Creating mosaics in *Drosophila*

NORBERT PERRIMON*

Department of Genetics, Harvard Medical School, Boston,USA

ABSTRACT **The ability to create mosaic animals allows the phenotypic analysis of patches of groups of genetically different cells that develop in a wild type environment. In *Drosophila*, a variety of techniques have been developed over the years to generate mosaics, and in this chapter, I review the techniques that our laboratory has developed. These include the "Dominant Female Sterile" technique which allows the analysis of gene functions to oogenesis and embryogenesis; the "Gal4-UAS" technique which allows the control of where and when specific genes are expressed; and, the "Positive Marked Mutant Lineages" technique which allows clones of cells to express a specific reporter gene.**

KEY WORDS: *Drosophila, mosaics, Flp, Gal 4*

Introduction

As a student at the University of Paris in early 1980, I heard a series of lectures on *Drosophila* Developmental Genetics organized by Didier Contamine and François Jacob at the Collège de France. There, I listened for the first time about the classic studies conducted by Antonio García-Bellido and his colleagues using mosaic animals. This series of lectures had a profound impact on my career as I decided afterwards to learn more about *Drosophila* as an experimental system. At that time what struck me the most about the *Drosophila* system was the ability, and ease, of creating *Drosophila* mosaic animals using X-rays. Soon after, I was fortunate to be accepted into the laboratory of Madame Gans at Gif/Yvette to begin my "Diplome d'Etude Approfondies". It was in Madame Gans' laboratory that I began my clonal analysis studies of the *ovo*^{D} Dominant Female Sterile mutations. During this time, I spent numerous hours reading Antonio García-Bellido's studies on imaginal discs development. Antonio's papers, and those of others, gave me full appreciation of the influence of novel technologies on scientific progress. In particular, the "Minute technique", which led to the "compartment hypothesis" (see García-Bellido *et al.,* 1979), was a clear example of the impact of novel technologies in Biology.

Over the years my colleagues and I have spent a fair amount of effort at improving and developing new techniques that facilitate the creation of mosaic animals. In this chapter, I review the contributions that we have made to the field and describe some of our ongoing efforts at perfecting these techniques.

Techniques of germline mosaics:
the "FLP-DFS" technique

Many genes involved in development are used at multiple times during development making it often impossible to detect their effects by simply looking at whole mutant animals. Genes that mutate to zygotic lethality represent almost 95% of the 5,000 loci in *Drosophila* that mutate to detectable phenotypes. The effect of these genes on development can be analyzed by generating clones of homozygous cells in an otherwise heterozygous animal. Thus, it is possible for example to examine the effect of mutations in essential genes on the development of adult structures by generating clones of homozygous mutant cells in an heterozygous animal, assuming of course that these clones are not cell lethal. Similarly, genes that mutate to zygotic lethality can be systematically examined for their roles during oogenesis and embryonic development by generating animals that carry a mutant germline (germline clones) in an otherwise wild type animal. Importantly, embryonic or larval phenotypes associated with the lethal phase of an essential gene may not at all reflect the full range of gene functions because of their maternal contributions, i.e., germline cells being necessarily heterozygous, may produce gene products that will accumulate in the oocyte, thereby rescuing in part the loss of function in the zygote.

One of the techniques used to generate germline clones is to transplant mutant pole cells into wild-type female embryos (Illmensee, 1973). However, this technique is of limited systematic application as it is cumbersome. More user friendly is the "dominant female sterile" (DFS) technique which utilizes germline-dependent DFS mutations (Wieschaus, 1980; Perrimon and Gans, 1983; Perrimon, 1984).

