Lecture 4. *Drosophila* transgenes and making mosaic flies.

18.10.24

The original *Drosophila* transformation method. (Rubin, Spradling, Gehring 1980s)

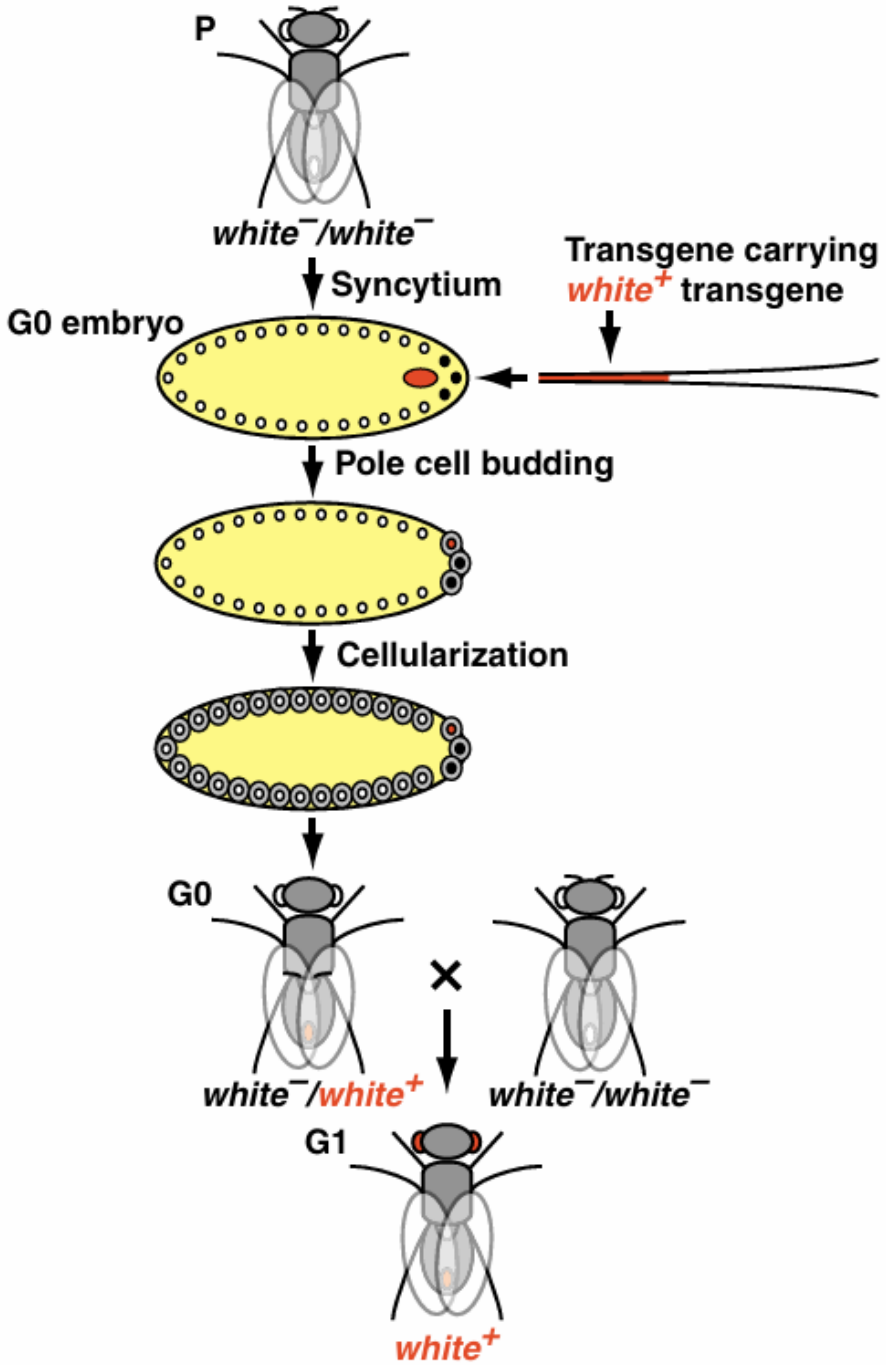

Fig. 1. *Drosophila* transgenesis. *white*⁺ transgene DNA (red) is injected into generation zero *Drosophila* embryos (G0) of less than 1 hour old, which have been obtained from a parental (P) generation. The early developmental stages of *Drosophila* embryos are characterized by rapid nuclear divisions that occur without accompanying cell divisions, creating a syncytium. Prior to cellularization, pole cells (black) bud off at the posterior end. For germ line transmission to occur, the transgenic DNA must be taken up into the pole cells that are fated to become germ cells. Transgenic DNA integrated into a pole cell (red pole cell) can be transmitted from one generation (G0) to the next (G1 progeny). The resulting integration events are identified using an appropriate