TRANPOSABLE ELEMENTS IN DROSOPHILA

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INTRODUCTION

Transposable elements were discovered by Barbara McClintock about forty years ago, but her work was first greeted with considerable skepticism. It has only been in the last two decades that there has been a general realization that transposons are widespread in both prokaryotes and eukaryotes and are not merely an eccentricity of cultivated maize.

STRUCTURE OF TRANSPOSABLE ELEMENTS

A transposable element is a defined DNA sequence which commonly, but not always, has inverted terminal repeats of up to 40 base pairs in length. The inverted repeats flank a central region containing transposition genes called transposases and other genetic determinants. Characteristically, a duplication of a few base pairs of target (host) DNA is found at the sites of insertion and appears as direct repeats that flank the inserted sequence.

Transposable elements can vary in size from a few hundred to a few thousand base pairs. These elements are typically present at multiple sites in the genome and the location of these sites often differs from one individual to another within a species. The copy number of most transposable element families does not increase without restraint. A number of mechanisms exist for maintaining copy number within a fairly narrow range about a mean which varies for each family. Mechanisms of transposition and excision can also vary widely between different families.

TRANSPONSON-MEDIATED EFFECTS ON HOST DNA

Transposable elements can alter both the organization and expression of genes at frequencies that can exceed those of mutation events due to other causes, both spontaneous and induced (Sankaranrāyanan, 1988). Transposon-mediated mutations have the potential for producing both major disruptions of a functional genome and quantum jumps in an otherwise slow and continuous evolution of DNA sequences. Transposable elements have the rare property essential for successful mutator elements (Leigh, 1973) in that there is complete linkage between the mutator and its induced effects.

A number of DNA changes result from transposon-mediated rearrangements including insertions, excisions, deletions, inversions, duplications and translocations. Insertions of transposable elements can occur into both the coding and regulatory regions of genes. A new insertion into the coding region of a gene frequently, but not always, leads to inactivation of that gene. Insertions into regulatory regions of genes are likely to be less drastic and can lead to changes of gene expression, including the new expression of cryptic genes. The effects of insertions can be reversed by precise excisions which result in the restoration of the wild type genotype and phenotype. Excisions can also be of the imprecise variety which may
leave remnants of some part of the original insertion in the host chromosome. Imprecise excisions can lead to a number of phenotypic effects depending on the extent and location of the sequences involved.

Deletions are a common type of mutation associated with transposable elements and they characteristically extend from the terminus of resident elements for varying distances into the host DNA. Two elements inserted in opposite orientation in host chromosomes can lead to inversion of parts of each of the elements and the intervening host DNA.

Mutations associated with active mobile sequences may be deleterious, advantageous or neutral but, like mutations in general, the majority of changes caused by mobile elements are deleterious (e.g., Fitzpatrick and Sved, 1986). However, a small minority of such changes may be advantageous to the host organism. For example, it has been shown that cells of *Escherichia coli* harboring the transposons *Tn5* or *Tn10* have a growth advantage in chemostat competition over strains that lack these element but are otherwise isogenic (Chao *et al.*, 1983).

**TRANSPOSABLE ELEMENTS IN DROSOPHILA**

Both the number and variety of known transposable element families in *D. melanogaster* is the largest of any species, probably because of the intensity with which this species has been studied. TEs are an important constituent of the middle repetitive DNA and comprise at least 10% of the genome (Young, 1979). Functional elements vary in size from about 2.6 to 8.8 kb. Depending on the family, they produce target site duplications of between 4 and 9 bp on insertion. Copy number per genome also tends to be characteristic for each family and varies between 6 and 80 with a mean of about 30. A detailed review of *D. melanogaster* transposable elements is provided by Finnegan and Fawcett (1986).

**Highly conserved elements:** *D. melanogaster* contains a number of transposable element families whose sequences are highly conserved. These include the *copia*-like elements which characteristically have long terminal repeats (LTRs). From an evolutionary point of view, the *copia*-like elements are of considerable interest because of their structural similarity to the DNA proviruses of vertebrate retroviruses (Finnegan and Fawcett, 1986). The similarities between proviruses and transposable elements provide a very strong argument that the two are evolutionarily fairly closely related. Temin (1980) has argued that retroviruses have evolved from transposable elements, but Finnegan (1985) suggests that transposable elements might equally well be degenerate proviruses and that the two possibilities are not necessarily mutually exclusive.

