Genetic mosaic techniques for studying *Drosophila* development

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Summary

Genetic screens for recessive mutations continue to provide the basis for much of the modern work on *Drosophila* developmental genetics. However, many of the mutations isolated in these screens cause embryonic or early larval lethality. Studying the effects of such mutations on later developmental events is still possible, however, using genetic mosaic techniques, which limit losses or gains of genetic function to specific tissues and cells, and to selected stages of development. A variety of genetic mosaic techniques have been developed, and these have led to key insights into developmental processes in the fly. Variations on these techniques can also be used to screen for novel genes that are involved in non-embryonic patterning and growth.

Introduction

Genetic mosaic techniques are those that induce genetic changes in a subset of cells or tissues in an individual organism. Such techniques have been crucial for our current understanding of a number of developmental processes in *Drosophila*. There are several reasons for this. Most importantly, the techniques provide a way of examining genetic changes that would be lethal if applied to the entire organism. For example, many of the mutations that completely remove the function of a given gene are homozygous lethal in embryonic or early larval stages of development. This is not a problem if one is studying early development; indeed, the isolation and characterization of embryonic lethal mutations was a landmark in *Drosophila* developmental genetics. However, if one is interested in studying the later stages of development, the only ways of circumventing early lethality are to use viable alleles or temperature-sensitive alleles (provided that they exist or can be generated), or to use genetic mosaic techniques. If the genetic change can be limited to small numbers of cells, or can be induced at later stages of development, early lethality can often be avoided. Our present understanding of the development of late-developing adult tissues in the fly, such as the appendages, compound eye, internal organs and oocyte, owes much to the use of genetic mosaic techniques.

Genetic mosaics also provide a powerful tool for teasing apart complex developmental interactions, no matter what developmental stage is being examined. If removing a gene prevents the development of a specific structure, is it because the precursors of that structure require the gene? Or is the requirement in some different group of cells? If the gene is expressed in both sets of cells, genetic mosaics provide the only means of answering these questions. Genetic mosaics also juxtapose wild-type and genetically altered cells, and this can be used to test the 'cell-autonomy' of a mutant phenotype. That is, are the mutant cells affected by the presence of neighboring wild-type cells? Can the mutant phenotype be rescued by wild-type cells and, if so, over what range? Conversely, can the mutant cells affect the development of their wild-type neighbors (sometimes referred to as 'domineering' non-autonomy)? Testing the cell autonomy of a mutation again helps to determine how directly a gene is involved in a given developmental decision. And for a novel mutation that affects signaling, one can determine whether it is required in the sending or receiving cells. For example, cell autonomy was used to determine that Notch was a receptor, and Delta its ligand, before any details were known about the Notch signaling pathway (Heitzler and Simpson, 1991)

Mosaic techniques have also provided much of what we know about normal cell lineages in *Drosophila* tissues. Although dye-tracing techniques can be used to follow cell lineages in the embryo (e.g. Vincent and O'Farrell, 1992; Bossing and Technau, 1994), the extensive cell proliferation that occurs between embryonic and adult stages dilutes lineage dyes to undetectable levels. Thus, researchers interested in later stages of development have relied upon genetic mosaics to mark wild-type cells and their progeny; the 'compartmental' lineage restrictions of adult appendages were discovered using these techniques (Garcia-Bellido et al., 1973; Garcia-Bellido et al., 1976).

In this article I briefly summarize the most popular techniques for generating genetic mosaics in *Drosophila*. I include not only those mosaic techniques used for examining mutations in endogenous genes, but also the techniques used to limit the expression of gene constructs to particular regions or stages of development.

Making mosaics the old-fashioned way

Surgical approaches

Before the advent of engineered constructs and the like, there were essentially two approaches to creating genetic mosaics in flies. The first involved transplantation between different fly strains, either of cells or nuclei (Geyer-Duszynska, 1967; Illmensee, 1968; Illmensee, 1973; Technau, 1986) [for techniques see Santamaria (Santamaria, 1986)] or whole tissues (such as imaginal discs, which form most of the adult epidermis) (Ephrussi and Beadle, 1936) [for techniques, see Ashburner (Ashburner, 1989)]. Although often useful, these surgical techniques have been underused of late, presumably because of the technical difficulties involved. The small size of

the fly embryo can make cell and nuclear transplantation difficult. Transplanting mature imaginal discs is easier; they will grow after being injected into an adult or larva, and will differentiate in pupae. However, it is difficult to get them to incorporate into the adult epithelium, as they usually differentiate as a crumpled mass inside the developing pupa, leaving some structures difficult to analyze. Immature imaginal discs from embryos or early larval stages are also small and difficult to isolate, although discs can develop from whole embryos after they have been transplanted into a host (Simcox, 1997). And, in many cases, one would like to study changes to only part of a disc.

Given the power of *Drosophila* genetics, it was therefore natural for researchers to use purely genetic techniques for creating mosaics. Initially, researchers relied on two techniques to study recessive mutations in flies: chromosome loss and the recombination of homologous chromosomes during mitosis.