marker, such as as *white*⁺. When used in a mutant *white*⁻ strain, this transgene marks transgenic flies by giving them a darker eye color (see Table 2 and Box 3 for more information on the markers used in fly transgenesis).

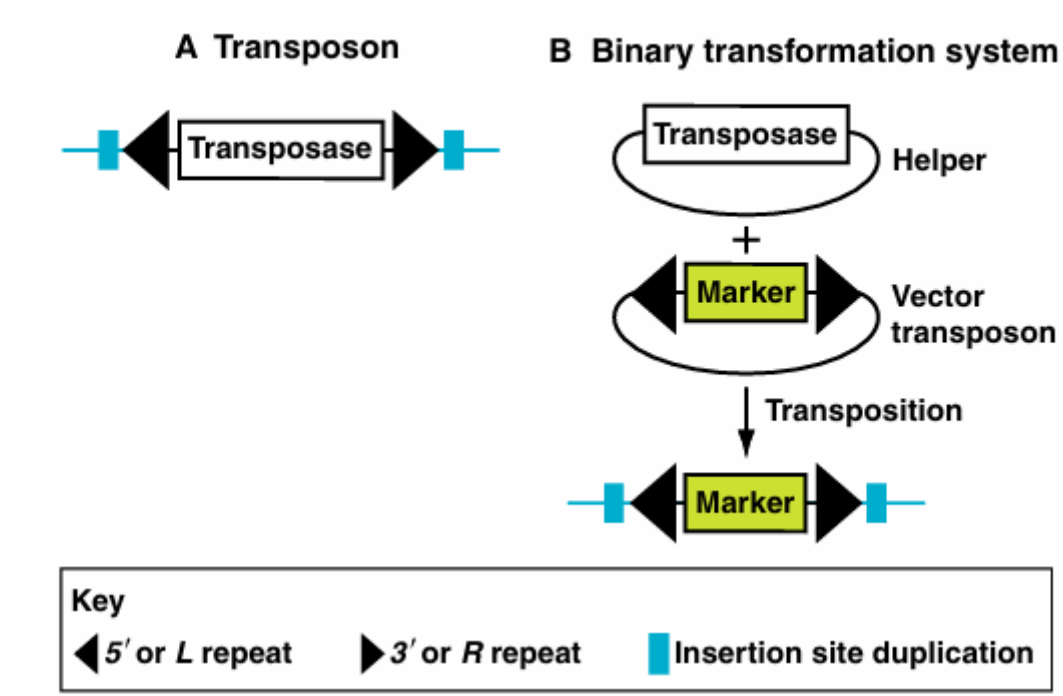

Fig. 2. Binary vector/helper transposon transformation system.
(**A**) Active transposons are mobile elements that consist of two inverted terminal repeats (black) that flank an open reading frame encoding a transposase. Both features are required for transposition. The inverted repeats are commonly called *5′* or *Left* (*L*) and *3′* or *Right* (*R*). Transposition results in a duplication of the insertion site (blue).
(**B**) Transposon and transposase can be separated, resulting in a binary vector/helper transposon transformation system that allows the regulated transposition of transgenes into the genome. Transposition events are identified by dominant markers (green, and see Table 2 and Box 3).

