P-element-mediated transformation

Transgenic fruit flies have contributed greatly to *Drosophila* developmental genetics. They are made by inserting a known sequence of DNA into the *Drosophila* chromosomal DNA, using as a carrier a **transposon** that occurs naturally in some strains of *Drosophila*. This transposon is known as a **P element**, and the technique as P-element-mediated transformation (Fig. S2.1).

P elements can insert at almost any site on a chromosome, and can also hop from one site to another within the germ cells, an action that requires an enzyme called a transposase. As hopping can cause genomic instability, carrier P elements have had their own transposase gene removed. The transposase required to insert the P element initially is instead provided by a helper P element, which cannot itself insert into the host chromosomes and is thus quickly lost from cells. The carrier and helper elements are injected together into the posterior end of the egg where the germ cells are made.

As well as the gene to be inserted, an additional marker gene, such as the wild-type *white*+ gene, is added to the P element. When *white*+ is the marker, the P element is inserted into flies homozygous for the mutant *white*− gene (which have white eyes rather than the red eyes of the wild-type *Drosophila*). Red eyes are dominant over white, and so flies in which the P element has become integrated into the chromosome, and is being expressed, can be detected by their red eyes.

In the first generation, all flies have white eyes, as any P element that has integrated is still restricted to the germ cells. But in the second generation, a few flies will have wild-type red eyes, showing that they carry the inserted P element in their somatic cells.

This technique has been used to increase the number of copies of a particular gene, or to introduce a mutated gene that has its control or coding regions altered in a known way, or to introduce new genes. It is also possible to introduce genes that carry a marker coding sequence such as *lacZ* (encoding the bacterial enzyme β-galactosidase), whose expression is detectable by histochemical staining (see Box 1D). The P element itself has also been used as a mutagen, as its insertion into a gene usually destroys that gene's function.

This approach has been adapted to large-scale screens that look systematically for genes whose overexpression or misexpression in a particular tissue causes a mutant phenotype (see Box 2D). This is known as misexpression screening. In this case, flies expressing Gal4 in the tissue of interest are crossed with large numbers of different lines of target flies carrying random insertions of the Gal4-binding site, and the progeny screened for a mutant phenotype. This approach is a useful complement to the more conventional genetic screens described in Box 2A, which generally detect loss-of-function mutations. If the target flies also carry a mutation in a known gene, misexpression screening can be used to identify genes whose overexpression enhances or
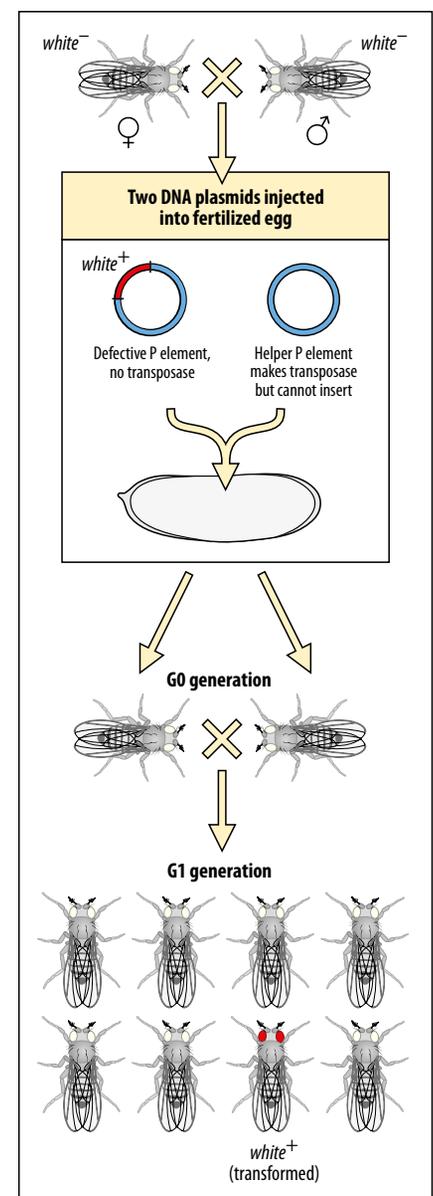

Fig. S2.1

suppresses the mutation. This approach can identify genes whose products interact directly or are part of the same pathway.

Like many other organisms, *Drosophila* can now also be genetically modified using the CRISPR-Cas9 technology (see Box 3D).

■ Further reading

Venken, K.J.T., Bellen, H.J.: **Transgenesis upgrades for *Drosophila* melanogaster**. *Development* 2007, **134**: 3571–3584.

■ Long form question

The generation of transgenic *Drosophila* using the P element transposon has played a complementary role to traditional mutation analysis in deciphering the genetic regulation of *Drosophila* development. Answer the following questions: Why is the P element important to this process? Why is the P element injected into the posterior of the egg, before any cellularization has occurred? Why are flies mutant for the *white* gene typically used as the hosts for these experiments? Last, what genetic crosses are necessary before the consequences of the P element injection can be observed?