Chromosome loss

Several mutations can induce the random loss of the three autosomes, the X chromosome or the Y chromosome during early cleavages of the fly embryo (reviewed by Ashburner, 1989). However, although individual flies with a single X or fourth chromosome are mostly viable, loss of a second or third chromosome usually causes death. Certain morphologically abnormal chromosomes are also unstable, such as the much-used ring-X chromosome. Loss of a ring-X occurs randomly during the first few divisions of the zygotic nuclei, and creates a gynandromorph, which has a mixture of female (XX) and male (XO) tissues. This chromosome has been used to study normal cell lineages and to study mutations on the X. However, nearly half of the resultant early embryo is XO, so if the X carries a lethal mutation, the embryo is unlikely to survive to later stages of development.

Mitotic recombination

A more generally applicable method is the induction of mitotic recombination between the arms of homologous chromosomes (Stern, 1936). As shown in Fig. 1, this can generate, from a heterozygotic parent cell, two daughter cells that are homozygous for everything distal to the site of recombination. A low level of mitotic recombination occurs spontaneously in flies, and this rate can be increased to a useful, although still low, level by exposing flies to X-rays or γ-rays (Patterson, 1929).

Irradiation has the advantage that the stage at which recombination occurs can be controlled. Timing cannot be exact, however. First, there is a delay between the recombination event and the production of the daughter cells. Moreover, the inheritance or ‘perdurance’ of wild-type mRNA and protein from the parent cell can block the expression of the mutant phenotype for several cell divisions. Irradiation can also, depending on the dose and stage of development, cause enough damage and cell death to slow subsequent development (reviewed by Ashburner, 1989).

Marking cells

One problem that chromosome loss and irradiation-induced mitotic recombination techniques share is that the location of homo- or hemizygous cells within the tissue is essentially random. These cells therefore need to be marked (reviewed by Ashburner, 1989; Lawrence et al., 1986). Identifying mutant cells in the adult epidermis is usually done by linking the mutation being studied to a benign marker mutation on the same chromosome arm; the marker mutation changes the color (*yellow*) or morphology (*forked*, *multiple wing hair*, etc.) of the cuticle that is secreted by a given cell. In the eye, pigmentation mutants or constructs, such as *white*, are used in a similar manner.

A variety of techniques have been used to mark the cells of internal organs, or cells at stages of development before cuticle or pigment are made. Initially, researchers used mutations in ubiquitously expressed enzymes, such as a temperature-sensitive mutation in *succinate dehydrogenase* (Lawrence, 1981). Homozygotic cells were identified by their failure to stain after appropriate histochemical reactions. More recently, constructs encoding several non-endogenous, histologically identifiable tags have been inserted into the genome; available tags include β-galactosidase (β-gal) (e.g. Blair, 1992), a Myc epitope (Xu and Rubin, 1993) and green fluorescent protein (GFP). In mitotic recombination-based approaches, the histological tags are usually located on the wild-type chromosome, so that the homozygous mutant cells are identified by the absence of the marker (Fig. 1). In some cases, the *marker*/*marker* sister of the *mutant*/*mutant* cell can also be identified; this ‘twin-spot’ is useful both as a control for the effects of the sister *mutant*/*mutant* cells, and as an indication of the location of the recombination event within the tissue (Fig. 1). However, it should be pointed out that *mutant*/*mutant* cells are occasionally difficult to identify because of the absence of the marker, especially if the cells are few in number or the tissue is complex. For one solution to this problem, see the Gal80 section below.

Clones

Even following irradiation, mitotic recombination is still a relatively rare event. However, this rarity has also led to the discovery of a useful feature of *Drosophila* development, that cell intermixing and migration are quite limited in *Drosophila* epithelia. The homozygous daughter cell produced by a single mitotic recombination event almost always forms a single, spatially coherent ‘clone’ of descendents (Fig. 1B). This is unlike the salt-and-pepper patterns that are commonly seen in genetic mosaics in vertebrates, and can simplify the analysis of phenotypes. The size of the clone observed in an adult depends on the developmental stage at which the larva was irradiated. As the percent of cells undergoing recombination is constant, the number of clones observed at later stages depends on the number of target cells that were present during irradiation. For example, clones induced earlier in imaginal disc development are large but infrequent, whereas those induced later are smaller but more frequent.