This technique has been especially fruitful using the X-linked DFS mutation *ovo*^{D1}. Females heterozygous for *ovo*^{D1} do not lay eggs and develop atrophic ovaries containing no vitellogenic eggs. A mitotic exchange occurring in the female germ cells results in recombinant daughter cells which have eliminated the DFS mutation and thus can produce eggs. *ovo*^{D1} is completely penetrant for the DFS phenotype and because this mutation affects germ cell development at an early
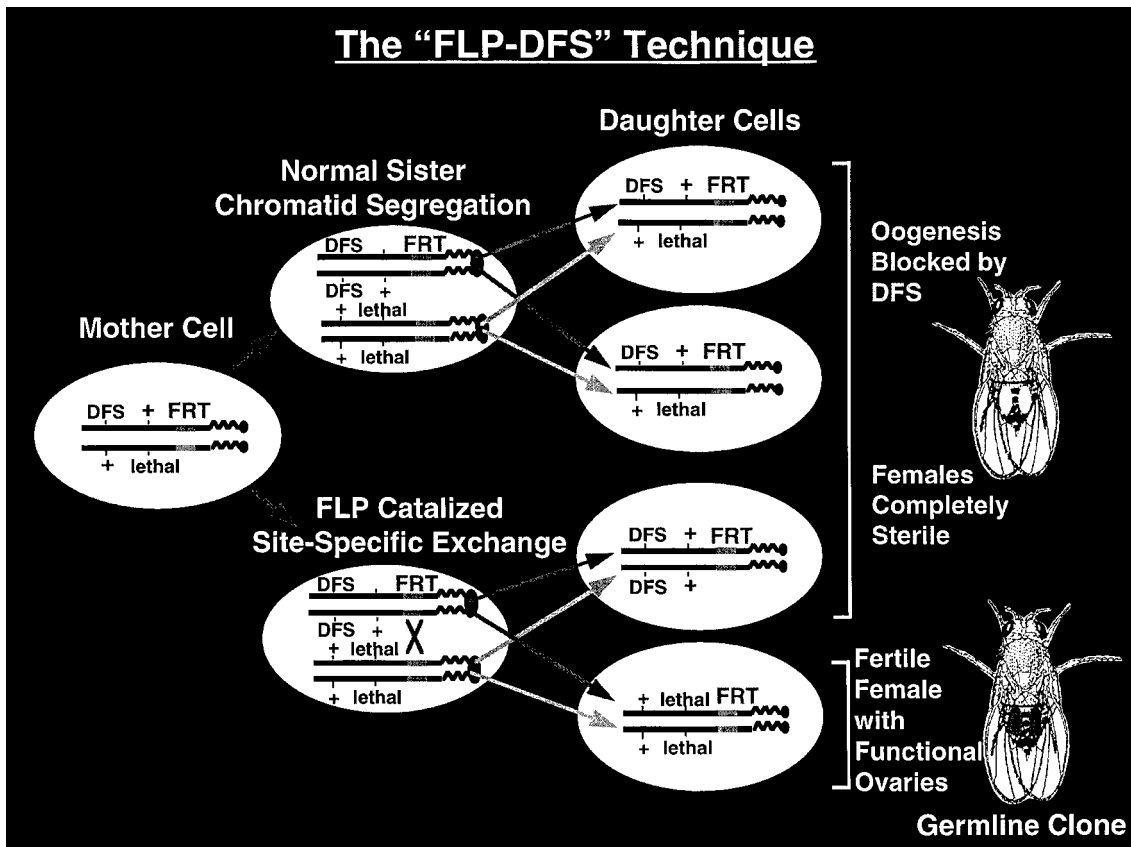

Fig. 1. The FLP-DFS technique. *Chromosomal exchange that occurs in the euchromatin of a fly of genotype DFS + FRT/+ lethal FRT;FLP/+ . The FRT insertion is located proximally to both DFS and lethal. hsp70-FLP from another chromosome site can provide recombinase activity following heat shock induction to catalyze site-specific chromosomal exchange at the position of the FRT sequences. FLP-catalyzed recombination result in the recovery of almost 100% of females with lethal/lethal homozygous germline clones (lowest branch). Adapted from Chou and Perrimon (1996). Atrophic ovaries are shown as empty ovals and ovaries with developed ovarioles as filled ovals. FLP-recombinase target sequences (FRT). Dominant female sterile (DFS). Recessive zygotic lethal mutation (lethal).*

stage, germ cells that have lost ovo^{D1} during the larval stages lead to large clones often populating the full ovary (Perrimon, 1984).

