

***Drosophila* FLP/FRT Screens and a model for cancer genetics**

I briefly mentioned how hypomorphic mutations (vulval development mutants in *C. elegans*) might identify essential genes that are involved in later aspects of development. Another strategy is to make clones of cells that are mutant for an essential gene in the tissue that you are studying. As we saw in the last lecture on mosaic analysis, the production of clones can be stimulated with X-rays. Today, we will discuss advances that use genes and sites that are involved in recombination in yeast to stimulate mitotic recombination in *Drosophila*. We will then discuss how these tools are used to screen specific chromosomal arms for mutations that disrupt targeting of the photoreceptor cells to the brain.

The yeast FLP recombinase and FRT sites

Where and when mosaic clones are produced during development can be controlled by using tissue specific enhancers to drive recombinase enzymes that recognize specific sites engineered into the genome. The yeast 2 μ m plasmid encodes an enzyme known as the FLP recombinase that recognizes two FRT sites and catalyzes reciprocal exchange between these sites. This recombination occurs during replication and results in an internal flipping the orientation of the regions flanking the two sites. This flipping results in an additional round of replication and is thought to be important to amplification of the 2 μ m plasmid, which exists in high copy number in yeast.

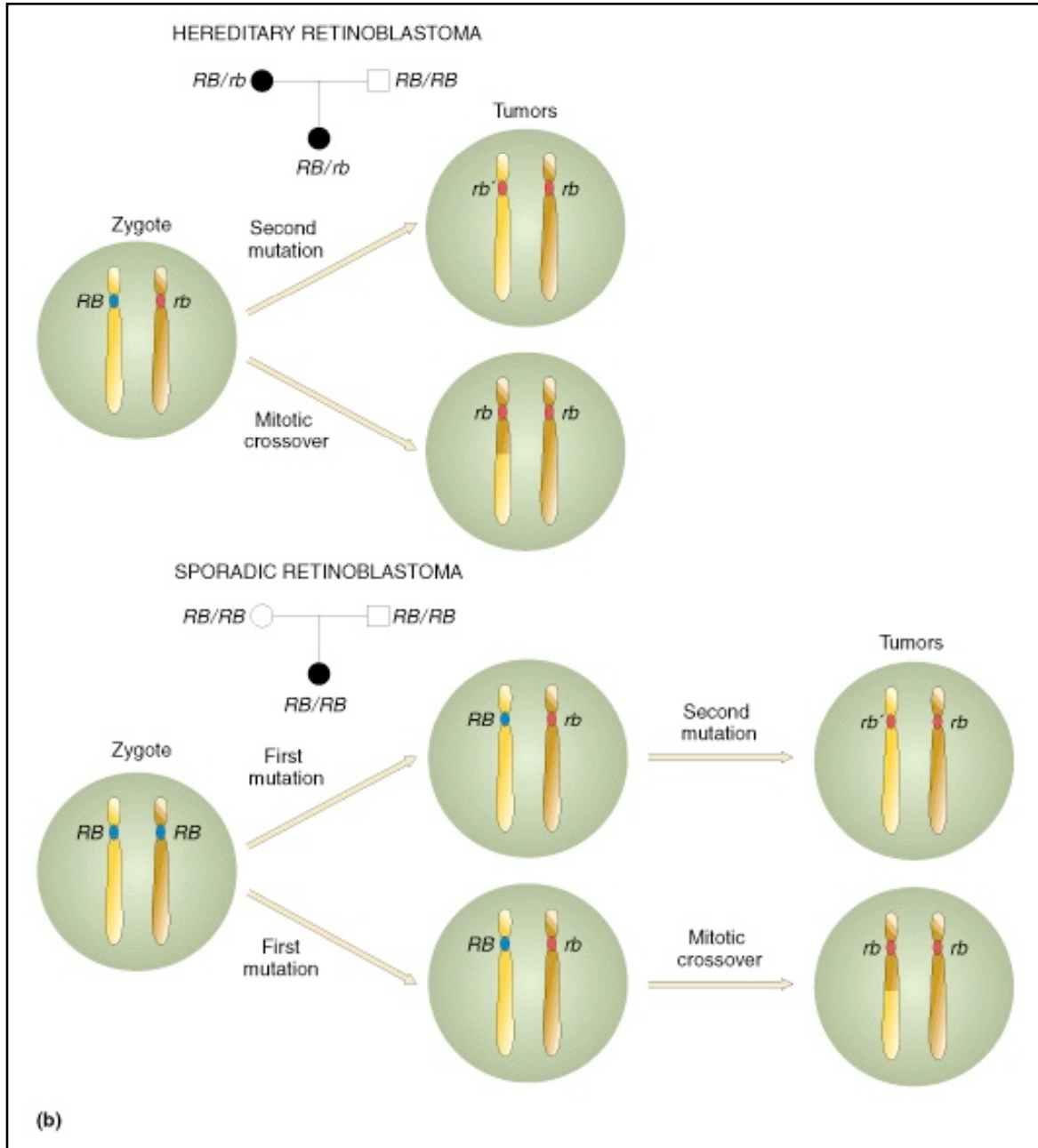
The FLP recombinase and FRT sites can also be used to stimulate reciprocal exchange in flies. The FRT sites, which are recognized and cleaved by FLP recombinase to initiate crossing over between the two sites, have been placed near the centromere on chromosomal arms using P element transposition. To stimulate exchange, FLP recombinase can be expressed in any tissue to stimulate mitotic recombination.