The *Minute* technique

In some cases, it is helpful to have mitotic recombinant clones that are as large as possible, for example, when looking for restrictions in cell migration, or when removing a broadly expressed signaling molecule. This can be accomplished using the *Minute* technique (reviewed by Ashburner, 1989; Lawrence et al., 1986) (Fig. 2). Dominant mutations at several different *Minute* loci slow cell division rates. Those *Minute* mutations that have been characterized disrupt ribosomal proteins. When

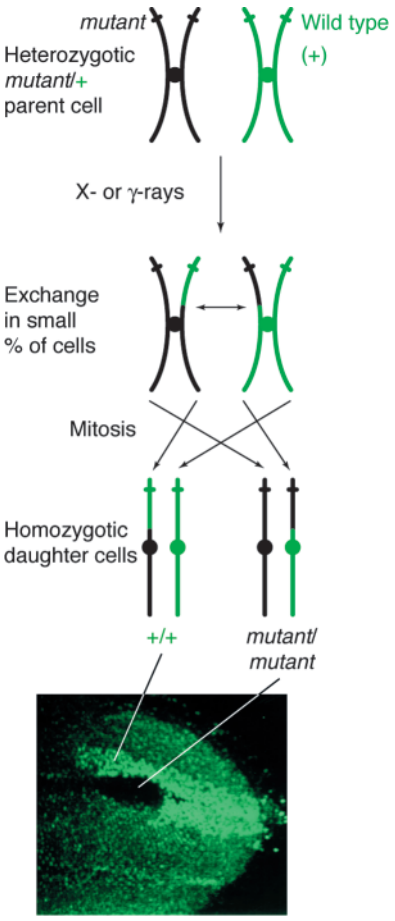

Fig. 1. Chromosome behavior during and after irradiation-induced mitotic recombination. The clones produced by the two homozygotic daughter cells of a mitotic recombination event are shown below. The photograph shows a *shaggy* mutant clone, which lacks anti-Myc staining (green), and its sister 'twin spot' (+/+), which has a double dose of the Myc epitope, in a pupal wing blade.

a wild-type (+/+) clone forms in *Minute*/+ tissue, the clone has a growth advantage over its slow-dividing neighbors. Interestingly, the abnormally large +/+ clones do not usually alter developmental patterning. The *Minute* technique can thus be used to increase the size of *mutant* clones. This technique can also increase the chances that a *mutant* clone survives. For example, mutant clones in imaginal discs are often lost because of a phenomenon called clone competition, where abnormal slow-growing cells are eliminated in some way by the surrounding wild-type cells [for recent work on this phenomenon, see Moreno et al. (Moreno et al., (2002)]. Giving the clone a growth advantage using the *Minute* technique often rescues it.

Germline mosaics

Another use of mitotic recombination is to induce homozygous mosaics in the developing germline. In this way, germline contributions can be removed even if the mutation is homozygous lethal in the parent. A powerful variation on this technique uses dominant *Female sterile* mutations to block the production of any but the homozygous mutant oocytes: the heterozygotic *Female sterile/mutant* oocytes die, but the *mutant*/*mutant* oocytes produced by recombination survive (reviewed by Ashburner, 1989). An advance that made *Female sterile* mutations available on every chromosome arm was the cloning of the gain-of-function allele *Ovo-D*^{1} and the insertion of constructs containing *Ovo-D*^{1} throughout the genome (Chou et al., 1993; Chou and Perrimon, 1996).

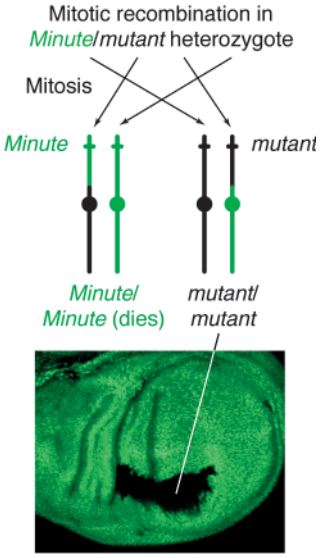

Fig. 2. The *Minute* technique. The homozygous *mutant* cells produced by mitotic recombination lack the *Minute* mutation, and thus divide more quickly than the surrounding heterozygotic cells. The homozygous *Minute* clone usually dies. The photograph shows a wild-type clone, lacking GFP (green), in a +/*RpS5*^{2} [also known as *Minute(1)o*^{sp}] late third instar wing imaginal disc.

Additions and improvements to mosaic techniques

The stable insertion of DNA constructs into the fly genome via engineered transposable elements (most commonly the *P* element) (Rubin and Spradling, 1982; Spradling and Rubin, 1982) has made several modifications of and additions to the early mosaic techniques possible (see Duffy, 2002). The initial insertion of a new *P* element construct into the genome is still somewhat laborious, requiring the injection of many embryos to generate one transformant. However, constructs that have already been incorporated into the genome can be remobilized, and thus 'hopped' from one position to another in the genome, by mating the transformed fly strain to another that carries a constitutively expressed transposase (Robertson et al., 1988).

The mosaic techniques discussed in the following sections use variations on two different systems, both derived from yeast. The first uses targeted DNA recombination at FLPase recombination targets (FRTs), which can be driven in flies by the FLP recombinase (FLPase) (Golic and Lindquist, 1989). The second uses the Gal4 transcription factor to drive the expression of constructs that are coupled to the UAS enhancer sequence (Brand and Perrimon, 1993). Flies carrying most of the constructs discussed below are available as community-wide resources through the Bloomington *Drosophila* Stock Center (http://flystocks.bio.indiana.edu/).