To induce the mitotic exchange between homologous chromosomes, female heterozygous for ovo^{D1} can be treated with X-rays. To generate germline chimeras of an X-linked zygotic lethal mutation *(lethal)*, using the "ovo^{D1}-DFS" technique, individuals trans heterozygous for both the ovo^{D1} and *lethal* mutations are treated with X-rays and *lethal/lethal* homozygous germline clones recovered. One technical problem of the ovo^{D1}-DFS technique is the low frequency of mosaic females recovered following X-ray irradiation (Perrimon, 1984). To overcome this problem we have used the properties of the yeast "FLP-FRT" site-specific recombination system. The yeast FLP-recombinase, and its recombination targets (FRTs), from the 2μm plasmid of *Saccharomyces cerevisiae*, were successfully transferred into the *Drosophila* genome (Golic and Lindquist, 1989). The heat-inducible FLP-recombinase gene, under the control of an *hsp70* promoter, recognizes and promotes recombination specifically at the level of the FRT sequences. Golic and Lindquist (1989) demonstrated that a *mini-white* gene flanked with FRT elements can be excised resulting in the production of mosaic eyes. In addition, Golic (1991) has shown that FLP-recombinase can catalyze site-specific recombination between homologous chromosomes. By combining the FLP-FRT recombination system with the "DFS" technique, we have developed the "FLP-DFS technique" (Fig. 1; Chou and Perrimon, 1992,1996; Chou *et al.,* 1993). Our laboratory has built specific chromosomes that allow this technique to be extended to the entire *Drosophila* genome (these stocks are available from the Bloomington *Drosophila* Stock Center; see Appendix in Chou and Perrimon, 1996).

The FLP-DFS technique can be used to systematically examine the contribution of zygotic lethal mutations to oogenesis and embryogenesis (Perrimon *et al.,* 1996). One of its limitations, however, is that at the present time, with the exception of mutations that are located on the same chromosomal arm, it is difficult to examine the phenotypes of embryos derived from germlines that lack multiple gene activities. To overcome this problem we are in the process of developing the "Multiple GermLine Clone" (MGLC) technique. The principle of this method is to generate males that carry multiple DFS mutations, located on different chromosome arms, in cis with appropriate FRT insertions. The problem in building such flies is that it is impossible to introduce two DFS mutations in the same male as a result of a cross. Thus, we are generating males that contain multiple, silent DFS mutations consisting of insertions of *ovo promoter - ovo*^{D} gene constructs separated by a "FLP-out" cassette (Struhl and Basler, 1993). Following expression of the FLP enzyme, the ovo^D DFS genes can be reactivated by removal of the transcriptional stop inserted in the "FLP-out" cassette. This technique will make possible the examination of phenotypes of embryos derived from germline cells that lack multiple gene activities.

Techniques of gene misexpression: the "Gal4-UAS" technique

Loss of function studies allow one to determine whether a specific gene is necessary to a developmental process. These studies need to be complemented by gain of function studies that determine whether a specific gene is sufficient to trigger the
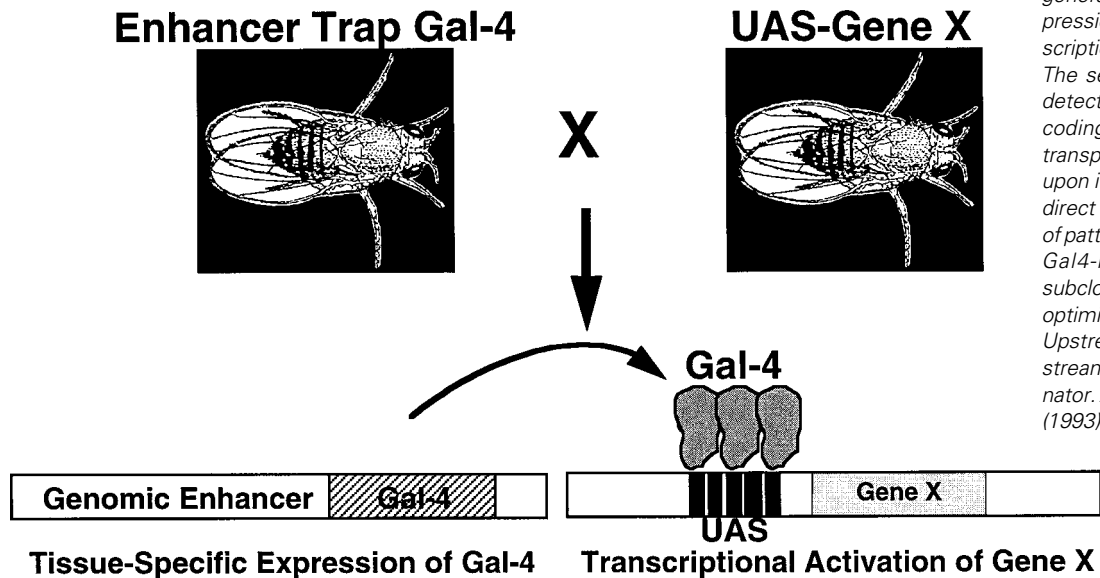
The Gal-4/UAS Technique

