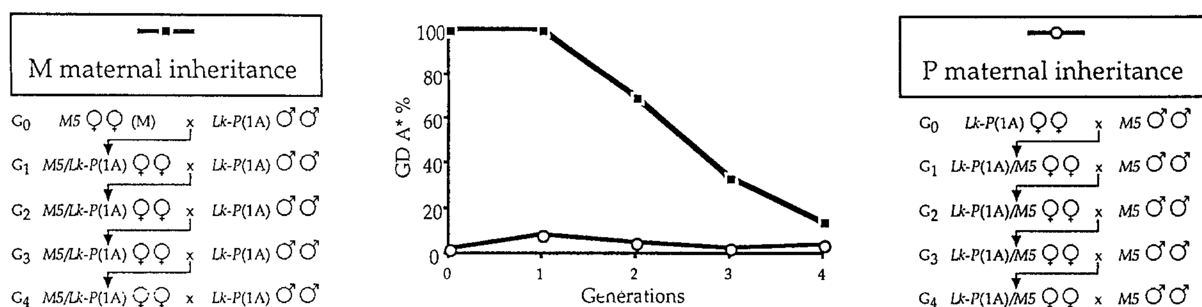

Fig. 3. Maternal inheritance of the P cytotype. Exclusion by the *M5* balancer was used to monitor the segregation of the *P*(1A) elements specifying the P cytotype. The *P*(1A) line used in these experiments contained the two autonomous *P* copies at 1A of the *Lk-P*(1A) line plus an additional *P* copy in 1B, that was not yet eliminated. In the left panel (M maternal inheritance) the repressor making elements were paternally derived at each generation. In the right panel (P maternal inheritance), they were constantly maternally derived. At each generation, a sample of females were crossed at 29 °C with males from the standard P strain, Harwich. The incidence of gonadal dysgenesis was estimated by dissection of ≥50 females from the progeny of this cross (GD A*%).

In Eurasian populations, the dynamics of *P* element spread might have been influenced by the presence of a high number of *KP* elements, a particular internally deleted *P* element (see further). The preponderance of this class of elements might be due to its repressing properties against hybrid dysgenesis, or to an advantage in transposition. Jackson, Black and Dover (1988) and Ronsseray, Lehmann and Periquet (1989) provided some evidence that internally-deleted *P* elements, in particular the *KP* element, interact with autonomous *P* elements and slow down or stop the spread of the latter in experimental populations. If a similar process has taken place in natural populations, the geographical differentiation observed in our first screening of natural populations in the mid-1980s should evolve very slowly. So, we are running out a new screening of natural populations (D. Higuet, S. Ronsseray, G. Periquet & D. Anxolabéhère, unpublished).

The hypothesis of recent loss of *P* elements in the strains maintained in laboratory conditions (Engels, 1981a) can be ruled out because it is very unlikely that a population, even with a weak effective size, eliminates 40 or 50 *P* copies by genetic drift and/or excisions. Moreover, this process would simultaneously provoke the loss of autonomous *P* elements and lead not only to empty strains but also to M′ strains, bearing non-autonomous, definitively immobile *P* sequences. Until today, such a loss has never been observed.

The hypothesis proposed by Kidwell (1983) of a recent invasion of natural populations of *Drosophila melanogaster* by the *P* elements is more likely. It is consistent with the temporal and geographical distribution of the *P* elements. This invasion probably took place in an American population before the 1950s and may have involved a neotropical species such as *Drosophila willistoni* (see next paragraph). This event cannot be precisely dated because *P* elements may have been maintained in small isolated populations during a long period before their spread to other populations. A hypothesis on the process of *P* element invasion may be postulated, given data on the evolution of experimental mixed populations (Anxolabéhère *et al.*, 1986; Periquet, Hamelin & Ronsseray, 1989). Anxolabéhère, Kidwell and Periquet (1988) proposed the model of 'innovative stepping-stone' invasion. According to this model, several phases of invasion, mixing and new colonization by resulting populations took place following the original contamination. At each step, *P* elements underwent internal deletions, leading to a reduced efficiency of the resulting population ability to contaminate a new population. This process first occurred during the initial invasion of American populations, leading to moderate P strains distributed in a gradual manner over the continent, as described by Kidwell and Novy (1985). The subsequent invasion of Eurasia followed the introduction of individuals from such populations into Western Europe and Eastern Asia. It led to stable weak P/Q populations after a transient period of instability. New deletions occurred during this process and resulted in a lower autonomous/non-autonomous elements ratio in the populations invading the rest of Europe and Asia. This led to the M′ strains found in these areas since the mid-1970s. The geographical differentiation of the Eurasian populations could be the consequence of two waves of *P* element migration, one coming from the west (Anxolabéhère *et al.*, 1985) and the other coming from the east (Anxolabéhère *et al.*, 1990), both issued from American populations. Molec-

ular analysis suggests that the two migrations have been currently stopped on both sides of the Russian-Popular Republic of China frontier (between Alma-Ata and Urumqi, a high mountainous area). Remarkably, a phase of high mutability, which could be due to *P* element transpositions, was observed in Russian populations in 1973 (Golubovski & Belyaeva, 1985). Similarly, Mukai *et al.* (1985) and Choo and Lee (1986) have analyzed the temporal variation of the detrimental load per chromosome in a Japanese population and a Korean population respectively. These results suggest that some mutators, such as the *P* elements, may have invaded these populations at the end of the 1960s.

Phylogenetic distribution of P *element* – P *element in* Scaptomyza pallida

Many data suggest that *P* elements invaded the *Drosophila melanogaster* genome in the 1950s, after a horizontal transfer from *Drosophila willistoni*: (i) the historical and geographical distribution of *P* elements in *Drosophila melanogaster* is consistent with their appearance in this species in South America, 40 years ago (see above); (ii) *P* sequences are completely lacking in the sibling species of *Drosophila melanogaster* (Brookfield, Montgomery & Langley, 1984), but they are found in many more distant species of the Drosophila genus (Daniels *et al.*, 1984; Anxolabéhère, Nouaud & Periquet, 1985; Lansman *et al.*, 1985; Daniels & Strausbaugh, 1986; Stacey *et al.*, 1986); (iii) a functional *P* element has been isolated from *Drosophila willistoni* whose sequence is identical to that of the autonomous *P* element of *Drosophila melanogaster* except for one base-pair (Daniels *et al.*, 1990).

P related sequences are present in most species of the four groups (*willistoni*, *saltans*, *obscura* and *melanogaster*) from the *Sophophora* subgenus. This suggests that *P* elements were present in the ancestor of this subgenus (reviewed in Daniels *et al.*, 1990).

P elements are also found in some more distant species from subgenera related to the *Sophophora* subgenus (Anxolabéhère, Nouaud & Periquet, 1985; Daniels *et al.*, 1990) in particular, they are present in the three species examined of the *Scaptomyza* genus: *Scaptomyza adusta*, *Scaptomyza elmoi* (Daniels *et al.*, 1990) and *Scaptomyza pallida* (Anxolabéhère, Nouaud & Périquet, 1985).