FRT-mediated mitotic recombination

FRTs have been inserted into proximal locations on each of the chromosome arms, and several stocks have been generated that express FLPase under the control of the hsp70 heat-shock promoter (hs-FLPase) (Chou and Perrimon, 1992; Golic, 1991; Xu and Rubin, 1993). If a fly has two FRTs in identical positions on homologous chromosomes, heat-shock-induced expression of FLPase can cause recombination between the FRT sites (Fig. 3). This technique has several advantages over irradiation-induced recombination. FRT-mediated mitotic recombination rates are much higher than those caused by irradiation, although they are still low enough to ensure that only a small percentage of cells will be homozygous. The site of the recombination is also controlled, so that one no longer

has to worry about recombination occurring in the chromosomal region distal to the mutation, or between the mutation and the marker. Heat shock also induces less cell death than irradiation. However, there are also disadvantages to FRT-mediated recombination. Mutations and markers must first be meiotically recombined onto the appropriate FRT-bearing chromosome before the technique can be used. Not only does this take time, it also prevents the technique from being used for extant mutations on the fourth chromosome, where meiotic recombination does not occur. Moreover, the technique cannot be used for those genes that are proximal to any available FRT insertion.

FRT-mediated FLPout constructs

Another use of FRTs is to remove stretches of DNA that are located between two FRTs. This technique was originally used to remove gene constructs (Golic and Lindquist, 1989), but it can also be used to induce the misexpression of selected genes and constructs in clones of cells via the FLPout technique (Struhl and Basler, 1993) (Fig. 4). The basic FLPout construct contains a strong, ubiquitously active promoter (often that of actin or tubulin) coupled to: an FRT; a marker gene; a transcription termination signal (either 5′ or 3′ to the marker gene); a second FRT; and, finally, the coding sequence to be misexpressed. In such constructs, the promoter is blocked from driving the expression of the downstream coding sequence by the termination signal. However, when the region between the FRTs is removed by FLPase-induced recombination (a 'FLPout'), both the marker gene and the termination signal are lost, and the downstream coding sequence is expressed. In most applications of this technique, recombination is driven by hs-FLPase; the timing of the FLPout and the percentage of cells undergoing recombination depend on the timing and levels of heat shock. Interestingly, the activation of the FLPout does not require mitosis, and can thus be used to drive gene expression in postmitotic tissues.

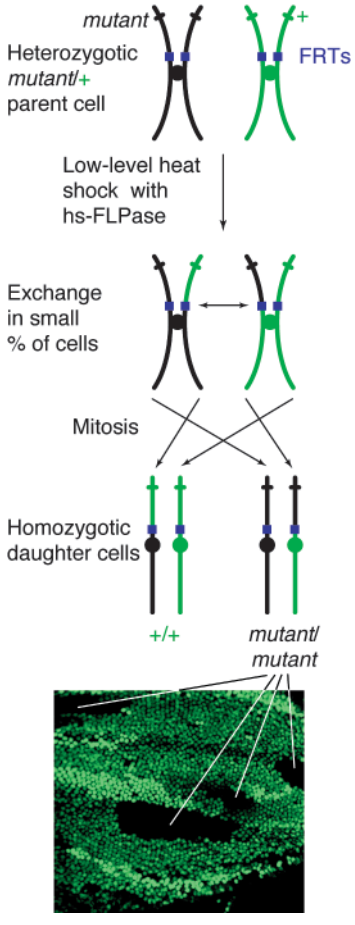

Fig. 3. FRT-induced mitotic recombination, catalyzed at FRTs by hs-FLPase. The photograph shows several *engrailed* clones, lacking anti-Myc staining (green), in a pupal wing blade. FRTs, FLPase recombination targets; hs-FLPase, heat-shock-induced FLP recombinase.

The Gal4 UAS system

The Gal4 UAS system exploits the sensitivity of the UAS enhancer element to the Gal4 transcription factor. If the coding region of a gene or construct is linked to UAS, the gene will be expressed in Gal4-expressing cells (Brand and Perrimon, 1993) (reviewed by Duffy, 2002). Thus, if one has a fly strain in which Gal4 expression is spatially restricted, this strain can be mated to a second strain that contains any chosen *UAS-gene* construct; the resultant offspring will be mosaics of cells that either do or do not express that gene (Fig. 5). *UAS-GFP* or *UAS-lacZ* can be used to simultaneously mark the region of Gal4 expression.

The trick to generating mosaics using this system is to restrict the expression of Gal4. In a few cases, this has been achieved by making constructs that employ promoters and enhancers from known genes (e.g. Staehling-Hampton et al., 1994) (Fig. 5A). However, most of the Gal4-expressing fly lines have been generated by enhancer trapping (Brand and Perrimon, 1993; Callaja et al., 1996) (Fig. 5B,D), a technique that has been previously used to detect genes with novel expression patterns (O'Kane and Gehring, 1987). In this approach, a weak promoter is linked to the Gal4-coding region, and the resulting construct is inserted randomly into the genome (or is re-mobilized from a previous insertion site). If the construct gets inserted near an active enhancer or promoter sequence, Gal4 can be expressed in a pattern that is identical to that of the neighboring endogenous gene. Screens for useful Gal4 lines continue, often aided by using UAS constructs that allow novel Gal4 expression patterns to be detected in adults (e.g. *UAS-yellow*) (Callaja et al., 1996).