Enhancer Trap Gal-4   ## UAS-Gene X

X

Gal-4

| Genomic Enhancer ▨▨▨ Gal-4 ▨▨▨ | | | | | UAS Gene X |

UAS

Tissue-Specific Expression of Gal-4   Transcriptional Activation of Gene X

Fig. 2. **The Gal4/UAS system.** *The principle of the technique is described in the text. Two approaches can be used to generate different patterns of Gal4 expression. The first is to drive Gal4 transcription using characterized promoters. The second is based on the "enhancer detection" technique whereby the Gal4 coding sequence is fused to the P-transposase promoter which, depending upon its genomic site of integration, can direct expression of Gal4 in a wide range of patterns in embryos, larvae and adults. Gal4-responsive target genes are subcloned behind a tandem array of five optimized Gal4 binding sites (UAS, for Upstream Activation Sequence), and upstream of the SV40 transcriptional terminator. Adapted from Brand and Perrimon (1993).*

developmental process under scrutiny. Several methods have been used for controlling ectopic expression in *Drosophila*. For example, expression of a specific gene can be driven in a tissue specific manner under the control of specific transcriptional regulatory sequences, or uniformly under the control of an heat shock promoter. These techniques, however, are limited by the availability of cloned and characterized promoters that can direct expression in a desired pattern and by the problems inherent to uniform expression, respectively (see Discussions in Brand and Perrimon, 1993; Brand *et al.,* 1994).

To overcome these problems we have developed the "Gal4-UAS" technique (Brand and Perrimon, 1993; Fig. 2), which allows the control of where and when specific genes are expressed. This mosaic technique is based on the transactivator properties of the Gal4 yeast protein (Fisher *et al.,* 1988). The Gal4 system separates the target gene from its transcriptional activator in two distinct transgenic lines. In one line the target gene remains silent in the absence of its activator; in the second line the activator protein is present but has no target gene to activate. Only when the two lines are crossed together is the target gene turned on in the progeny, and the phenotypic consequences of misexpression be conveniently studied. In contrast to the original enhancer-trap method, the Gal4-UAS method is designed to generate lines that express a transcriptional activator, rather than an individual target gene, in numerous patterns. Any gene of interest, *xyz*, can then be activated in different cell- and tissue-types merely by crossing a single line carrying a UAS*xyz* construct to a library of activator-expressing lines. Numerous activator-expressing lines have been generated allowing target genes to be expressed in numerous distinct patterns. In the past few years this technique has been used extensively to address various questions of developmental genetics. These include the targeted expression of wild-type and dominant negative genes, toxins and cellular markers such as the green fluorescent protein.

The Gal4-UAS system has been combined successfully with the FLP-FRT technology to develop novel ways to create mosaics. By using the Gal4 system to control the expression of the yeast recombinase, FLP, in a spatial and temporal fashion, we have shown that it is possible to efficiently and specifically target loss-of-function studies for vital loci to any tissues (Duffy *et al.,* 1998). Using this approach, simple and extremely efficient F1 adult phenotypic screens can be carried out to identify gene functions involved in the development of a specific tissue.

The "Positive Marked Mutant Lineages" (PMML) technique

The analysis of the functions of signaling pathways at the cellular level in various cell types requires the availability of efficient techniques of marking mutant clones. At present the technique commonly utilized is that of Xu and Rubin (1993) where mutant cells, induced in heterozygous animals using the FLP-FRT system, are identified by their lack of expression of a marker gene. One difficulty with this method is that clones of mutant cells are detected by the absence of a marker, which can be problematic for the detection of small clones in some tissues.