We have analyzed *P* elements present in different strains of *Scaptomyza pallida* (Simonelig & Anxolabéhère, 1991, 1994). This species contains about 15 *P* elements, including three to four full-length elements as well as shorter, deleted elements. All elements are divergent from one another. Most of them appear to be immobile. These data indicate that *P* elements are old components of the *Scaptomyza pallida* genome. Moreover, the presence of *P* sequences in species closely related to *Scaptomyza pallida* suggests that they have had a long evolutionary history in the Scaptomyza genus. We have also found that most *P* elements of *Scaptomyza pallida* are located in the pericentromeric heterochromatin. This confirms other studies which show that in the course of their evolution, transposable elements tend to accumulate into pericentromeric heterochromatin where they become immobile and non-coding (Vaury, Bucheton & Pélisson, 1989).

In addition, two of the full-length *P* elements of *Scaptomyza pallida* (PS2 and PS18) are coding. PS2 and PS18 are 4% divergent from one another, and they are 23% (for PS2) and 24% (for PS18) divergent from the canonical *P* element of *Drosophila melanogaster*.

At least one of them (PS18) is active since, when introduced into *Drosophila melanogaster* germ-line, it is able to transpose and to mobilize a *Drosophila melanogaster* defective *P* element. Comparison of sequences between the complete *P* elements of *Drosophila melanogaster* and *Scaptomyza pallida* reveals that the structural characteristics are maintained: PS2 and PS18 contain terminal inverted repeats and internal repeats very similar to those of the *Drosophila melanogaster P* element. In addition, the noncoding regions at both ends necessary for transposition are more conserved than the coding sequences. Two domains found in the *Drosophila melanogaster P* transposase (helix-turn-helix and leucine zipper) are well conserved in the putative proteins encoded by PS2 and PS18.

A full-length *P* element from *Drosophila bifasciata*, a species of the *obscura* group, has been sequenced (Hagemann, Miller & Pinsker, 1992). The authors have reported that the phylogeny of the *P* elements from *Drosophila bifasciata*, *Drosophila melanogaster* and *Scaptomyza pallida* differs from the species phylogeny: *P* elements from *Drosophila bifasciata* and *Scaptomyza pallida* are less divergent (10%) than *P* elements from *Drosophila bifasciata* and *Drosophila melanogaster* (22%). In a recent work (Simonelig & Anxolabéhère, 1994) we have compared the coding sequences of the elements present in several species of the *obscura* group with the corresponding sequences in PS2 and PS18. Our results confirm that the *Drosophila bifasciata P* sequence does not have a common ori-

gin with *P* elements of other species of the *obscura* group and reinforce the hypothesis of a horizontal transfer of *P* sequences between *Scaptomyza pallida,* or a closely related species, and *Drosophila bifasciata.* Recently Clark, Madison and Kidwell (1994) analyzed a *P* sequence from another species of the *Scaptomyza* genus (*Scaptomyza elmoi*) and conclude that this sequence forms with PS2 and PS18 a clade that clusters with the *P* sequence from *Drosophila bifasciata.*

P elements are therefore old components of the Drosophilidae family and are even anterior to the Drosophilidae divergence. In fact, *P*-like sequences have also been found outside of this family (Perkins & Howells, 1992; Anxolabéhère & Periquet, 1987). But why are *P* sequences present in some lineages and absent in others with which they share common ancestors?

Transmission of elements among species could be strictly vertical (mating-dependent) or could occur horizontally between species that are reproductively isolated. In the vertical mode of transmission, the descendants of an ancestral species that contained these elements should also contain homologs of them, unless they were subsequently lost in some lineage during diversification. The sequence conservation of the elements should reflect the phylogenetic relationship of the species in which they reside. In the horizontal mode of transmission, the distribution of the elements may be discontinuous and their sequence conservation is not necessary related to the phylogeny of the species which harbor them. However, other mechanisms, like evolution rate of transposable elements, can lead to a discrepancy between species phylogeny and element phylogeny (Capy, Anxolabéhère & Langin, 1994).

An explanation of *P* phylogeny including at least some horizontal transfer events is probably reasonable (Clark, Madison & Kidwell, 1994). However, the frequency of such events is unknown. Lateral transfers of *P* sequences are probably rare events since *P* elements are absent in *Drosophila simulans*, the sibling species of *Drosophila melanogaster*, which is also a cosmopolitan species. We know that *Drosophila simulans* is able to tolerate *P* element in its genome when introduced by microinjection and to maintain functional *P* elements (Scavarda & Hartl, 1984; Montchamp-Moreau, 1990). In our laboratory, transformed lines of *Drosophila simulans* have retained about 15 *P* elements per haploid genome after seven years. However these lines present a weak *P* activity and incomplete *P* repression. Thus the regulation mechanism of *P* element transposition could be host dependent. The majority of Drosophila species without *P* element are probably able to tolerate this genetic parasite.

P transposition

P elements in *Drosophila melanogaster* display almost no sequence polymorphism but are diverse with respect to size. Many defective elements are present in P strains. Unlike complete *P* elements, defective elements are non-autonomous but can be mobilized in *trans* by active elements (Engels, 1984). These defective elements are variable in size and are derived from larger elements principally by internal deletions that arise in a dysgenic background during the excision-transposition process (Engels, 1989). Deletion breakpoints frequently occur within short direct repeats, from 2 up to 6 bp (O'Hare & Rubin, 1983; Engels, 1989).

In vitro *transposition*

Kaufman and Rio (1992) have developed an *in vitro* assay for *P* element transposition. Transposition products were recovered by selection in *E. coli*, and contained simple *P* element insertions flanked by 8 bp target site duplications as observed *in vivo*. Transposition required partially purified *P* element transposase, Mg^{+2} and used GTP as a cofactor. Linear, precleaved donor transposon DNAs were functional in their assay but those lacking 3′-hydroxyl groups were inactive. Their results suggest a direct transfer of donor element DNA into a target site with no requirement of the maintenance of covalent bonds between the transposon and flanking donor DNA. Therefore *P* element transposition occurs *in vitro* via a 'cut and paste' mechanism, and this is consistent with the genetic data.

Gap repair models

Taking advantage of the observation that the rate of precise excisions of a *P* element is dependent on the nature of the homologous chromosome, Engels *et al.* (1990) proposed a conservative 'cut and paste' mechanism for *P* element transposition. Excision of the donor element would result in a double-strand gap at the donor site. This gap would be subsequently repaired by a process similar to gene conversion: the broken ends use a homologous duplex (either the sister chromatid or the homolog) as a template for DNA synthesis. Genetic information is copied from the template duplex to the excision site. This gap repair mechanism

involves an intermediate structure with two Holliday junctions flanking the filled-in gap. These junctions would be resolved according to the Hastings model to account for the rarity of cross over products and the lack of backwards conversion, defined as the transfer of sequence information from the broken strand to the template (Hastings, 1988). According to the nature of the template (containing or not an identical *P* element insertion), the repair of the gap results either in restoration or loss of the element at the donor site. In the last case, the gap repair regenerates the wild type sequence (precise excision). The model further proposes that elements with internal deletions are generated by interruptions of the repair process.

