## Trasposable elements: P elements

In 1938 Marcus Rhodes provided the first genetic description of an unstable mutation, an allele of a gene required for the production of pigment in maize. This instability resulted in a variegated phenotype. Variegation describes irregular patterns of pigmentation and reflects changes in the expression of genes that control pigment production. Rhodes defined two genes required for this instability: the unstable allele defined one gene, and a dominant mutation called Dotted that controlled this instability mapped to a separate locus, defining a second gene. In the 1940s and 1950s Barbara McClintock showed that this type of instability was caused by genetic elements that transposed from one locus to another. It was not until the description of transposable elements in bacteria two decades later that McClintock's work was truly appreciated. McClintock won the Nobel Prize for her work on transposable elements in 1983.

*Drosophila* P elements were also identified by genetic criteria. The ability of geneticists to regulate the movement of P elements has led to the use of these elements as powerful genetic tools.

### Hybrid dysgenesis is caused by the mobilization of *Drosophila melanogaster* P elements

P elements move at a high frequency in the germlines of the progeny of certain crosses causing new mutations and sterility. Crosses between different *Drosophila melanogaster* strains from different parts of the world produce normal, fertile progeny. However, if females from a lab strain, called the M strain, mate with males taken from the wild, called the P strain, the F1 progeny are abnormal. If the F1s are raised at $29^{O}$C, all are sterile, due to a failure of germ cells to develop properly. If raised at $22^{O}$, some of the flies are fertile, but there is a high rate of mutations induced in the gametes of the F1 animals. This type of sterility is called gonadal dysgenesis, and the phenomenon of producing it when mating two different strains is called **hybrid dysgenesis**, and happens when a lab strain is mated as a female with a male from any strain recently isolated from the wild. Another important feature of hybrid dysgenesis is the negative effects are limited to the germline. The somatic tissues of the F1 progeny are normal. Finally, when new mutations were isolated from hybrid dysgenic cross, they were unstable, reverting to wild type at a high frequency. This observation suggested that the mutations were induced by transposable elements.

This germline sterility is specific to M female to P male crosses. It doesn't occur in M female x M male, P female x P male, or P female x M male crosses. Hence the nomenclature. M and P refer to the maternal and paternal contributions, respectively, to hybrid dysgenesis. These observations raised two important questions. Could transposable elements be the source of the sterility and high mutagenesis rates? Why do the mutations arise in the germline, but not somatic tissue?

Gerry Rubin and his colleagues identified the transposable elements responsible for hybrid dysgenesis. The *white* gene had recently been isolated molecularly, providing an opportunity to characterize the mutations caused by hybrid dysgenesis. Mutations in the *white* gene, which lead to a white-eyed phenotype, were induced by hybrid dysgenesis. M females were crossed to P males at $20^{o}$, the F1 males were crossed to white females where the white mutation was on a balancer chromosome, and the F2 females were screened for white eyes. (The scheme was a bit more complicated, but there is no reason to o though it here.) Several white-eyed F2 females were produced, and they also reverted to wild-type spontaneously. If hybrid dysgenesis is caused by transposable elements, the *white* gene of the mutant flies should contain these insertions. Molecular analysis showed that the *white* gene of the mutants contained insertions that varied in size depending on the allele being studied. The largest insertions were 2.9 KB, containing inverted repeats at the ends and the gene encoding transposase. The presence of the insertions also correlated with the mutant phenotype. When the mutants reverted to wild type, the insertion was lost.

Remarkably, P strains contained many copies of the related insertions, and M strains have none. We now know that P elements produce inhibitors of transposition. These are responsible for the lack of transposition in the P strains and in M/P hybrid somatic tissues. In the germline, the inhibitor is provided maternally, which explains why P females, but not M females, are protected from the effects of P elements.

**The 2.9 KB element provides tranposase**

Rubin and his colleague Spradling showed that the 2.9 KB P element was capable of providing transposase activity for other elements in the genome. One particularly sensitive assay for P element activity is a weak allele of the *singed* gene on the X chromosome, known as $singed^{weak}$ or $sn^{w}$ for short (see Figure below for genetic assay). In M

strains, $sn^w$ produces a stable slightly bent bristle phenotype, but the phenotype is highly unstable in hybrid dysgenic crosses, resulting in a wild-type bristle ($sn^+$) phenotype or a more extreme phenotype with severely bent bristles, known as *singed*$^{extreme}$ ($sn^e$). The $sn^w$ promoter contains two P elements; when one excises, the $sn^+$ is produced, and when the other excises, the $sn^e$ is produced.

$sn^w$ / $sn^w$ M females x $sn^+$ / Y P male

$sn^w$ / $sn^+$ F1 female x $sn^+$ / Y M male

$sn^{w?}$ / Y males and $sn^+$ / Y (50% are $sn^+$ or $sn^e$ in phenotype)

**Use of the $sn^w$ allele to detect transposition**

Rubin and Spradling used the $sn^w$ allele to show that the 2.9 kb element could mobilize P elements. They injected into *Drosophila* $sn^w$ / Y male embryos plasmids containing the 2.9 P element. These males were then crossed to *singed* mutant ($sn^-$) females and the F1 females scored for the $sn^+$ and $sn^e$ phenotypes. Both phenotypes were produced and correlated with the excision of one or the other of the P elements in the *sn* gene. They also found that these strains often contained stable tranposase activity and this correlated with the presence of an integrated 2.9 kb element.