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Transgenesis upgrades for *Drosophila melanogaster*

Koen J. T. Venken[1] and Hugo J. Bellen[1,2,3,4]

Drosophila melanogaster is a highly attractive model system for the study of numerous biological questions pertaining to development, genetics, cell biology, neuroscience and disease. Until recently, our ability to manipulate flies genetically relied heavily on the transposon-mediated integration of DNA into fly embryos. However, in recent years significant improvements have been made to the transgenic techniques available in this organism, particularly with respect to integrating DNA at specific sites in the genome. These new approaches will greatly facilitate the structure-function analyses of *Drosophila* genes, will enhance the ease and speed with which flies can be manipulated, and should advance our understanding of biological processes during normal development and disease.

specific genomic docking sites (see glossary, Box 2) via the use of different recombinases and integrases (Groth et al., 2004; Oberstein et al., 2005; Horn and Handler, 2005; Bateman et al., 2006; Venken et al., 2006; Bischof et al., 2007). Many of these advances have their origins in mouse molecular genetics (Seibler and Bode, 1997; Bethke and Sauer, 1997; Bouhassira et al., 1997; Groth et al., 2000; Thyagarajan et al., 2001) and have been very useful for developing new fly transgenic techniques, as discussed below.

Here, we summarize many of the current methods that are used to generate transgenic flies. We first review classical transposon-mediated transgenesis and site-specific integration methods, before describing a plethora of recent improvements that have their basis in site-specific integration systems.

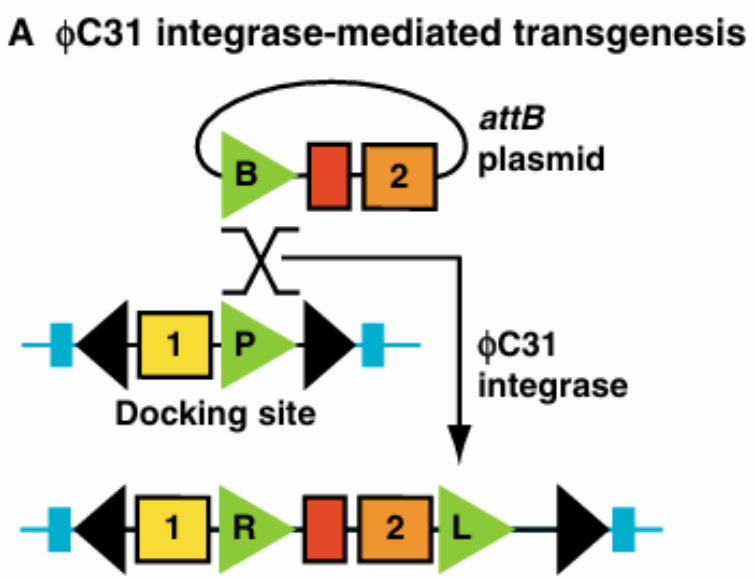
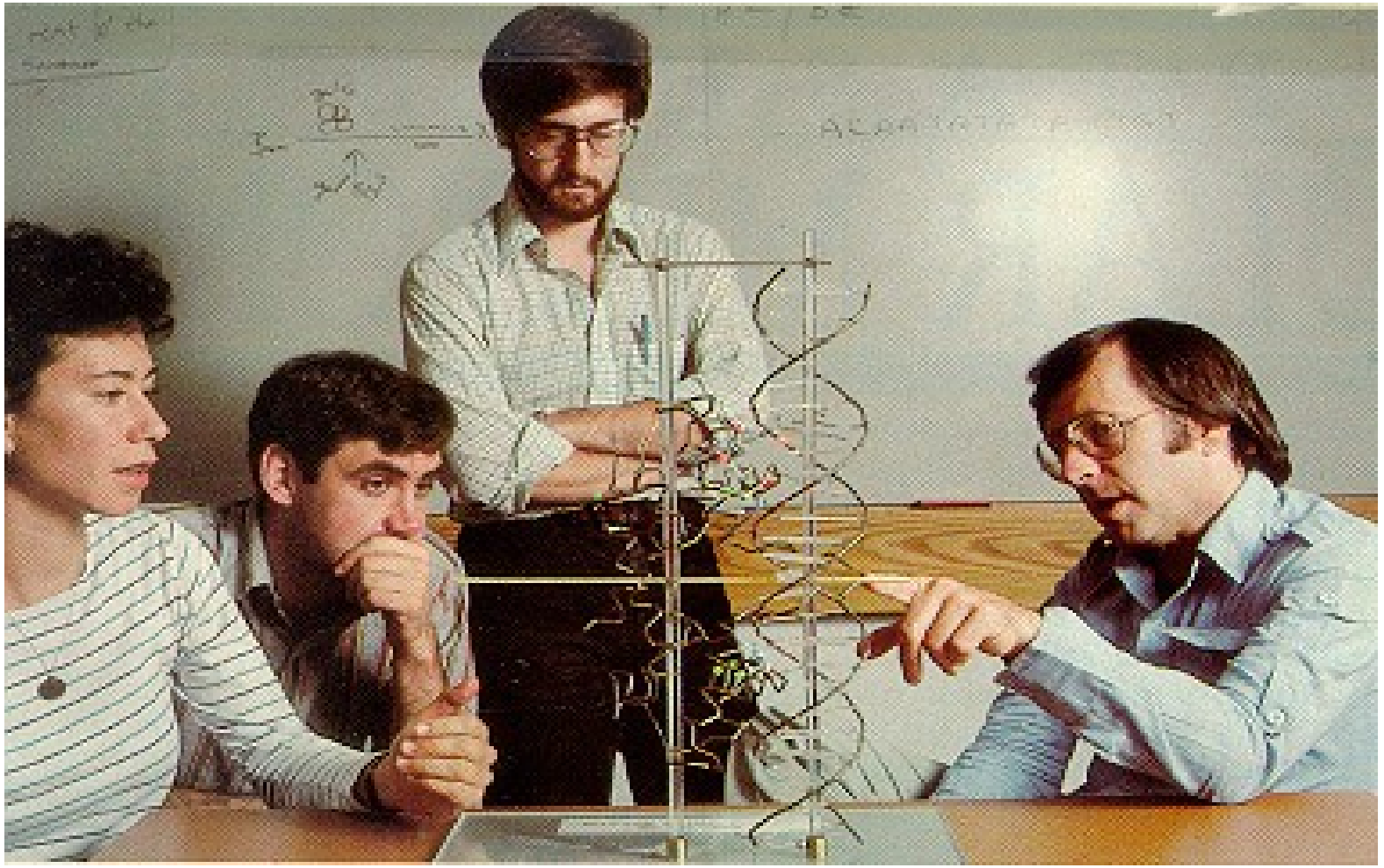