Recently, analysis of conversion tracts from partially heterologous templates led Nassif *et al.* (1994) to propose a synthesis-dependent strand annealing model (SDSA) similar to the one studied by Formosa and Alberts (1986). It involves the release of the growing strand behind a migration bubble. After *P* element excision by double strand breaks, the repair is initiated by each terminus finding and invading a homologous sequence. The two ends may locate different homologous sites. DNA synthesis proceeds independently from each 3′ end, with the newly-synthesized DNA being immediately displaced from its template and the process yields two overlapping single-stranded sequences. The single strands then pair, and synthesis is completed by each using the other as a template.

Local transposition

In several experiments of *P* transgene mobilization from diverse genomic locations, it was shown that *P* element has a great tendency to transpose near the starting site (Daniels & Chovnick, 1993; Tower *et al.*, 1993; Zhang & Spradling, 1993; M. Delattre, D. Anxolabéhère & D. Coen, in preparation). Tower *et al.* (1993) and Zhang and Spradling (1993) described a great number of new insertions located within 1–180 kb of the starting site on a minichromosome. Many of these 'local transpositions' landed very close to or within the donor *P* element. They observed more local insertions in the progeny of females than in the progeny of males. This general property of *P* element transposition is likely to be useful to significantly enhance the efficiency of insertional mutagenesis by using a starting *P* element located near the target of interest.

We have used the properties of $zeste^{1}$-*white* interactions (transvection) to monitor phenotypically such events on a *P*[*w*] transgene (M. Delattre, D. Anxolabéhère & D. Coen, in preparation). Indeed, the $zeste^{1}$ repression on the $white^{+}$ gene expression is dependent on the number of *zeste* binding sites (ZBS) present in close proximity (for a review see Pirrotta, 1991). In agreement with the works of other authors, we observed many double insertion mutants containing a second element integrated into or close to the original insert, detected by a more repressed *white* gene expression (lighter eye color). It appears that secondary insertions present a strong directional and orientational preference with regard to the starting element. The second insertions are localized more frequently within the starting element or in the 5′ flanking genomic DNA, rather than in the 3′ flanking sequences. In addition, this tendency is associated with a strong orientation preference with regard to the donor insert. In our study, all of the elements (18/18) that inserted into the 5′ side of the donor element are in opposite (head to head) orientation. Tower *et al.* (1993) and Zhang and Spradling (1993) observed the same tendency, although at a lower rate. To explain this, they propose the existence of factors bound to *P* element termini that might frequently tether the excised element to the unexcised copy on the sister strand in a fixed orientation, leading to a local insertion event.

We propose an alternative hypothesis. To explain the preponderance of reverse duplication on the 5′ side, the double strand breaks at the two ends of *P* element could be non-simultaneous. There would be a first break on the 5′ end, and later, but not always, a second break on the 3′ end. This 5′ break would be repaired according to a 'hairpin model' that exclusively produces duplication of elements in opposite orientation (Coen, Carpenter & Martin, 1986). This mechanism involves ligation of the free ends and further single strand cut at the other end of the insert.

Deletions between direct repeats

Unusual kinds of rearrangements within direct repeats internal to a *P* element were observed in dysgenic background. It appears that *P* transposase induces a high rate of precise deletions and amplifications between repeated sequences contained in a *P* transgene. These events occur with tandemly arranged repeats of different size: 350 bp of ribosomal 5S gene (Pâques & Wegnez, 1993) or 3.44 kb 5′ regulatory sequence of *white* gene (M. Delattre, D. Anxolabéhère & D. Coen, in preparation). The works of Kurkulos *et al.* (1994) show that deletions can also occur on duplicated sequences separated by several kilobases

of unrelated sequence: between the directly repeated 276-nucleotide *copia* long terminal repeats (LTRs) and between the 599-nucleotide repeats of FRT elements separated by 5.7 kb (Kurkulos *et al.*, 1994). With a *P*[*w*] transgene, containing 1.4 kb repeats separated by 450 bp of unique sequences, we have observed precise amplifications with creation of new junctions (M. Delattre, D. Anxolabéhère & D. Coen, in preparation). Consequently, *P* transposase mediated direct repeats deletions and amplifications seem to be a general phenomenon. These events occur at both original and new insertion sites and necessitate the integrity of *P* element extremities (M. Delattre, D. Anxolabéhère & D. Coen, in preparation). Consequently internal deletions and amplifications are a direct or indirect consequence of *P* element transposition.

The gap repair models predict that this kind of rearrangement could occur in the case of interruption of the repair process before complete reparation: the two partially extended 3′ ends might pair with each other at repeated sequences. Unequal pairing between repeats lead to deletion or amplification events (Pâques & Wegnez, 1993; Kurkulos *et al.*, 1994). In our study, we observe that the rate of deletion ($\approx$20%) is much higher than the rate of amplification ($\approx$1%). The gap repair models do not account for this excess. Consequently, we propose the existence of a *Drosophila melanogaster* homolog of the *E. coli* RuvAB protein (Lovett *et al.*, 1993). This protein would have the capacity to realign the intermediates to a more favorably paired configuration, leading preferentially to deletions. Besides, it is conceivable that different mechanisms could work concurrently on the gap opened by excision and then we cannot eliminate the possibility of an intramolecular recombination process producing only deletion events.

P regulation

Germ-line restriction of P *transposition*

P transposition is limited to the germ-line by the restriction of the splicing of the 2–3 intron (IVS3) to this tissue (Laski, Rio & Rubin, 1986). The fully spliced mRNA produced by complete *P* elements encode a 87 kDa transposase, while in the absence of splicing they code for a 66 kDa protein (Rio, Laski & Rubin, 1986; Fig. 2). This protein has been shown to have repressor properties (see further). *In vitro* engineered *P*[Δ*2–3*], a *P* element precisely excised *in vitro* for the IVS3 at the DNA level, is able to transpose in the soma (Laski, Rio & Rubin, 1986) and to produce in this tissue the 87 kDa transposase (Rio, Laski & Rubin, 1986). The IVS3 splicing is restricted to the germ-line by (an) inhibitor(s) present in the somatic cells (Siebel & Rio, 1990; Chain *et al.*, 1991)

The P *cytotype and the measurement of* P *repression*

P transposition and the accompanying dysgenic phenomena occur at high rates in the germ-line of the progeny of M female × P male crosses. The virtual absence of transposition and dysgenic traits in the progeny of P females (crossed either to P or M males) demonstrates maternal repression of *P* (Fig. 1). This maternal effect is attributed to a cytoplasmic condition not permissive for *P* element transposition called the *P cytotype* (Engels, 1979a). This P cytotype state can be maternally inherited for several generations (Engels, 1979a; Fig. 3). Both the maternal effect and the maternal inheritance are ultimately specified by the genomic *P* elements themselves (reviewed in Engels, 1989; Rio, 1990).