To overcome this problem we have developed the "PMML" technique which uses a ubiquitous enhancer and an FRT on one chromosome, and an FRT and reporter gene on the homolog (Fig. 3; Harrison and Perrimon, 1993). A recombination event at the FRT site, caused by a pulse of FLPase expression, places the reporter gene downstream of the enhancer, thus allowing expression of the reporter gene in a subset of the daughter cells. This technique can be used to follow specific cell lineage. For example, Margolis and Spradling (1995) used this technique to conduct a clonal analysis of follicle cells. In addition, this technique can be used to label patches of homozygous mutant cells in an otherwise heterozygous animal. For these experiments, the mutation to be analyzed

Using FLP Recombinase-Mediated Intermolecular Recombination for Generating Positively Marked Mitotic Clones
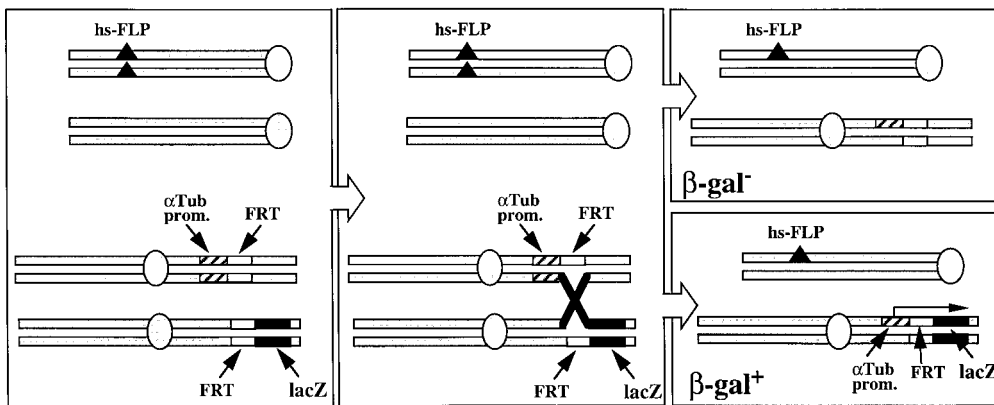

Fig. 3. **The PMML system.** *Chromosome segregation associated with FLP-mediated mitotic exchange. Chromosomes of mother cell (on the left) are shown following DNA synthesis. Chromosomes of daughter cells (to the right) are shown with the normal diploid content following mitosis. Only cells that have reconstituted an alpha-tubulin promoter-FRT- lacZ gene, as the result of a FLP-catalyzed recombination at the level of the FRT element, will stain for β-Galactosidase activity. Adapted from Harrison and Perrimon (1993).*

is recombined distally to the distal portion of the marker cassette (FRT-lacZ). Once recombination at the level of the FRT occurs, clones of homozygous mutant cells will express the marker. It is important to notice, however, that depending on the pattern of x and z chromosomal segregation (see Pimpinelli and Ripoll, 1986), not all positively marked cells may become homozygous for the mutation. This problem can be overcome by the addition of an additional cellular marker, distally and in cis, with the proximal portion of the marker cassette (promoter-FRT).

The PMML technique that we originally developed (Harrison and Perrimon, 1993) has two shortcomings which have limited its general use. First, the fragment of the α-tubulin promoter that was used in our experiment did not drive expression of the *lacZ* gene uniformly in all cell types. Second, the β-Galactosidase protein generated by our construct contains a nuclear localization signal such that the marker does not allow the phenotypes of some cell lineages to be scored appropriately.

We are in the process of adding a number of refinements to this technique. First, a ubiquitous promoter such as *actin 5C* instead of α-tubulin is being used. Second, instead of lacZ as a reporter gene, we have decided to use Gal4. A recombination event at the level of the FRT element will lead to a group of cells that express Gal4 clonally. The ability of these cells to express Gal4 will allow a wide variety of markers such as lacZ, GFP, tau-GFP to be expressed in the Gal4-expressing cells.

Concluding remarks

The ability to examine the phenotypes associated with a patch of genetically different cells that develop in a wild type context is critical to the analysis of gene functions during development. In the past 15 years, a number of sophisticated techniques in *Drosophila* and other animals have been developed to achieve these goals. However, additional improvements on the existing techniques are required to further facilitate our analysis of animal development. For example, techniques that allow one to precisely control the level at which a specific gene is expressed in a mosaic patch do not yet exist. In addition, methods to generate mosaic animals that carry patches of cells that express multiple gene activities, or that have lost multiple gene activities need to be developed.

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