Many UAS constructs have been generated using cloned genes. Another way of driving the expression of UAS-gene constructs that is more random, but is proving to be increasingly useful, uses EP constructs (Rorth, 1996; Rorth et al., 1998) (Fig. 5D). EP constructs contain multiple UAS sequences that are coupled to a weak promoter; as with enhancer trap constructs, and have been hopped around the genome. If an EP construct lands in a favorable position it can, in the presence of Gal4, drive the expression of the neighboring gene (see Fig. 5E). Many of these EP insertions have been precisely mapped in the genome, and thus provide a ready-made resource for the misexpression of wild-type genes (the insertion sites are mapped at http://flybase.bio.indiana.edu, or available by BLAST at http://www.fruitfly.org/blast/).

The Gal4-UAS system can also be used to generate loss-of-function mosaics. The genes in UAS constructs can be engineered to ones liking; and thus dominant-negative constructs can be used. Another, increasingly popular way of generating loss-of-function mosaics uses UAS-hairpin constructs to drive expression of double-stranded (ds) RNA (Kennerdell and Carthew, 2000; Fortier and Belote, 2000; Piccin et al., 2001) (Fig. 5F). dsRNA has not been effectively

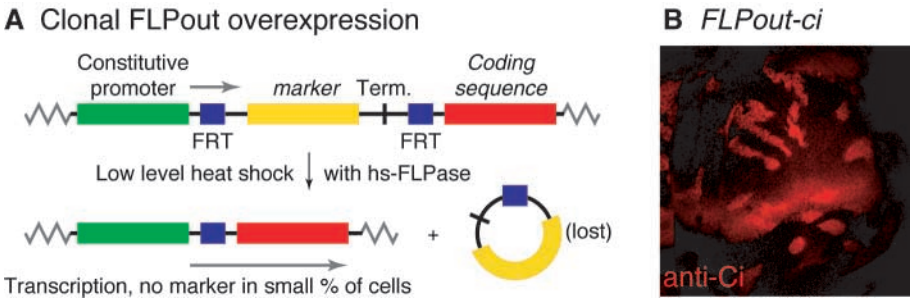

Fig. 4. The FLPout technique. (A) hs-FLPase catalyzes the removal of an FRT, a *marker* gene and a transcriptional termination signal (Term.) from the FLPout construct, allowing the constitutive promoter to drive the expression of the downstream gene sequence. (B) The photograph shows several clones generated using a *FLPout-cubitus interruptus* (*ci*) construct (Hepker et al., 1997), which can identified by the high levels of anti-Ci staining (red), in a late third instar wing imaginal disc. FRT, FLPase recombination target; hs-FLPase, heat-shock-induced FLP recombinase.

delivered into *Drosophila* except by injecting embryos, and the effects of such injections do not last until late stages of development (Kennerdell and Carthew, 1998). UAS-hairpin constructs, when coupled with appropriate Gal4 drivers, provide another way of generating dsRNA. The hairpin constructs contain forward and reverse coding regions, which are coupled end to end by a short spacer sequence. The RNA transcribed from these constructs folds back upon itself to generate dsRNA, which knocks down the expression of the corresponding endogenous gene. Such knockdowns are often incomplete (Kennerdell and Carthew, 2000). Nonetheless, this approach provides a powerful way of analyzing genes for which no mutants are available.

Combinations and variations

Given the utility of the FRT and the Gal4 UAS systems, it was almost inevitable that they would end up being united. This has been achieved in several ways.

FLPout-Gal4

Previously, researchers wanting to drive the mosaic misexpression of a gene had to choose between building a FLPout or a UAS construct. This is no longer the case, as several FLPout lines have now been developed that express high levels of Gal4 (de Celis and Bray, 1997; Ito et al., 1997; Pignoni and Zipursky, 1997). Thus, a researcher can simply build a *UAS-gene* construct, and then drive the expression of that gene in FLPout clones expressing Gal4, or in the regions defined by any other Gal4 driver. Many of the FLPout-Gal4 chromosomes also carry UAS-GFP or a *lacZ* marker, providing a positive marker for the clones.