The study of the P cytotype repression mechanism and inheritance in natural strains or in lines harboring engineered *P* element(s) can be tested by measuring directly the repression of *P* mobilization or of the deleterious effects of this mobilization. Assays can be performed in the germ-line, using complete *P* element as transposase source, or in the soma, using *P*[Δ*2–3*] (see before).

Repression of transposition and/or excision. *P* excisions events can be detected by using a hypermutable allele of *singed* called *singed-weak* (sn^w) (Engels, 1979b, 1981b, 1984; Roiha, Rubin & O'Hare, 1988). This allele is due to the insertion of two defective *P* elements at the *singed* locus and presents a slight malformation of the bristles. Excision of any one of the two *P* elements results in a change of phenotype (sn^e, sn^+). *P* transgenes marked with a reporter gene (e.g. *P*[*white*$^+$]) (Laski, Rio & Rubin, 1986) can be also used to detect excisions and transpositions by measuring germ-line and/or somatic mosaicism.

Repression of gonadal dysgenesis (GD). The deleterious effects of *P* mobilization in the germ-line (Fig. 1) result in gonadal dysgenesis (GD) sterility (temperature dependent agonadal sterility in both sexes; Kidwell, Kidwell & Sved, 1977). When developed at 29 °C, the F_1 progeny of a cross of M females with

P males results in a high level of GD sterility (90–100%). On the contrary, individuals issued from a P female do not present GD sterility in any crosses (Fig. 1). The capacity of a given female to repress dysgenesis is thus determined by measuring the percentage of GD sterility in the progeny of the A* cross (tested females × reference P strain males).

Repression of pupal lethality. The deleterious effect of *P* mobilization in the soma is pupal lethality. The combination of the numerous defective *P* elements from an M′ strain with the *P*[Δ*2–3*] element 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 and the rearing temperature. It also depends on the regulatory state and can be fully suppressed by the P cytotype. Rescue of pupal lethality therefore provides an assay for *P* element regulation (Gloor *et al.*, 1993).

Effect on mutations or transgenes sensitive to the P *background.* A second set of regulation assays involves cytotype dependent mutations. Several mutated alleles of genes are due to *P* insertions and have a different phenotype in a P or in an M background. This property can be used to test the ability of a line to exert some *P* repression. The females homozygous for the *singed-weak* allele are sterile only in the P background (Robertson & Engels, 1989). On the contrary, the phenotypic effect of a *P* insertion at the *vestigial* locus ($vg^{21.3}$) is suppressed by the P cytotype while the phenotype is extreme in the M background (Williams, Pappu & Bell, 1988). A line carrying the *P*[w^{d1}9.3](19DE) transgene insertion shows an eye coloration weaker in a P than in an M background (Coen, 1990; see further).

Effect on P-lacZ *fusion genes. P-lacZ* fusion genes, where the *E. coli lacZ* gene is fused in frame with the *P* element transposase gene, are widely used to detect *cis*-regulatory regions ('enhancer-trap'). Their use has made it possible to show that the *P* promoter is ubiquitously expressed and is very sensitive to interactions with flanking sequences (position effect) resulting in time and tissue (or cell type) specific expression (for a review on enhancer trap see Wilson, Bellen & Gerhing, 1990).

We have used these enhancer trap constructs as *in vivo* reporters for *P* promoter activity and assayed *P-lacZ* expression in P and M backgrounds. This can be done in any tissue or cell type, and notably in somatic as well as in germ-line tissues, by choosing insertion lines with appropriate patterns of expression (Lemaitre & Coen, 1991; Lemaitre, Ronsseray & Coen, 1993; see further).

The results of all these assays tend to be positively correlated (Lemaitre, 1992; Gloor *et al.*, 1993) but lines can be found which display strong regulatory properties in some assays and undetectable properties in others (*P*[*Sal*I](89D), (Robertson & Engels, 1989); *Lk-P*(1A), (Ronsseray, Lehmann & Anxolabéhère, 1991)).

Type of repressor making P *elements*

In vitro *modified* P *elements. In vitro* modified elements having lost the coding capacity of the last exon by frameshift mutations, splice site mutations (Robertson & Engels, 1989; Gloor *et al.*, 1993) or deletion (Misra & Rio, 1990; Misra *et al.*, 1993) have been studied. They are therefore unable to produce a protein other than the 66 kDa truncated transposase. Depending strongly on chromosomal position, some insertions of these elements displayed strong repression capacities in the soma but only weak or partial repression capacities when germ-line tests were used.

Naturally occurring repressor making P *elements.* A deleted element unable to code for transposase but able to code for the 66 kDa protein has been genetically isolated from a natural population (Nitasaka, Mukai & Yamazaki, 1987). A *P* element deleted for the last exon (between positions 2025 and 2416) was found in a line deriving from the P strain π_2, althougth it is not present in the π_2 strain itself (Gloor *et al.*, 1993). It probably occured by a *de novo* deletion during the synthesis of the line. This element (named A12) had regulatory capacities using somatic tests but was not tested in the germ-line.

A geographically widespread element named *KP*, bearing a deletion that removes exon 2 and part of exons 1 and 3 (between 807 and 2561), has also been shown to exert some repression in somatic and germline assays (Black *et al.*, 1987; Jackson, Black & Dover, 1988; Biémont *et al.*, 1990; Simmons *et al.*, 1990; Higuet, Anxolabéhère & Nouaud, 1992; Rasmusson, Raymond & Simmons, 1993; W.R. Engels & C. Preston, personal communication). However, strains can be found which contain more than 20 *KP* copies and which have very weak or no regulatory properties (Biémont *et al.*, 1990).

Another small element (0.5 kb) called *SP* deriving from natural chromosomes has also been found to have partial regulatory properties (Rasmusson, Raymond & Simmons, 1993).

Complete autonomous P *element.* The regulatory properties of a number of lines harboring only complete *P* elements, after genetic transformation, suggested that this type of element is not able to establish the P cytotype (Steller & Pirrotta, 1986; Anxolabéhère *et al.*, 1987; Daniels *et al.*, 1987; Preston & Engels, 1989). Gloor *et al.* (1993) have analyzed a large number of lines harboring one single *de novo* insertion of a complete *P* element, generated by transposition. In some of them, they have detected some repression properties, using assays performed in the soma (repression of pupal lethality or suppression of $vg^{21.3}$). However, none of these insertions showed any regulatory properties in the germ-line (G.B. Gloor, personal communication). The analysis of the *P* element complement of a natural P strain (π_2) has also suggested that the complete *P* element could play a role in the P cytotype determination of this strain, since determinants of P cytotype have been genetically located on all major chromosomes and the complete *P* element is the only type of element also present on all chromosomes (O'Hare *et al.*, 1992).