In *Drosophila*, most detectable spontaneous mutations appear to result from the insertion of a widely distributed class of retroviral-like elements (Sankaranarayanan, 1988) and these mutations appear to account for a
significant portion of the allelic variation present in natural populations. Insertion mutants have been associated with new developmental and/or tissue-specific patterns of expression. Further, McDonald (1989) argues that this class of elements may play a role in the production of evolutionary novelties and in catalyzing the formation of new species which may be especially pronounced under the special conditions existing in peripheral populations.

Unstable elements and those with long inverted repeats: There are two classes of elements in D. melanogaster that have long terminal repeats: the fold-back and TE elements. Fold-back elements have long, inverted repeat sequences of varying length and constitute about three percent of the total genome of D. melanogaster. TE elements are the largest known eukaryotic transposable elements and can be so large as to be detected as visible insertions in polytene chromosomes. TEs are highly unstable and may change their genetic content in association with transposition.

Elements that produce hybrid dysgenesis: The term "hybrid dysgenesis" was first introduced (Kidwell et al., 1977) to describe a syndrome of unusual phenotypic traits that were induced by outcrossing males from a number of D. melanogaster strains with females from long-established laboratory strains. Two families of transposable elements, the P elements (Bingham et al., 1982) and the I elements (Bucheton et al., 1984) are responsible for the P-M and the I-R systems of hybrid dysgenesis, respectively. Subsequently, hobo, a third family of transposable elements has been described whose activation produces a number of phenotypic manifestations that have some of the characteristics shared by the P-M and I-R systems of hybrid dysgenesis (reviewed by Blackman and Gelbart, 1989).

Hybrid dysgenesis was originally described on the basis of the unusual phenotypic properties and modes of inheritance which were observed to be associated with the P-M and I-R systems. The most important of these are the associated induction of a number of dysgenic traits, including high frequencies of insertion and excision mutations, transmission ratio distortion, male recombination, chromosomal aberrations, hybrid dysgenesis and temperature-sensitive sterility.

Reciprocal cross differences occur which are due to the maternal inheritance of element-encoded repressors. In the P-M system, this represents one aspect of an autoregulation system by which P elements regulate copy number (Engels, 1989).

The activation of P and I elements is normally restricted to the germ line. The germline specificity of P element transposition is due to differential splicing of the complete P element transposase transcript in germ line and soma (Laski et al., 1986). From an evolutionary standpoint, the restriction of transposition to the germline reduces the deleterious effects of somatic transposition on the host.
APPLICATIONS OF TRANSPOSABLE ELEMENTS

Two important ways in which \( P \) elements have been used in biological research are: (1) as vectors for gene transfer using germline transformation and (2) for mutagenesis.

**Germline transfer**: \( P \) element DNA can be used as an effective vehicle for transferring genes from one fly to the germ line of another (Spradling and Rubin, 1982). \( P \) element transformation has provided an important new tool for many purposes including the study of development. The \( P \) element is used as a vector for introducing foreign or modified genes into the germline of Drosophila embryos and for subsequently studying the expression and regulation of such transferred genes under different conditions. Bacterial plasmids carrying the sequences of interest within a partially deleted \( P \) element are injected into early embryos together with "helper" plasmids carrying almost complete \( P \) sequences that provide the transposase enzyme necessary for movement and integration into the DNA of the recipient embryo.

In addition to the success achieved with \( P \) element transformation in \textit{D. melanogaster}, it has also been possible, using the same techniques, to transfer \( P \) elements from one \textit{Drosophila} species to another (e.g., Daniels et al., 1989).

**\( P \) element mutagenesis**: The use of active transposable elements for mutagenesis comprises a novel method that does not involve the incidental dangers associated with chemicals and ionizing radiation. Two types of \( P \) element mutagenesis, primary and secondary, may be distinguished (Kidwell, 1986). Primary \( P \) element mutagenesis involves the insertion of a \( P \) element into genes or chromosomal regions that were previously unoccupied to produce an identifiable phenotype. Secondary mutagenesis involves the secondary activation of a resident \( P \) element.

One of the most important uses of primary \( P \) element mutagenesis is in the method of "transposon tagging" for cloning genes. The aim is to tag genes, that have been identified on the basis of genetic analysis, by inducing insertion mutations with a transposable element whose sequences had previously been cloned.