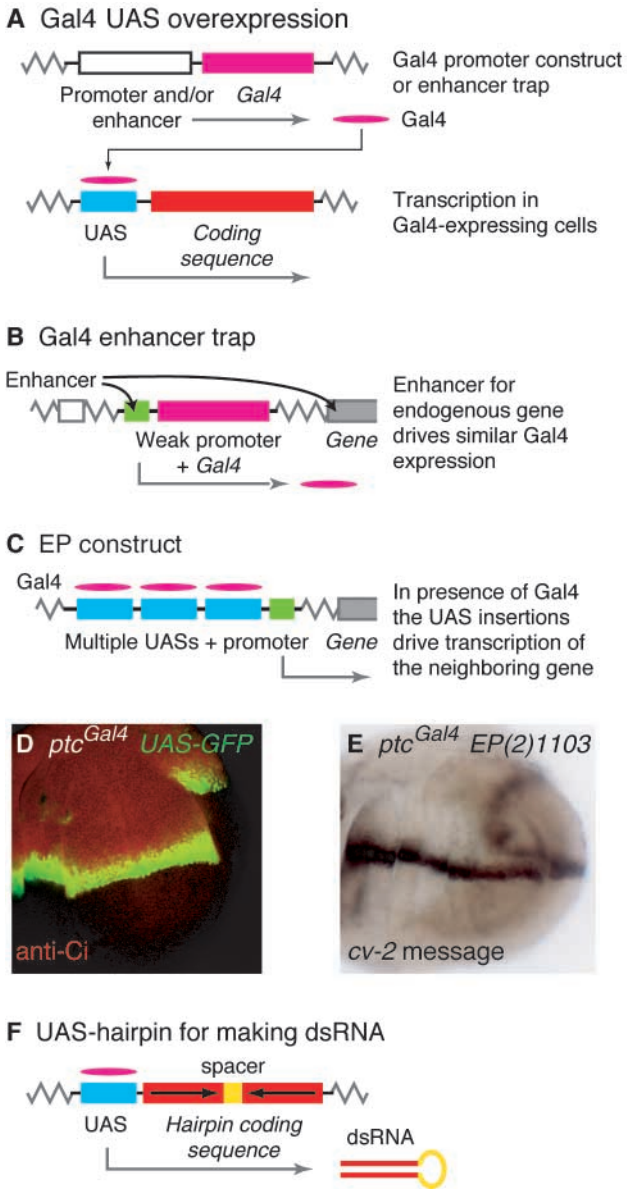

Fig. 5. (A) The Gal4 UAS system. A construct that contains a known promoter or enhancer coupled to the *Gal4* gene drives region-specific expression of Gal4. Gal4, in turn, stimulates the transcription from a construct that links the UAS sequence to a chosen coding sequence. (B) The Gal4 enhancer trap. When the enhancer trap construct falls near an active endogenous enhancer, that enhancer drives *Gal4* expression. (C) The EP construct. In the presence of Gal4, the UAS and promoter in the inserted UAS-EP construct drive the expression of neighboring genes. (D) A late third instar wing disc showing the region-specific expression of *UAS-GFP* (green) driven by a ptc^{Gal4} enhancer trap. Strong anti-Ci (Cubitus interruptus) staining (red) shows the anterior compartment. (E) In situ hybridization of a late third instar wing disc showing the ectopic expression of an endogenous gene driven using an EP insertion. ptc^{Gal4} drives the expression of *crossveinless 2* (*cv-2*), which is located near the *EP(2)1103* insertion. Reproduced, with permission, from Conley et al. (Conley et al., 2000). (F) Generating dsRNA using a UAS-hairpin construct. Gal4 drives the expression of a UAS construct containing two inverted coding regions placed head to head (red, arrows), separated by a short spacer sequence. The mRNA produced by the construct folds back upon itself to form dsRNA, which will interfere with expression of the corresponding endogenous gene.

UAS-FLPase

FRT-based mitotic recombination or FLPouts can be induced in spatially restricted domains in the fly by crossing flies that express Gal4 in a restricted manner to those that carry a UAS-FLPase construct (Duffy et al., 1998). Interestingly, this provides a way of tracing the history of a gene's expression patterns during development (Weigmann and Cohen, 1999) (Fig. 6). In this approach, a given Gal4 line is used to drive the expression of FLPase under the control of UAS. These flies also contain a FLPout-*lacZ* construct, and the FLPase-induced recombination of this construct causes the irreversible expression of *lacZ*. As a result, *lacZ* is expressed in the descendents of all of the cells that ever expressed Gal4, even if they are no longer expressing Gal4. The only difficulty here is telling whether some unexpected pattern of *lacZ* expression is due to previously undetected gene expression, or due to the leakiness or inaccuracy of the Gal4 driver. For example, the *patched Gal4* enhancer trap (ptc^{Gal4}) is thought to mirror accurately the anterior compartment-specific expression of the endogenous *ptc* gene (Hinz et al., 1994; Speicher et al., 1994) (Fig. 5D). However, when ptc^{Gal4} is used to drive UAS-FLPase and a FLPout-construct, FLPout clones are often found in the 'wrong' (posterior) compartment (Fig. 6B).