P elements derived from natural chromosomes have also been genetically isolated. Two full-length *P* elements inserted at the tip of the *X* chromosome (site 1A) are sufficient to elicit a complete P cytotype in the germ-line of the *Lk-P*(1A) line (Ronsseray, Lehmann & Anxolabéhère, 1991). By *Δ2–3* induced excisions, two lines were generated, each one harboring one or the other of the two *P* copies of the *Lk-P*(1A) line. Both display partial P cytotype, including maternal effect and maternal inheritance, *and* the ability to produce transposase (S. Ronsseray, M. Lehmann & D. Anxolabéhère, in preparation). This demonstrates that the complete *P* element can elicit the true maternally inherited P cytotype and shows that it can produce both the transposase and a repressor (see further).

In conclusion, *P* elements with different structures are probably involved in *P* regulation. The regulatory properties of an element are strongly dependent on the insertion site. In addition, it must be pointed out that, among the large number of insertions studied, the maternal *effect* typical of the P cytotype was found only in a *P*[66 kDa] element (Misra *et al.*, 1993) and with the *Lk-P*(1A) elements (Ronsseray, Lehmann & Anxolabéhère, 1991). The maternal *inheritance* (influence of this maternal effect over more than one generation) was found only with the *Lk-P*(1A) strain harboring only complete autonomous *P* elements (Ronsseray, Lehmann & Anxolabéhère, 1991; S. Ronsseray, M. Lehmann & D. Anxolabéhère, in preparation).

Mechanism of P *repression*

P *repressor represses the* P *promoter.* We have used various *P–lacZ* fusion genes to investigate the mechanism of *P* repression. *P* repression was monitored by histological localization of β-galactosidase (X-gal staining) or by quantitative measurements of β-galactosidase activity on the same *P-lacZ* insertion in an M or in a P background. For all the *P-lacZ* insertions tested (11 germ-line specific and 28 expressing the *P-lacZ* fusion in somatic tissues), the β-galactosidase activity is repressed in a P background (Lemaitre & Coen, 1991; Lemaitre, Ronsseray & Coen, 1993). This repression occurs in all tissues and cell types, germinal or somatic, and at all developmental stages. However, using standard P strains, the repression was consistently more important (in fact almost total) in germ-line tissues.

This repression results in a reduced level of the specific *P-lacZ* fusion transcript (Lemaitre & Coen, 1991). This and the fact that *P* repressors are able to act on heterologous promoters (see further) are strong arguments in favor of a direct repression of the *P* promoter activity in a P background.

The maternal effect of the P *cytotype is restricted to the germ-line.* All the observations on somatic tissues have failed to detect a maternal P cytotype effect, with all types of regulatory elements, including those from standard P strains (Engels *et al.*, 1987; Williams, Pappu & Bell, 1988; Robertson & Engels, 1989; Coen, 1990; Misra & Rio, 1990; Lemaitre & Coen, 1991; Ronsseray, Lehmann & Anxolabéhère, 1991; Lemaitre, Ronsseray & Coen, 1993). This is true also for somatic ovarian tissues (Lemaitre, Ronsseray & Coen, 1993).

Conversely, the maternal effect is observed for all the germ-line based tests, when standard P strains were used and with the *Lk-P*(1A) lines and its two single insertion derivative lines: repression of germ-line transposition, repression of GD sterility induction and repression of the expression of germ-line specific *P-lacZ* insertions. *P* repression is observed in the germ-line only when *P* elements from the P strain are maternally derived. The maternal effect of P cytotype is therefore limited to the germ-line and is exerted only

by specific *P* insertions (see further).

The 87 kDa transposase protein has no detectable repressing capacity in vivo. The purified *P* transposase protein has been shown to bind *in vitro P* sequences encompassing in 5′ the sequences thought to constitute the *P* promoter (Kaufman, Doll & Rio, 1989). Moreover, it represses transcription from this promoter in an acellular transcription assay (Kaufman & Rio, 1991).

We have tested the repressing capacity of the transposase *in vivo* on *P-lac*Z insertions that are readily repressed in a P background but that fail to excise at high frequencies in dysgenic conditions. In the F_1 of dysgenic crosses involving a strong P strain, no repression was observed in the germ-line where transposase is expressed at maximum levels (Lemaitre, Ronsseray & Coen, 1993). When transposase is ubiquitously expressed from a *P*[*Δ2–3*] insertion, no *in vivo* repression was detected in the soma (Lemaitre & Coen, 1991) or in the germ-line (Lemaitre, Ronsseray & Coen, 1993).

The 'pre-P cytotype', a component of the P cytotype transmitted strictly extra-chromosomally. Engels (1979a) has shown that the cytotype determination of an individual involves both chromosomal *P* elements and maternally inherited factors. The transmission of cytotype over several generations involves, at least partially, a maternal inheritance: the two F_1 females from the M female × P male and P female × M male crosses will have and transmit to their daughters different cytotype properties (Engels, 1979a; Kidwell, 1981). The influence of this initial maternal inheritance can be detected for up to five generations (Fig. 3).

Sved (1987) has shown that cytotype determination does not persist in the absence of chromosomal *P* elements, even for one generation. Nevertheless, we have shown that P mothers transmit to the eggs they lay an extra-chromosomal component that we call '*pre-P cytotype*' (Ronsseray, Lemaitre & Coen, 1993). This component will promote P cytotype determination, provided that chromosomal regulatory *P* elements are present in the zygote. The P cytotype determination of an individual therefore requires the inheritance of both the pre-P cytotype (maternally) and chromosomal regulatory *P* elements (maternally or paternally). Like the P cytotype, the pre-P cytotype cannot persist in a zygote which has no chromosomal *P* copies. Therefore, the pre-P cytotype determinants are not auto-replicative and their transmission to the following generation requires the presence of chromosomal *P* elements. We have proposed that the molecular basis of the pre-P cytotype could be the accumulation of the 66 kDa protein in the oocytes of P females (Ronsseray, Lemaitre & Coen, 1993; see further). A strong argument for the positive feedback of the P repressor on its own production proposed by O'Hare and Rubin (1983) comes from the fact that certain autonomous complete *P* elements, like those present in the *Lk-P*(1A) strain, are able to specify either the 87 kDa transposase, which has no repression capacities *in vivo*, or a repressor that we think is the 66 kDa protein (see further).