**TE-INDUCED QUANTITATIVE GENETIC VARIATION**

Divergent artificial selection has been practised for several quantitative genetic traits including abdominal and sternopleural bristle number in populations founded by crosses designed to activate the P-M and I-R systems of hybrid dysgenesis (Mackay, 1987, 1988; Frankham et al., 1991). Activated \( P \) elements are more effective than activated I elements for generating quantitative genetic variation in bristle traits (Pignatelli and Mackay, 1989). Accelerated response to selection attributable to TE-induced mutational
variance often occurs. The induction of P-M hybrid dysgenesis increases total variation by a factor of 1.86 for fitness, 14.02 for viability, 2.43 for fertility, 1.37 for abdominal bristle number, 1.21 for sternopleural bristle number and 1.63 for female productivity.

Spontaneous muational heritabilities for bristle traits are significantly higher than those produced by 1000r X-rays per generation (Mackay, 1988) confirming that the P element is a powerful biological mutagen and a highly useful experimental tool for the investigation of the genetic basis of both single locus traits and those subject to quantitative genetic variation.

**DISTRIBUTION AND EVOLUTION OF P ELEMENTS IN DROSOPHILA**

The patterns of distribution of *Drosophila* TE families varies widely. Some families have very limited distributions, others are quite widespread. The distribution of P sequences within the genus is quite patchy and, in many cases, not congruent with phylogenetic groupings (Stacey et al., 1986). This pattern is consistent with P elements having been transferred interspecifically several times in the history of the genus and with the hypothesis that these elements have only recently been introduced into the *D. melanogaster* genome (Kidwell, 1983). The recent determination that a complete P element from *D. willistoni* has a nucleotide sequence essentially identical to that of complete *D. melanogaster* P elements (Daniels et al., 1990) provides additional support for the hypothesis of interspecific transfer and strongly suggests that *D. willistoni* was the donor species. We have identified one potential vector of this transfer, a mite, *Proctolaelaps regalis* DeLeon, whose morphology, behavior and association with *Drosophila* are consistent with the properties necessary for such a vector. Using Southern blot hybridization, PCR amplification and DNA sequencing, we have determined that samples of *P. regalis* associated with a P strain of *D. melanogaster* carried P element sequences.

**REFERENCES**


Question: A.E. Freeman

Can you control the movement of terminal repeat sequences and the enclosed transposable elements from one chromosome to another, that is, can the transposable element be manipulated?

Response: M.G. Kidwell

Transposable elements can move from one genomic site to another (in either the same or a different chromosome), when they are activated by the presence of an appropriate enzyme (transposase) which is often encoded by the elements themselves. Transposable elements are usually found in multiple copies per genome.

Question: A.W. Norskog

Margaret, can you draw on analogy from your work with Drosophila mites, relative to mites that infect chickens as to their possible effect on evolutionary development?

Response: M.G. Kidwell

Our observations that the mite P.regalis can carry Drosophila sequences is the first of its kind to be reported. It is too early to know whether this property is restricted to a single species or genus, of mites or whether it is a more general phenomenon. The possible evolutionary implications are unknown.

Question: Bob McKay

Could the appearance of P elements in D. meleanogaster be attributed to man, because of errors in the lab?

Response: M.G. Kidwell

Even if flies from a P-bearing species were inadvertently mixed with D. melanogaster flies, a vector would still be required to make the transfer between reproductively isolated genomes. It seems far more likely that man played a role in facilitating the spread of P-bearing flies around the world in conjunction with international trade in fruits.
Question: M. Boichard

How do you explain the difference in expression of the P element between the reciprocal crosses P male X M female and M male X P female?

Response: M.G. Kidwell

Repressors of P element transposition are transmitted maternally by P female, but not by M female. Repressors are encoded by P elements themselves in addition to P transposase. M flies are therefore lacking in both repressors and transposase and the progeny of M mothers are unable to repress paternally-derived P elements.

Question: R. Shuman

1. How do P-elements contribute to the overall process of speciation?
2. Are the mites, which you speculated to be involved as a P-element vector, species-specific?

Response: M.G. Kidwell

1. This is an interesting question, but the answer is presently unknown. No-one has yet demonstrated either theoretically or empirically, that transposable elements can result in a barrier to gene flow between populations.
2. The mite P. regalis is an omnivorous semi parasite that can live on a variety of organic material including the immature stages (egg, pupae, etc.) of a number of Drosophila species.

Question: B. Gowe

Can you describe in more detail how you think the mites transferred the P elements?

Response: M.G. Kidwell

We have shown that P. regalis mites associated with Drosophila, themselves carry P element sequences. We speculate that the mites employ preoral digestion of food. This process may result in the injection of the DNA from P fly embryos into M fly embryos by the mouth parts of the mite. An additional vector such as a bacterium cannot presently be excluded.