UAS-Gal4

This construct provides another way of driving the irreversible expression of Gal4 (Hassan et al., 2000). Once activated by the region-specific expression of a given Gal4 insertion, the UAS-Gal4 construct adds positive feedback, driving its own expression. Therefore, the descendents of the cells expressing the region-specific Gal4 will continue to express Gal4, even if the region-specific insertion no longer expresses Gal4.

Gal80

The Gal4 inhibitor Gal80 provides yet another way of controlling the Gal4 UAS system (Lee and Luo, 1999). When Gal80 expression is driven with a tubulin promoter (tub-Gal80), it can inhibit the activity of a tubulin promoter-Gal4 construct. If Gal80 is removed (see below), Gal4 is disinhibited and drives the expression of a UAS construct.

In the MARCM (mosaic analysis with a repressible cell marker) technique, the tub-Gal80 is removed using FRT-mediated mitotic recombination (Lee and Luo, 1999) (Fig. 7). The advantage of this technique over FLPouts is that it simultaneously generates a mitotic recombinant clone. This can be used, for example, to generate a clone of homozygous mutant neurons that also express a membrane-associated GFP, thus marking the mutant axons; in fact, this technique can be used in all cases where one needs to positively mark homozygous mutant clones. Moreover, this technique can also be used to generate clones that are not only homozygous for a given mutation, but also simultaneously express any chosen UAS construct.

Regulating the timing of Gal4 activity

For many experiments, it would be useful if the timing of gene misexpression could be regulated. The timed generation of FLPout clones provides one way to do this, but it is often impossible to generate clones of the needed size and in the necessary positions. Moreover, once the FLPout is activated, it cannot be turned off. In some cases, the cold-sensitivity of Gal4 can be used to minimize UAS-driven gene expression at chosen stages of development. However, even at 18°C, Gal4 still retains considerable activity.

Therefore, several laboratories have developed ways of regulating the Gal4 UAS system by building hormone or drug sensitivity into the Gal4 or UAS, or even adding a FLPout cassette after the UAS sequence (reviewed by Duffy, 2002). However, all of these techniques require using novel Gal4 or UAS constructs, and thus cannot be used with the lines that were previously generated. A new technique that solves this problem uses a temperature-sensitive form of Gal80 (Gal80ts) (R. Davis, personal communication). Fly lines carrying Gal80ts under the control of a tubulin promoter have been generated and crossed to lines containing Gal4 and UAS constructs. At low permissive temperatures, the Gal80ts blocks the effectiveness of Gal4, while at higher restrictive temperatures, it fails to inhibit Gal4.

Genetic screens

Mitotic recombination can be used to make not only known mutations homozygous, but also mutagenized chromosomes.

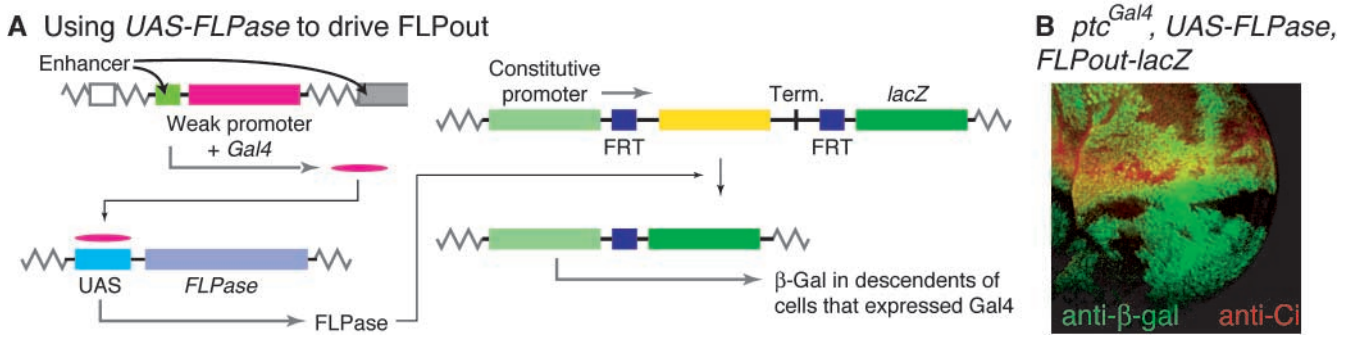

Fig. 6. Using *UAS-FLPase* and *FLPout* constructs to mark the descendents of Gal4-expressing cells. (A) Gal4 drives expression from the *UAS-FLPase* construct. The *FLPase* in turn activates the *FLPout* construct by deleting the sequences between the FRTs, leading to the irreversible expression of β-gal in the descendents of cells that expressed Gal4. (B) Anti-β-gal staining (green), showing the FLPout clones in a late third instar wing disc containing ptc^{Gal4}, *UAS-FLPase* and *FLPout-lacZ*. Anti-Ci staining (red) labels the anterior compartment. Note the presence of clones in the posterior compartment, which were not present in the ptc^{Gal4} *UAS-GFP* disc (Fig. 5E). β-gal, β-galactosidase; Ci, Cubitus interruptus; FLPase, FLP recombinase; *ptc*, *patched*.