A model for the P cytotype and its maternal inheritance
Although *P* transcription is almost completely repressed in the germ-line of P females, a small quantity of repressor made during oogenesis could be sufficient to specify and transmit the P cytotype. To achieve that, the repressor encoded by autonomous *P* elements should exert a positive feedback on its own synthesis, as proposed by O'Hare and Rubin (1983). O'Hare *et al.* (1992) proposed that this positive feedback could be due to the incomplete splicing of the IVS3 from *P* pre-mRNAs as a result of the presence in germ-line cells of a low concentration of the same repressor that inhibits IVS3 splicing in somatic cells (Siebel & Rio, 1990; Chain *et al.*, 1991) and that high levels of pre-mRNA would *titrate out* this repressor, making the IVS3 splicing possible.

Alternatively, we have proposed that the *splicing efficiency* itself may depend upon the *P* pre-mRNA concentration, following the hypothetical curve presented in Fig. 4A. At low *P* pre-mRNA concentrations, there would be a low efficiency of the IVS3 splicing from complete *P* element transcripts. This would result in the production of the 66 kDa regulatory protein translated from the unspliced *P* mRNA and no (or almost no) 87 kDa transposase (which is translated from the spliced messenger). By contrast, at higher *P* pre-mRNA concentrations, large amounts of the 87 kDa transposase, which has no regulatory capacities, would be synthesized due to the higher efficiency of the IVS3 splicing.

In the progeny of a dysgenic cross (M female × P male, Fig. 4B), the paternally-transmitted *P* elements are strongly transcribed. The transcripts would be therefore efficiently spliced. This would lead to the production of high levels of transposase and to no (or a very low level of) 66 kDa repressor in the germ-line, and thereby to high rates of *P* transposi-

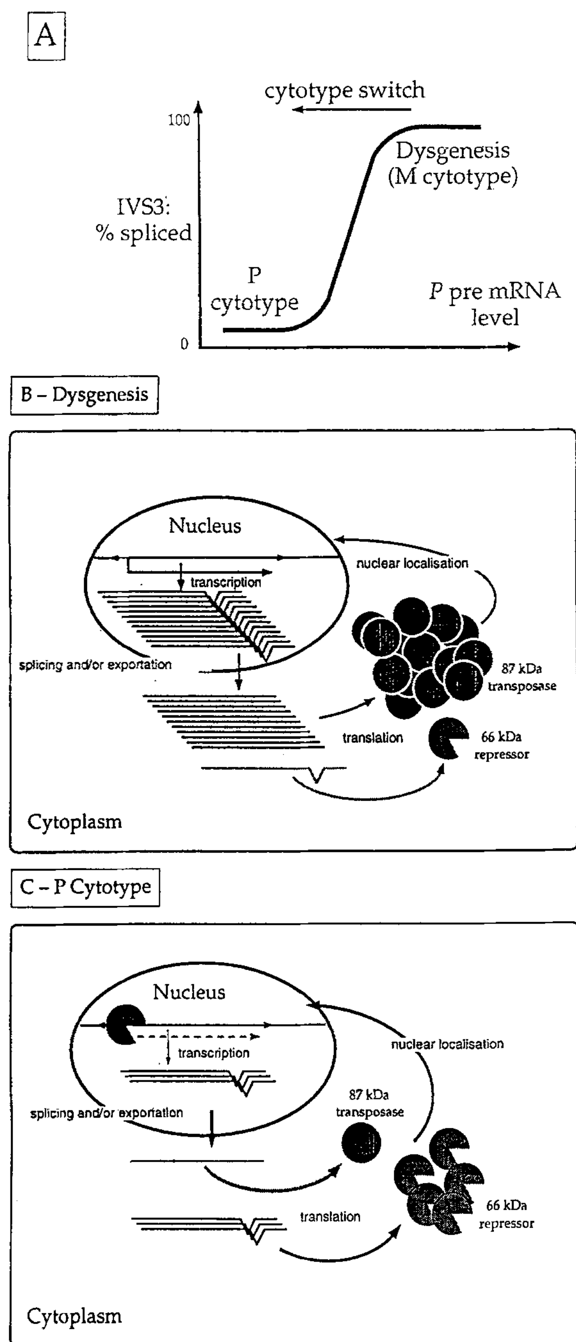

Fig. 4. A model for the P cytotype determination: positive feedback of the IVS3 on its own splicing. See text for explanations.

tion and to hybrid dysgenesis. Because the 87 kDa transposase lacks an *in vivo* regulatory capacity, this state of strong transcription will be maintained and the cytotype switch from M to P (Fig. 4A) would occur only when sufficient quantities of repressor have been accumulated to establish a low steady state level of *P* transcription (this would take several generations).

Then, in the germ-line of a P cytotype female (which possesses numerous complete *P* elements) the *P* element promoter activity is strongly repressed. As a result, the small amount of newly synthesized *P* pre-mRNA would be incompletely spliced (Fig. 4A). This will lead almost exclusively to the formation of the 66 kDa repressor (Fig. 4C). This state of low transcription with preferential formation of the repressor over the transposase would be readily transmitted through succeeding generations because the presence of repressor maintains the low transcriptional level over generations. Thus even in the situation in which transposase producing *P* elements are paternally derived, the level of maternally inherited repressor (pre-P cytotype) would be sufficient to repress these paternally transmitted *P* elements, and hence to maintain the P cytotype.

In the somatic tissues, irrespective of the direction of the cross, complete *P* elements code for the 66 kDa regulatory protein, due to the lack of splicing of the IVS3 in these tissues (Laski, Rio & Rubin, 1986). This could account for the lack of maternal effect in these tissues.

The features of this model match most of the observations concerning P cytotype determination. It explains why most if not all strains eliciting the maternal effect *and* the maternal inheritance of P cytotype are the strains containing full length *P* elements which can be differentially spliced. The *Lk-P*(1A) line contains only two full length *P* elements (Ronsseray, Lehmann & Anxolabéhère, 1991), which are both autonomous (S. Ronsseray, unpublished), and displays the germ-line characteristics of P cytotype strains (maternal *effect* and *inheritance*). Conversely, most of the strains which carry only deleted *P* elements (e.g. *KP*, *P*[*Sal*I](89D), or *P*[66kDa]) display biparental repression in the germ-line as well as in the soma (Black *et al.*, 1987; Robertson & Engels, 1989; Misra & Rio, 1990; Lemaitre, Ronsseray & Coen, 1993). Misra *et al.* (1993) have observed a *P*[66 kDa] element insertion which displays a maternal effect, presumably due to the presence of a maternal enhancer close to the insertion point. However, as these elements can only code for the same regulatory products in the somatic and germ-line tissues, regardless of the direction of the cross producing the F_1, we predict that none of them will be able to display maternal inheritance, as it is postulated to be determined by differential splicing efficiencies. Therefore this maternal inheritance would

only be observed when there are regulatory *P* elements that can be differentially spliced: one species of the transcript would encode the repressor, and the other would produce a protein with no repressing activity (transposase or else).