We will consider a specific example of the use of FLP recombinase and FRT sites to screen for mutations that disrupt the pattern of photoreceptor axon targeting in the fly brain.

Tumor suppressor genes

Tumor suppressor genes usually encode proteins that inhibit cell proliferation. Although more rare, Tumor suppressor genes can also encode proteins that inhibit apoptosis. Mutated forms of these genes can be inherited dominantly, but a second event must occur before the mutant contributes to cancer. The wild-type copy of the gene must be lost, and this can occur by spontaneous mutation, chromosomal loss (not shown above) or mitotic recombination. This “loss of heterozygosity” leads to a cell that

is no longer inhibited from proliferating or a surviving cell that is precancerous and normally fated to die. The diagram showing how both spontaneous and inherited forms of retinoblastoma occur when mutations lead to a mutant Rb locus. In inherited forms of Rb, the one mutant allele is inherited, but the wild-type allele must be lost before retinoblastoma tumor is formed. This trait is inherited in a dominant fashion, but the at the level of the cell the cancer only forms when both copies are mutant.



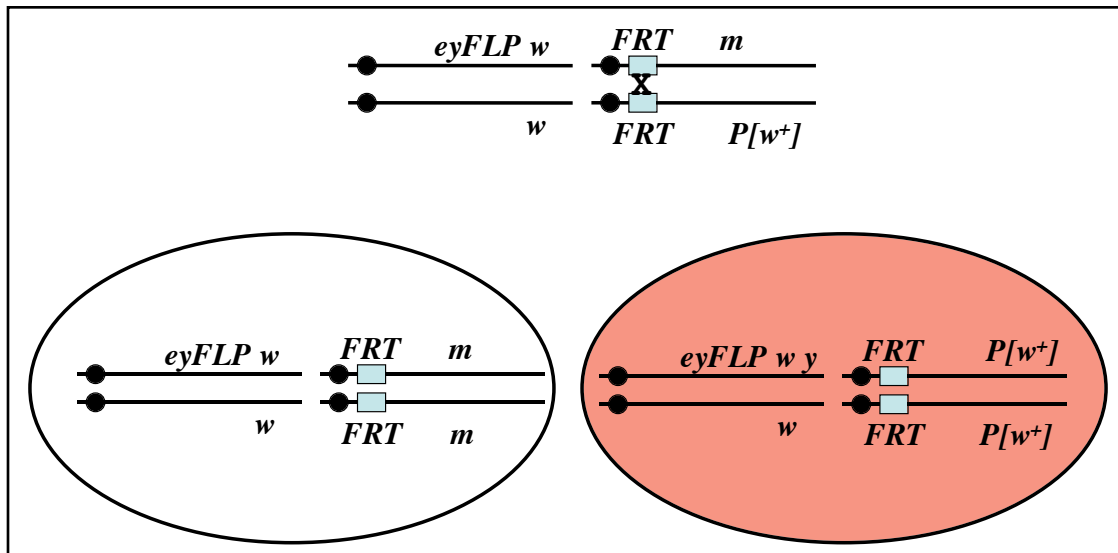
FLP/FRT screens for tumor suppressor genes in *Drosophila*

Iswar Hariharan and his coworkers have developed screens to identify tumor suppressor genes in the fly. The strategy is to generate mutations in a single chromosome and then to stimulate recombination to generate clones that are homozygous for the mutation. If the mutation is in a tumor suppressor gene, these cells should proliferate at the expense of cells containing the wild-type gene.