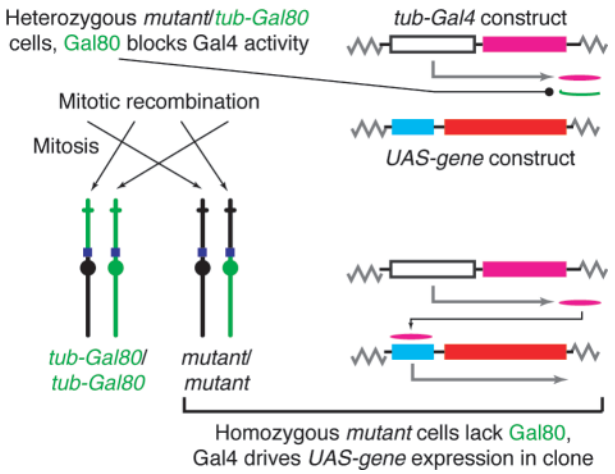

Fig. 7. The mosaic analysis with a repressible cell marker (MARCM) technique. Both *Gal80* and *Gal4* expression is driven using *tubulin* promoters (*tub*). In the parent cells, the Gal80 inhibits the activity of Gal4. Mitotic recombination creates a clone of homozygous *mutant* cells that lack the *tub-Gal80* construct. The Gal4 generated by the *tub-Gal4* construct (purple) is now free to drive expression from the *UAS-gene* construct (blue).

An advantage of this technique is that the screening can be done in the first (F1) generation, reducing the labor involved. In such a screen, mitotic recombination is used to generate homozygous cells in developing F1 flies that are each heterozygous for a different mutagenized chromosome. F1 adults that have clones with interesting phenotypes are recovered and a stock established. This technique was used even before the advent of FRT-based recombination, but the high rate of recombination that can be achieved using FRTs makes the technique much more efficient (Xu and Rubin, 1993). In the developing wing disc, for example, the appropriate induction of hs-FLPase can generate several clones in every wing blade of every F1 adult. Using UAS-FLPase and an appropriate Gal4 driver can further increase the rate of recombination and direct it to a particular tissue or subregion. Variations on this technique have been used to isolate several mutations that affect cell differentiation and growth patterns in the adult, such as *slimb* (which encodes a ubiquitin ligase that regulates Hedgehog and Wingless/Wnt signaling) (Jiang and Struhl, 1998; Theodosiou et al., 1998) and *warts* (which encodes a Lats family tumor suppressor) (Justice et al., 1995; Xu et al., 1995).

The use of FRT-based mitotic recombination in mosaic screens does have a few problems, however. First, the FRT and FLPase constructs are all inserted via *P* elements. This means that *P*-element-based insertional mutagenesis cannot be used in these flies without remobilizing the constructs. Therefore, most screens to date have used chemical mutagens or irradiation to generate mutations, which greatly slows down the subsequent identification and molecular analysis of the mutations. In the future, it should be possible to solve this problem by using other types of transposable elements for mutagenesis, especially those, like the *piggyBac* element, whose rate of mobilization in *Drosophila* can be as high as that of *P* elements (Horn et al., 2002; Häcker et al., 2003). Second, if chemical mutagens are used, they cause germ line mosaicism in the F1 flies; even though a particular mutagenized chromosome is present in the somatic cells, it might be lost in the germ line. Finally, the F1 adults must be kept alive, and this limits the types of phenotypes that can be screened. Thus, some laboratories have used mitotic recombination in F2 screens on individual mutant stocks.

A very different use of mosaics is to screen for novel genes whose misexpression causes interesting phenotypes. This is done using any appropriate Gal4 driver, including FLPout-Gal4 for mixexpression in clones, and mating these to different EP lines (Rorth et al., 1998). As noted above, EP constructs contain multiple UAS sequences, and these constructs have been inserted randomly around the genome. In the presence of Gal4, an EP insertion may drive the expression of a neighboring gene. After screening, those EP insertions that generate interesting phenotypes are mapped, and neighboring genes are tested for their developmental functions.

The future of mosaic techniques

New variations to and combinations of these techniques are constantly being developed and being made available to the *Drosophila* community. Given the ever-increasing power of these methods, anyone wanting more could with some justification be accused of ingratitude.

Nonetheless, development is an extremely complex and, in some cases, an annoyingly redundant process, and it is as yet difficult to manipulate more than a few genes at once using mosaic techniques in flies. A traditional way around the problem of redundancy and complexity in *Drosophila* is to screen for mutations that act as enhancers and suppressors of mutant phenotypes, and these techniques can be applied to mosaics (e.g. Parker et al., 2002). Still, in the age of genomics, one cannot also help being envious of the ease with which mixtures of mRNAs or dsRNAs can be used to disrupt embryogenesis in *Drosophila* and other organisms. It is not yet clear how complex mixtures of constructs could be delivered to late-developing tissues in *Drosophila*. Solving that problem could open up entirely new frontiers for mosaic analyses.

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