P interactions with host genes

Interaction of host genes with P *transposition and its regulation*

Several *Drosophila* encoded proteins playing a role in restricting the splicing of the IVS3 to the germ-line (see before) have been biochemically identified (Siebel & Rio, 1990; Chain *et al.*, 1991). It is presumable that, in addition to their role in the IVS3 splicing regulation, they play a role in the regulation of some Drosophila gene expression. However, this role is presently unknown.

Rio and Rubin (1988) have identified a Drosophila protein that binds specifically to the terminal 31-base-pair inverted repeats of *P*. The function of this protein is presently unknown, as is wether or not it plays a role in the transposition process.

Semi-dominant mutations of the *X*-linked *ovo* gene have been shown to partially suppress the incidence of the gonadal dysgenesis specifically in females (Wei, Oliver & Mahowald, 1991).

A subline of the Ica strain has been shown to have strong regulatory properties, linked to a *KP* insertion at 47D1 (Higuet, Anxolabéhère & Nouaud, 1992). The repressing capacity of this line has recently been separated from the 47D1 insertion (by $P[\Delta 2\text{–}3]$ induced excisions). This raises the possibility that the capacities of *P* dysgenesis repression of this strains could be due to a mutation in a Drosophila gene (D. Higuet, unpublished).

Interaction of P *repression with heterologous promoters*

P insertion mutations displaying phenotype sensitivity to cytotype have been described: sn^{w}, $vg^{21.3}$ (see before). We have previously shown that *P* repressor(s) may be able to interact with heterologous promoters contained inside *P* ends.

Interaction with $P[w^{d1}]$. $P[w^{d1}]$ is a *P* transgene including a *white*$^{+}$ gene harboring a tandem duplication of its regulatory sequences. An insertion of this transgene in 19DE was found to be sensitive to *P* repression (Coen, 1990). The repression was due to an enhancement of the *zeste*^{1} repression: it was ineffective in a *zeste*$^{+}$ background and greatly diminished in flies harboring a *Su(z)2* mutation. The repression was also strongly dependent on the insertion chromosomal position: only two new insertions of $P[w^{d1}]$ displayed the sensitivity to *P* repression, among $\approx$100 tested. The *P* repression was also dependent on the number of *white* regulatory regions present in the transgene (Delattre, Anxolabéhère & Coen, in preparation). Finally, no fusion transcript initiated at the *P* promoter or in the flanking sequences and encompassing *white* sequences was detected. The repression resulted in a diminution of the amount of normal *white*$^{+}$ transcript in adult heads in a P background and therefore resulted presumably from a long-distance *cis*-interaction between the *P* repressor interacting with *P* sequences and the *white*$^{+}$ regulatory sequences interacting with the *zeste*^{1} protein (Coen, 1990).

Interaction with HZ. We have used the *H162* line, harboring *HZ*, an enhancer-trap construction harboring, inside *P* ends, an *hsp70* promoter-*lacZ* coding sequence fusion instead of the *P-lacZ* fusion (Y. Hiromi, personal communication), as a control for the specificity of *P* repression. With this line, we have observed an activation of the β-galactosidase expression instead of a repression. This is observed with various P lines (Lemaitre & Coen, 1991) and with a line harboring the *P*[*Sal*I](89D) insertion (Table 1), which produces *P* repressor in the soma (Robertson & Engels, 1989). Certain lines harboring one *KP* insertion are also able to produce this effect (Lemaitre, 1992). We also observed that this activation does not depend on factors like age, sex, tissue (head or body) and rearing temperature (Lemaitre, 1992). In addition we have tested the effect of heat shock on this insertion: this resulted in a two to three times increase of the β-galactosidase level in either M or P backgrounds, but did not suppress the activation of the *HZ* transgene (Lemaitre, 1992; Table 1).

The insertion site did not influence drastically the *P* activation effect, as it was observed on two other lines (*H214* and *H243*) harboring one insertion of the *HZ* transgene although the overall levels of activity were greatly different between the various lines (Lemaitre, 1992; Table 1).

Two types of models are commonly invoked to account for this type of interaction between promoters:

(i) Transcriptional interference (Hausler & Somerville, 1979; Adhya & Gottesman, 1982; Cullen, Lomedi-

Table 1. Influence of heat shock, insertion and promoters orientation on the effect of *P* repressor on *P*[*hsp-lacZ*] transgenes.

	M		*P*[*SalI*](89D)	
H162 (no heat shock)				
Male	132.4	(30.0)	218.6	(30.9)
Female	190.4	(31.7)	292.4	(26.0)
H162 (Heat shock)*				
Male	287.75	(12.5)	433.9	(52.7)
Female	579.0	(105)	683.6	(24.9)
Effect of insertion position				
H214 (*HZ*)				
Male	30.6	(3.0)	50.0	(3.0)
Female	33.3	(10.4)	47.7	(3.8)
H243 (*HZ*)				
Male	10.4	(0.6)	12.0	(1.8)
Female	7.7	(0.6)	11.0	(1.0)
Effect of *P* and *hsp* promotors mutual orientation				
8402 (*HZ50*)				
Male	3.8	(0.6)	7.3	(0.3)
Female	3.3	(0.6)	4.8	(0.6)

Cultures were reared at 25 °C. Three measurements were done for each β-galactosidase activity determination, on crude protein extracts as described in Lemaitre, Ronsseray & Coen (1993). Means are given in nmol/min per mg of proteins, with the standard deviations in parenthesis.
* Flies were heat shocked 90 min at 37 °C. Protein extraction was performed 3 hours after the heat shock. Each if the H162, H214 and H243 stocks harbor one insertion of the *HZ* transgene at a different chromosomal site (Y. Hiromi, personal communication). The 8402 stock harbors one insertion of the *HZ50* construct. *HZ50* is identical to *HZ* with the exception that the *hsp-lacZ* is in the opposite orientation as compared to the *P* promoter orientation (Y. Hiromi, personal communication).

co & Ju, 1984; Gay, Tybulewicz & Walker, 1986; Proudfoot, 1986; Bateman & Paule, 1988; Corbin & Maniatis, 1989(a, b); Wu *et al.*, 1990) is observed if two promoters are tandemly arranged and if the transcript initiated at the upstream promoter comprises the downstream promoter.

(ii) Competition for an enhancer has been invoked to explain the expression of the β-globin gene and repression of the ϵ-globin gene (embryonic) in the adult chicken (Choi & Engel, 1988).