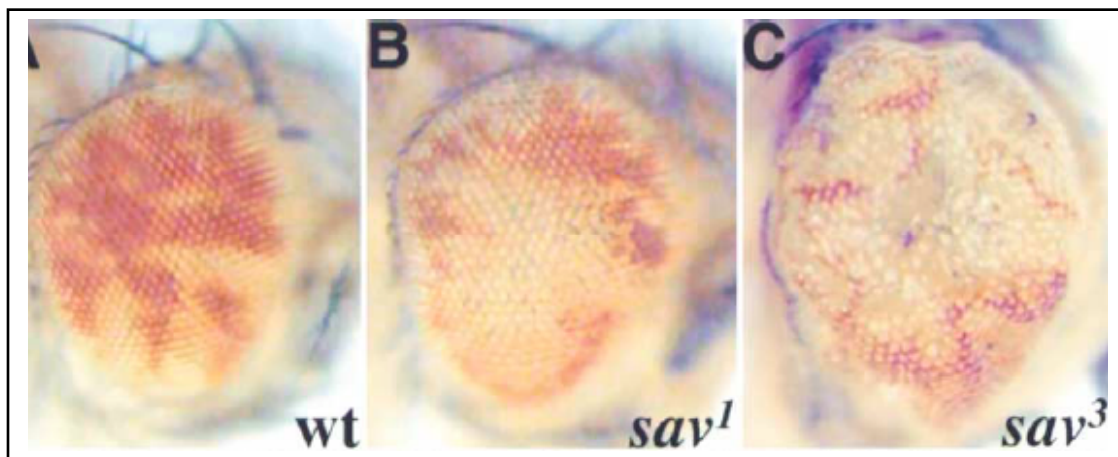
Several tools are used in these screens. First, the chromosome arm screened contains an FRT site near the centromere, inserted into the chromosome using P-element mediated transformation. Chromosome arms are screened one at a time, and the screens will only identify mutations that are distal to the FRT site. These sites are where homologous recombination occurs and are necessary to generate clones that are homozygous for the mutation in the tumor suppressor gene. Second, FLP recombinase is expressed in a particular tissue using a specific promoter. This drives recombination in that tissue alone and avoids the possible lethality associated with the loss gene function in other tissues. In this case the *eyeless* promoter is used, which drives expression of the recombinase only in the developing eye. The *eyeless-FLP* transgene is inserted into a chromosome using P-element mediated transformation. Finally, a cell autonomous marker is used to identify the clones that are homozygous for the mutation. These are usually a P element-transposed wild-type *white* gene. In a *white* mutant background, the $P[w^+]$ is placed on the chromosome not containing the mutation. In this way, white clones that lack the $P[w^+]$ transgene will be homozygous for the mutation.

The rationale for the screen is described in the Figure below. The idea is to make a strain that is homozygous mutant for *white* but also has a transgene containing a copy of the wild-type *white* gene ($P[w^+]$) on the autosome that contains the *FRT* sites. The *eyFLP* is on the X chromosome. It's here to mark the X containing *eyFLP*, but don't worry about it here. Let's consider the situation now where the autosome lacking $P[w^+]$ contains a mutation in a tumor suppressor gene (labeled *m*). Before recombination the cells are heterozygous for *m* and hence normal (remember that tumor suppressor genes are recessive at the level of the cell—they must be homozygous to form a tumor) and heterozygous for $P[w^+]$ and hence are red. In the developing eye, FLP is expressed and this stimulates mitotic recombination between the *FRT* sites. Some of these recombination events will lead to cells that are homozygous for the *m* tumor suppressor mutation. These cells (on the left) will be white and continue to proliferate because of the mutation, whereas the cells that are

not homozygous for the mutation will be red and won't divide in an uncontrolled fashion.

