Trasposable elements: Uses of P elements
Problem set B at the end

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.

Problem set B

1. A wild-type *ry+* (*rosy*) gene was introduced into a ry mutant using P element-mediated gene transformation, and a strain containing a stable *ry+* gene was established.

If a transformed male is mated to an M strain female, would you expect the P element construct used in this experiment to transpose to another site in the genome of the F1?

If a transformed male is mated to an P strain female, would you expect the P element construct used in this experiment to transpose to another site in the genome of the F1?

2. One form of male sterility in corn is maternally transmitted. Plants of a male-sterile line crossed with normal pollen give male-sterile plants. In addition, some lines of corn are known to carry a dominant nuclear restorer gene (Rf) that restores pollen fertility in male-sterile lines.

Research shows that the introduction of restorer genes into male-sterile lines does not alter or affect the maintenance of the cytoplasmic factors for male sterility. What kind of genetic results would lead to such a conclusion?

A male-sterile plant is crossed with pollen from a plant homozygous for Rf. What is the genotype of the F1? The phenotype?

The F1 plants from part b are used as females in a testcross with pollen from a normal plant. What would be the result of this testcross? Give genotypes and phenotypes, and designate the kind of cytoplasm.

The restorer gene already described can be called Rf-1. Another dominant restorer, Rf-2, has been found. Rf-1 and Rf-2 are located on different chromosomes. Either or both of the restorer alleles will give pollen fertility. Using a male-sterile plant as a tester, what would be the result of a cross where the male parent was

Heterozygous at both restorer loci?

Homozygous dominant at one restorer locus and homozygous recessive at the other?

Heterozygous at one restorer locus and homozygous recessive at the other?

Heterozygous at one restorer locus and homozygous dominant at the other?

3. In a screen for P element mutations in the miniature wings gene on X, you cross a male to a female that has a recessive lethal mutation on one X chromosome. The other chromosome is a balancer X chromosome containing an inversion, a recessive miniature wings

mutation, a different recessive lethal mutation and the dominant Bar mutation. How would you have generated the male?

What will be the phenotypes most of progeny from the cross to generate new alleles of miniature wings?

What is the phenotype and genotype of the animals that you will be screening for?

4. The snail Lymnea peregra is a hermaphrodite that can either self or cross breed. In this snail the cytoplasm is transmitted through the egg. You are given true-breeding sinistral (shell spirals left) and true-breeding dextral (shell spirals right) lines, and decide to determine the mode of inheritance of shell asymmetry by crossing the two lines.

P1	Dextral egg x Sinistral sperm	Sinistral egg x Dextral sperm
F1	all Dex	all Sin
F2(from self)	all Dex	all Dex

Is dextral recessive or dominant? What is the mode of inheritance? What are the genotypes of the F2 progeny.

What phenotypes would you expect to see in the F2 self progeny?

5. A fly geneticist carries out an enhancer screen and identified several lines that express *lacZ* only in a subset of photoreceptor cells and nowhere else in the fly. The investigator suspects that mutations in these genes will not lead to a lethal phenotype because of the limited expression of the genes, but might impair the ability of the mutant flies to see. One of the P elements is upstream of a gene encodes a rhodopsin-related molecule and *lacZ* is expressed specifically in the photoreceptor cell used to detect UV light. There is a simple assay to test whether flies can detect UV. What would you do to test whether this molecule is required for flies to detect UV?