To discriminate between these two models, we have carried on two lines of investigation:

Firstly we have studied transcription of the *HZ* transgene in the *H162* line in M and P backgrounds. Northern blots of poly-A$^+$ RNAs were probed successively with a *lacZ* riboprobe (Fig. 5, panel A) and a 5′ *P* riboprobe (Fig. 5, panel B). The results show clearly that there is a higher level of the *lacZ* transcript initiated at the *hsp70* promoter in a P background (Fig. 5, lanes A1 and A2) than in an M background (Fig. 5, lane A3). This confirms the conclusions obtained with β-galactosidase activity measurements and shows that the increase of expression results from an activation of the *hsp70* promoter. We were unable to detect any transcript initiated at the *P* promoter present in the *HZ* transgene (Fig. 5, lane B3). Such transcripts were not detected either in much

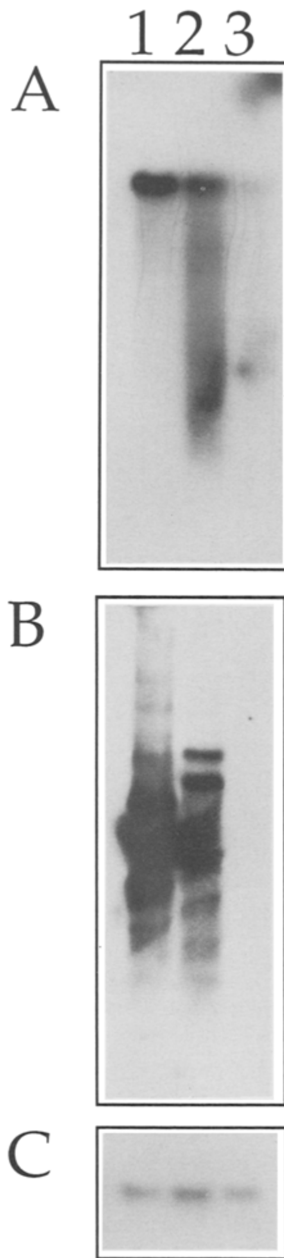

Fig. 5. Northern analysis of H162 (*P[hsp-lacZ]* insertion) in M and P backgrounds. Poly-A+ RNAs were electrophoresed, transferred on a nylon filter and hybridized with probes synthesized as described in Lemaitre and Coen (1991). Lane 1: H162 in a Harwich (P) background. Lane 2: H162; *P[SalI]*(89D). Lane 3: H162 in an M background. The same filter was successively probed with a *lacZ* riboprobe (panel A), a 5′ *P* riboprobe (panel B) and a *ribosomal-protein49* (*rp49*) probe as a control for the amount of RNA (panel C). On panel A, the only transcript detected in all lanes is the *hsp-lacZ* fusion which is clearly more abundant in lanes 1 and 2 (P backgrounds). Panel B was purposely over-exposed to ascertain the absence of any band in lane 3 (M background). In lanes 1 and 2, abundant *P* transcipts derived from Harwich *P* elements (1) or of the *P[SalI]*(89D) element (2) are detected.

longer exposures or with the use of a probe derived from the *HZ* construct itself and containing *P* element and untranscribed *hsp70* promoter sequences (Lemaitre, 1992). Hybridization of Northern blots of total RNAs failed also to detect such a transcript (Lemaitre, 1992). Therefore, if such a transcript exists, it is either unstable or at a level which is beyond detection with this method.

Secondly, we have analyzed the β-galactosidase activity of the *8402* line which contains an insertion of the *HZ50* transgene. *HZ50* is similar to *HZ* except that the *hsp70-lacZ* fusion is in the opposite orientation as regards to the *P* promoter present in the 5′ end of the vector. Although the β-galactosidase activity was much lower than in the *H162*, this activity is clearly higher in a P background than in an M background (Table 1). This shows that the type of interaction that we observe does not require a direct tandem arrangement of the promoters involved.

Taken together these results argue strongly against the transcriptional interference model and are in favor of a model involving long distance *cis*-interactions (e.g. the model of Choi & Engel, 1988). Elucidation of this phenomenon will help in understanding the mechanism of the interaction of the *P* repressor with the *P* transcription and the interaction of *P* insertions with neighbouring genes, as well as the sensitivity of certain *P* insertion mutations to *P* repression.

In addition, the observation of an interaction of *P* regulation, mediated by the 66 kDa protein as well as by the *KP* putative product, with the expression of transcripts having no homology with the *P* transcript (the *white*$^+$ transcript or the *hsp70-lacZ* fusion transcript) driven by heterologous promoters (the *white*$^+$ promoter or the *hsp70* promoter) is a strong argument in favor of an effect on transcription initiation rather than a post-transcriptional effect.

Theoretical approach of the dynamics of *P* regulation at the population level

The regulatory capacities of a line depend on both the chromosomal location and the nature of the *P* element(s) it contains (nature and tissue-specificity of repression, maternal effect vs. zygotic repression). Strains from natural populations may contain one, several or all of the different types of repressor-producing *P* elements that have been described. Therefore they

could exhibit different types of regulation. This raises some questions:

- What are the behavior and the mutual interactions of those mechanisms at the population level? Are they exclusive or synergetic?
- How have they influenced the *P* invasion process of the species?
- What is their respective role in P, Q and M′ populations?

To answer these questions, computer simulation of artificial populations has been used to analyze the dynamics of the installation of (a) state(s) in which *P* element transposition is nonexistent (repressed state(s) or loss of autonomous *P* element).

We have found that, in these populations, the different mechanisms of *P* regulation evolve together when they are submitted to the selective pressure of the dysgenic deleterious effects of the *P* element activity. We have followed the dynamic of appearance of *P* element(s) insertions eliciting zygotic repression (bi-parental, e.g. *KP* elements) and of insertions of element(s) acting according to the retroaction model based on splicing efficiency described above (P cytotype with maternal effect *and* maternal inheritance, e.g. *P*(1A) elements). In addition we have studied the consequences of the association of these two types of elements in the same genome on the evolution of the artificial populations (H. Quesneville & D. Anxolabéhère, in preparation).

We have found that several modes of regulation could coexist in populations at equilibrium. They are characterized by:

a) polymorphism of insertion of autonomous *P* element(s) at site(s) with properties similar to those of the 1A site, described above (*Lk–P*(1A)) eliciting a complete P cytotype;
b) presence of variable numbers of (i) autonomous *P* elements, (ii) deleted elements with repressing capacities but no transposase activity (*KP*-like) of (iii) deleted elements with neither repressing properties nor transposase activity;
c) polymorphism of insertion sites for all these types of elements.

The various final equilibrium states that are observed in these artificial populations correspond to P, Q and M′ populations.

In addition, we have observed a hitch-hiking effect of the regulatory insertions (*P*(1A)-like) on the adjacent insertion sites, which in turn influenced the dynamic and the equilibrium state reached (H. Quesneville & D. Anxolabéhère, in preparation).

This simulation procedure will be used to test the properties of the suspected mechanisms of the worldwide invasion of *Drosophila melanogaster* populations by *P* elements by recurrent waves ('innovative stepping-stone' invasion) and the obtained results will be compared to the results of our survey of natural populations (D. Higuet, S. Ronsseray, G. Periquet & D. Anxolabéhère, unpublished).

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