P-elements have revolutionized the way Drosophila geneticists conduct their research. Here, we will discuss just a few of the approaches facilitated by P elements.

**Cloning**

P elements play an important role in identifying genes that are defined by mutation. As you will learn in subsequent lectures,

genetic organisms like yeast, *C. elegans* and *Drosophila* are the genetic workhorses for understanding basic problems in cell biology, developmental biology and neurobiology. Genes identified by mutation are involved in particular processes, for example, how pattern is generated during development. To gain a molecular understanding of how a gene functions, it is first cloned and sequenced. P elements have proved crucial to this process in several ways. First, because P elements are mutagens, alleles of a particular gene are isolated in a background expressing transposase. These genes are "tagged" by a P element, allowing the rapid isolation of sequences flanking the P element and hence the mutant gene. This process of producing P-element induced mutations and subsequent isolation of the mutated gene is referred to as "transposon tagging." One of the goals of the Berkeley *Drosophila* Genome Project (BDGP), which has sequenced the Drosophila genome, is to isolate P element induced mutations. In 1999, Spradling, Rubin and their colleagues published a paper describing 1045 strains with P-element induced mutations defining 25% of the genes that are essential for adult viability. If an investigator has mutations in an essential gene, the person can request from the Bloomington Stock Center fly strains containing P-elements that map to the same region and conduct complementation tests. Because the sites of the P-element insertions in these strains are known, if the investigator's mutations and a P-element induced mutation fail to complement, the mutated gene is immediately identified. Cloning of a mutated gene would then consist of mapping, followed by a series of complementation tests with a series of P-element induced mutations in each gene of the region.

"Mutant rescue" can confirm that a specific gene is in fact the mutated gene. In this type of experiment, the wild-type gene is cloned between P-element inverted repeats and injected into embryos containing active transposase and the mutated gene. Rescue of the mutant phenotype by the inserted wild-type gene confirms that the mutation causing the phenotype is in the suspected gene.

**Enhancer trap screens**

Geneticists historically have studied gene function by making mutants and characterizing their phenotypes. An alternate approach is to study where genes are expressed. Neurobiologists, for example, are interested in understanding how cells are specified to become particular types of neurons. As you will see in a few lectures, geneticists have studied this problem by isolating mutants defective

in specifying photoreceptor cell fates of the *Drosophila* eye. An alternate approach is to identify genes expressed in the photoreceptor cells of the fly eye, and this can be done by an approach called enhancer trapping.

Several regulatory elements are involved in gene expression. RNA polymerase binds to a promoter region, where it initiates transcription. Transcription factors bind to other sequences, known as enhancers, that can be 5' to the coding region of the gene, within introns of the gene, or 3' to the coding sequences. The binding of these factors stimulate transcription by RNA polymerase. The strategy of enhancer trapping is to insert near a gene DNA that contains a minimal promoter that will normally not drive expression of *lacZ*, the *E. coli* gene that encodes beta-galactosidase, unless it is near enhancer elements that confer expression. DNA containing the white gene and a minimal promoter 5' to *lacZ* is inserted between P-element inverted repeats and introduced into embryos that express transposase, resulting in the insertion of the sequences into random sites within the genome. These experiments are done in white mutants, and fly lines containing these insertions are established (they have red eyes) and stained for lacZ expression. (X-gal is a substrate for beta-galactosidase and its cleavage results in an intense blue color.) Investigators interested in photoreceptor development look for the production of blue color in photoreceptor cells. Most insertions will not be near genes expressed in the fly eye and hence the eyes will not be blue, but in the rare instances where the DNA is inserted into or near a gene expressed in the eye, eye tissue will stain blue. These genes can then be studied using other approaches address their function in photoreceptor cells.

**The Gal4-UAS system**

The yeast transcriptional activator GAL4 has been used to regulate expression of specific genes in *Drosophila* by inserting the sequences that GAL4 binds (upstream activating sequence or UAS) next to the gene one wants to express (your favorite gene or YFG). Using P element transformation, the GAL4 gene has been inserted at random sites in the genome. These GAL4 insertions are expressed under the control of nearly enhancers that regulate expression of the gene that they control. A large number of these insertions now exist, expressing GAL4 in the entire nervous system, in parts of the nervous system, in the eye, in the legs---the list is long. What this means is that simply by crossing flies containing these transgenes to flies containing a transgene that contains UAS-YFG, you can express YFG in any tissue

that you want. YFG can be GFP, for example, and you can use this to label your favorite cells with GFP. Some investigators are interested in asking the question of what happens when they express their favorite gene ectopically in cells that would normally not express the gene. One of the most striking examples of this is when the eyeless gene is expressed ectopically. Eyeless encodes a transcription factor necessary for eye development. Mutants lacking the eyeless gene have no eyes. This is a conserved gene that is also required for normal eye development in vertebrates. Using the GAL4-UAS system, when eyeless is expressed etopically in different tissues, ectopic eyes would form there. Expression of eyeless in leg tissues caused eyes to form on the legs. Eyeless is thus the key gene for eye development since is it both necessary and sufficient.

As we explore Drosophila as a genetic system in more detail, we will consider additional uses of P elements.