



## P element in the *Drosophila melanogaster*

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Transposable elements propagate in eukaryotes as successfully as in prokaryotes. The genetic background of eukaryotes includes a variety of elements that possess the ability to move to randomly selected new locations within the genome in which they reside.

Eukaryotic transposable elements can be divided into two general groups :

One group of elements is comparable to bacterial transposons. The elements end in short inverted repeats and generate short direct repeats of target DNA at the site of insertion. Like bacterial transposons, these elements have no life outside the genome. Whether valuable components playing a role in cellular survival or selfish parasites concerned only with their own survival, they have no independent existence and do not generate free molecules of DNA. The best characterized are the controlling elements of maize and the P elements of *D.melanogaster* discussed.

The paradigm for another type of transposition event is provided by ability of **retroviruses** to insert DNA copies (proviruses) of an RNA viral genome into the chromosomes of a host cell.

Certain strains of *D. melanogaster* encounter difficulties in interbreeding. When flies from two of these strains are crossed, the progeny display “dysgenic traits”, a series of defects including

mutations, chromosomal aberrations, distorted segregation at meiosis, and sterility. The appearance of these correlated defects is called *hybrid dysgenesis*.

Two systems responsible for hybrid dysgenesis have been identified in *D. melanogaster*. In the first, flies are divided into the types I (inducer) and R (reactive). Reduced fertility is seen in crosses of I males with R females, but not in the reverse direction. In the second system, flies are divided into the two types P (paternal contributing) and M (maternal contributing).

Dysgenesis is principally a phenomenon of the germ cells. In crosses involving the P-M system, the F1 hybrid flies have normal somatic tissues. However, their gonads don't develop. The morphological defect in gamete development dates from the stage at which rapid cell divisions commence in germ the line.

Any one of the chromosomes of a P male can induce dysgenesis in a cross with an M female. The construction of recombinant chromosomes shows that several regions within each P chromosome are able to cause dysgenesis. This suggests that a P male has a large number of **P factors**, sequences occupying many different chromosomal locations. The locations differ between individual P strains. The P factors are absent from chromosomes of M flies.

The events responsible for the induction of mutations in dysgenesis were identified by mapping the DNA of w mutants found among the dysgenic hybrids. All the mutations result from the insertion of DNA into the locus. The inserted sequence is called the **P element**. The P insertions from a classic transposable system. Individual elements vary in length but are homologous in sequence. All P elements possess inverted terminal repeats of 31 bp, and generate direct repeats of target DNA of 8 bp upon transposition. The longest P elements are ~2.9 kb long and have four open reading frames. The shorter elements arise, apparently rather frequently, by internal deletions of a full-length P factor. At least some of the shorter P elements have lost the

capacity to produce the transposase, but may be activated in *trans* by the enzyme coded by a complete P element.

A P strain carries 30-50 copies of the P factor, about a third of them full length. The factors are absent from M strains. In a P strain, the factors are carried as inert components of the genom. But they become activated to transpose when a P male is crossed with an M female.

Chromosomes from P-M hybrid dysgenic flies have P factors inserted at many new sites. The chromosome breaks typical of hybrid dysgenesis occur at hotspots that are the sites of residence of P factors. The average rate of transposition of P elements to M chromosomes ~1 event per generation.

Activation of P elements is tissue-specific: it occurs only in the germ line. But P elements are transcribed in both germ line and somatic tissues. The primary transcript extends for 2.5 or 3.0 kb, the difference probably reflecting merely the leakiness of the termination site. Two protein products can be produced:

\* In somatic tissues, only the first two introns are spliced, creating an mRNA consisting of ORF0-ORF1-ORF2. Translation of this RNA yields a protein of 66.000 daltons. This protein is a repressor of transposon activity.

\* In germline tissues, an additional splicing event occurs to remove intron 3. This connects all four open reading frames into an mRNA that is translated to generate a protein of 87.000 daltons. This protein is the transposase.

Two types of experiment have demonstrated that splicing of the third intron is needed for transposition. First, if the splicing junctions are mutated *in vitro* and the P element is reintroduced into flies, its transposition activity is abolished. Second, if the third intron is deleted, so that ORF3 is constitutively included in the mRNA in all tissues, transposition occurs in somatic tissues as well as the germ line.

So whenever ORF3 is spliced to the preceding reading frame, the P element becomes active. This is the crucial regulatory event, and usually it occurs only in the germ line. What is responsible for the tissue-specific splicing? Somatic cells contain a protein that binds to sequences in exon 3 to prevent splicing of the last intron. The absence of this protein in germline cells allows splicing to generate the mRNA that codes for the transposase.

Transposition of a P element requires ~150 bp of terminal DNA. The transposase binds to 10 bp sequences that are adjacent to the 31 bp inverted repeats. Transposition occurs by a nonreplicative “cut and paste” mechanism resembling that of Tn10.

It is interesting that, in a significant proportion of cases, the break in donor DNA is repaired by using the sequence of the homologous chromosome. If the homologue has a P element, the presence of a P element at the donor site may be restored (so the event resembles the result of a replicative transposition). If the homologue lacks a P element, repair may generate a sequence lacking the P element, thus apparently providing a precise excision (an unusual event in other transposable systems).

The dependence of hybrid dysgenesis on the sexual orientation of a cross shows that the cytoplasm is important as well as the P factors themselves. The contribution of the cytoplasm is described as the **cytotype**; a line of flies lacking P elements has M cytotype. Hybrid dysgenesis occurs only when chromosomes containing P factors find themselves in M cytotype, that is, when the male parent has P elements and the female parent does not.

Cyotype shows an inheritable cytoplasmic effect; when a cross occurs through P cyotype (the female parent has P elements), hybrid dysgenesis is suppressed for several generations of crosses with M female parents. Thus something in P cyotype, which can be diluted out over some generations, suppresses hybrid dysgenesis.

It depends on the ability of the 66K protein to repress transposition. The protein is provided as a maternal factor in the egg. In a P line, there must be sufficient protein to prevent transposition from occurring, even though the P elements are present. In any cross involving a P female, its presence prevents either synthesis or activity of the transposase. But when the female parent is M type, there is no repressor in the egg, and the introduction of a P element from the male parent results in activity of transposase in the germ line. The ability of a P cyotype to exert an effect through more than one generation suggests that there must be enough repressor protein in the egg, and it must be stable enough, to be passed on through the adult to be present in the eggs of the next generation.

Strains of *D. melanogaster* descended from flies caught in the wild more than 30 years ago are always M strains descended from flies caught in the past 10 years are almost always P. P elements are indeed highly invasive when introduced into a new population; the source of the invading element would have to be another species. Because hybrid dysgenesis reduces interbreeding, it is a step on the path to speciation. Suppose that a dysgenic system is created by a transposable element in some geographic location. Another element may create a different system in some other location. Flies in two areas will be dysgenic for two (or possibly more) system.. If this renders them intersterile and the populations become genetically isolated, further separation may occur. Multiple dysgenic systems therefore lead to inability to mate-and to speciation.

## References

- Benjamin Lewin, Genes V.

- The evolutionary significance of transposons was considered by Camphell, Ann. Rev. Microbiol.35

- *Drosophila melanogaster* P elements have been summarized by Engles, Ann. Rev. Genet.17.