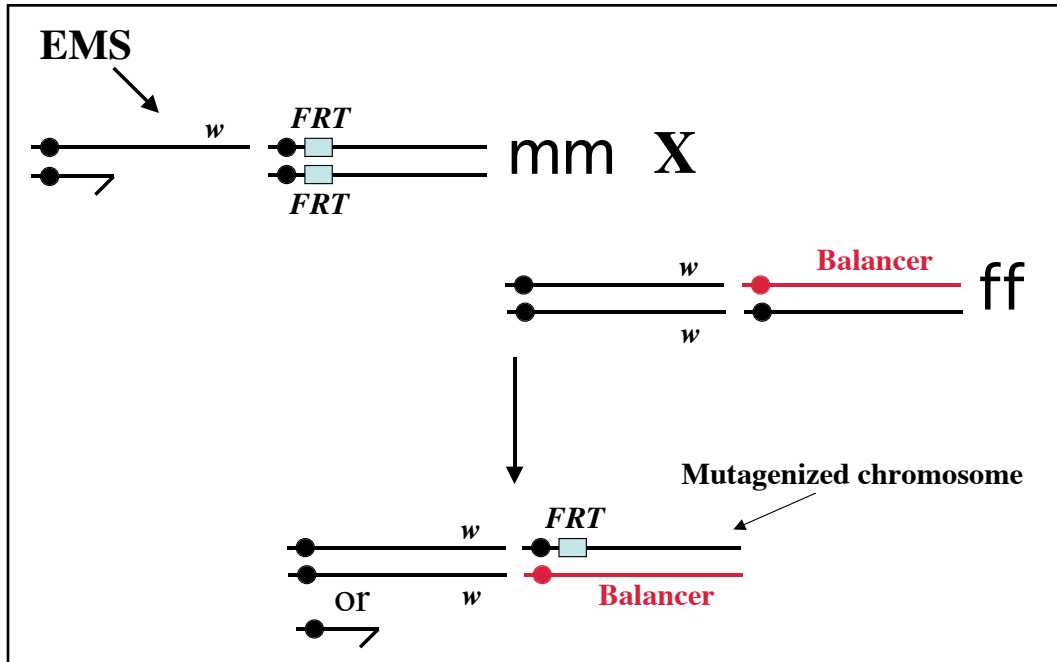


This situation leads to the expansion of white cells in a mosaic eye. Below are mosaic fly eyes that don't contain a tumor suppression (left) or have mutations in a tumor suppressor gene called *sav* (middle and right). The mutant allele on the right is more severe than the allele in the middle, so there are more white cells and the eye is deformed because too many cells are made.

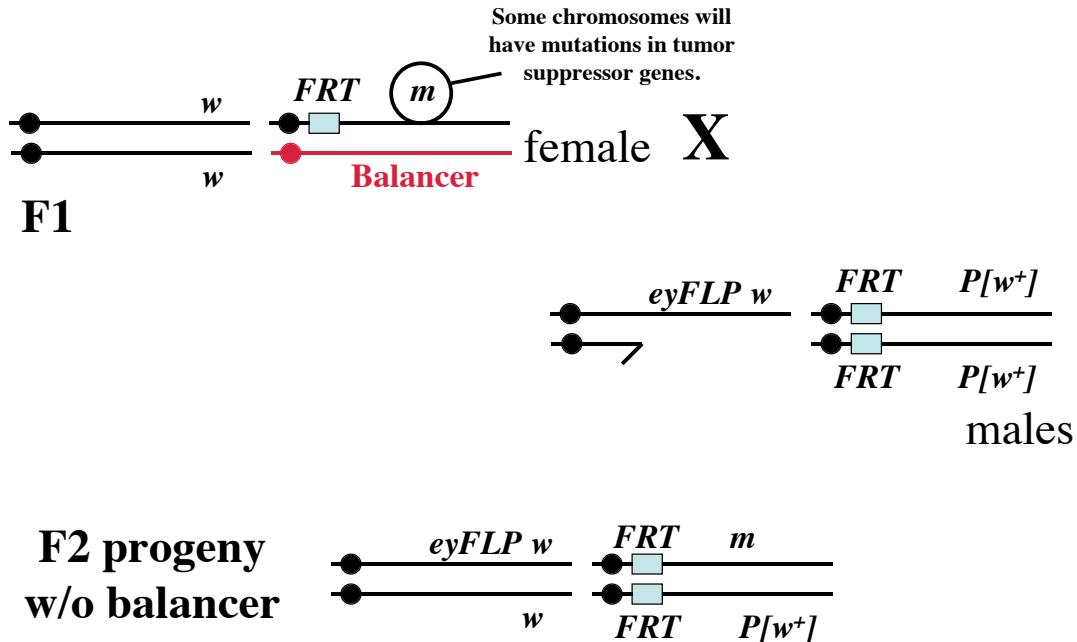


The last thing that we need to consider now is how the mutant screen was carried out, and that is illustrated in the two boxes below. First, males hemizygous for *white* and homozygous for *FRT* sites on an autosome are treated with the mutagen EMS. These males are then crossed

with females homozygous for *white* and heterozygous for a balancer for the autosome being screened. All of the progeny from this cross will have white eyes. The animals of interest contain the mutagenized chromosome with the FRT site and the balancer chromosome. These animals can be identified by the presence of the phenotype caused by the dominant mutation on the balancer.



What needs to be done now is to generate animals with the mutagenized chromosome that are also homozygous for the FRT sites and express FLP in the eye. To do this, the F1 females are crossed with males that have the *eyFLP* transgene and that are homozygous for the FRT sites and *P[w⁺]*. The female progeny from this cross that lack the dominant phenotype caused by the balancer chromosome have *eyFLP* transgene, are homozygous for the FRT sites and have the *P[w⁺]* on the nonmutagenized chromosome. If the mutagenized chromosome contains a mutation in a tumor suppressor gene, then these females should have expansion of white tissue in the eye.



Using this approach, Hariharan and his colleagues identified 23 genes involved in the regulation of proliferation and apoptosis in the eye. These genes have homologs in humans, and tumors were screened to see if any of them were mutant for the homologs. It turns out that these genes are also tumor suppressors in humans. This work identified the first three molecules, Hippo, Warts and Salvador, that defined a conserved pathway for growth control.