Fig. 7. Site-specific integration in *Drosophila*. (**A**) ΦC31 integrase-mediated transgenesis using single *attP* docking sites. Docking sites are transposons, such as *P* elements (Groth et al., 2004), *piggyBac* (Venken et al., 2006) or *Mariner* (Bischof et al., 2007), that contain a single *attP* recombination site and a marker 1, and that are integrated into the genome. A plasmid containing an insert, marker 2 and an *attB* recombination site, can then integrate into the docking site when ΦC31 integrase is provided. Correct recombination events between *attP* and *attB* are identified using marker 2. They result in two hybrid sites, *attL* and *attR*, that are no longer a substrate for ΦC31 integrase – the reaction is therefore irreversible. (**B**) Cre- and FLP-mediated RMCE.

GAL4-UAS binary expression system in flies

Yeast *GAL4* gene encodes a positive regulatory protein that turns on transcription of *GAL* genes to grow on galactose



Mark Ptashne with graduate students Cynthia Wohlberger, Liam Keegan, Ed Giniger at Harvard, 1982

Profile of Norbert Perrimon

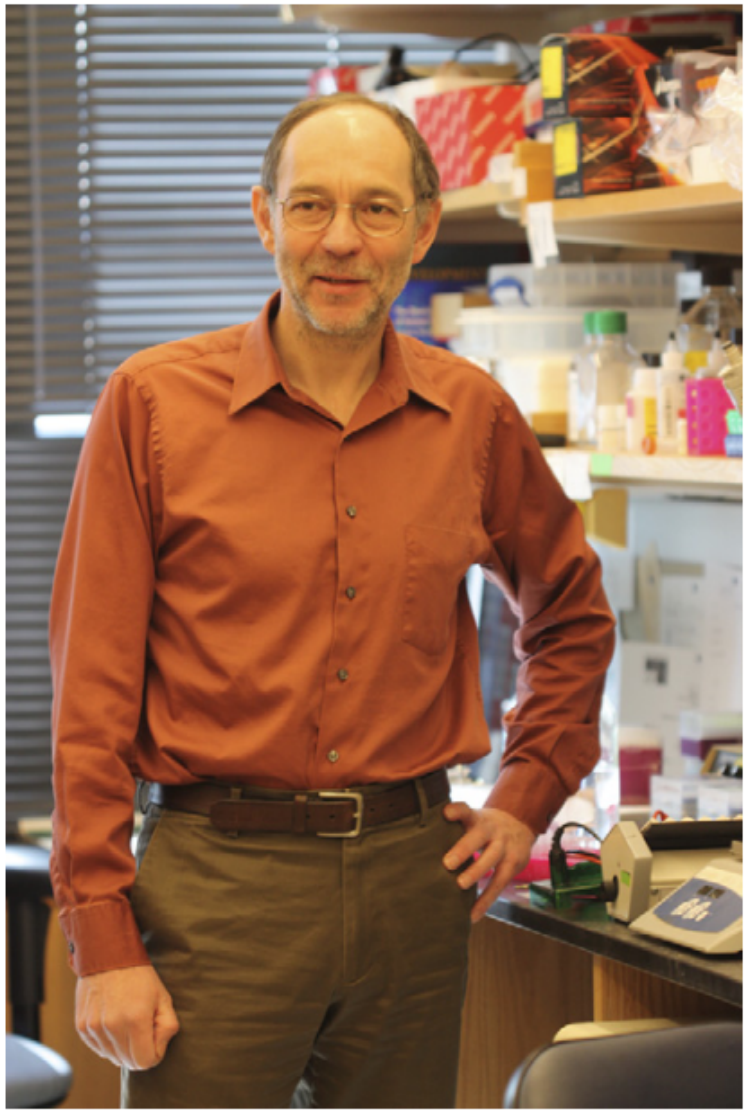
Sandeep Ravindran
Science Writer

As a child, one of Norbert Perrimon's first introductions to the scientific method came from his father. Perrimon's father was interested in geology. "Often we would go to various areas in Normandy, where I grew up, and collect different types of soils, rocks, and fossils. I always found it quite interesting to see how from the collection of different bits and pieces, you could draw complex geological maps," Perrimon says. Many years later, Perrimon would use a similar process to make his mark not in geology, but as a biologist working on the fruit fly, *Drosophila*.

Over a long and distinguished career, Perrimon combined different pieces of data collected from the study of various fruit fly mutants to map complex developmental and signaling pathways. In recognition of his discoveries in the field of *Drosophila* development and signaling, and for developing important genetic tools for studying fruit flies

genes could control developmental decisions, suggesting that characterization of these genes would lead to a molecular understanding of patterning. This was the kind of science I wanted to do," Perrimon says.

During his doctoral work, which he completed with Anthony Mahowald at Case Western Reserve University in Cleveland, Perrimon became interested in developing ways to isolate and characterize mutations that affected embryonic development. He developed a technique, called the "dominant female sterile method," to study the maternal effect of mutations in genes that are essential for flies (2). "You can't look at embryos derived from females that are homozygous mutant for a gene that is essential for viability because those flies are dead, so you need to create mosaics," Perrimon says. Mosaic organisms contain cells with two or more different genotypes, and the dominant female



Norbert Perrimon. Photo courtesy of Norbert

portant genetic tools for studying fruit flies, Perrimon was elected to the National Academy of Sciences in 2013. Now a professor of developmental biology and genetics at Harvard Medical School, Perrimon first started developing tools to study fruit fly genetics as a graduate student at the University of Paris.

Creating Mosaics

It was in the early 1980s that Perrimon first became interested in genetics as a tool to tackle questions in developmental biology and embryology. "I was looking for an organism where I could use genetics to study the complexity of the way animals form," he says. "I started working in *Drosophila* because it is a great model system to apply genetics." In 1981, he started his doctoral research studying fruit fly oogenesis in the laboratory of Madeleine Gans at the University of Paris.

At the time, researchers were beginning to use fruit flies to identify genes involved in early development. "This was an exciting time because a number of important findings regarding patterning and the way animals develop had just been made," Perrimon says. In particular, genetic screens conducted by developmental biologists Christiane Nüsslein-Volhard and Eric F. Wieschaus had shown that a single mutation could have specific effects on the development of the embryo (1). "It became quite clear that some

ferent genotypes, and the dominant female sterile method allows researchers to produce female flies that do not express an essential gene product during oogenesis, Perrimon says. "So we were able to look at embryos, which were derived from eggs that were completely depleted for an essential gene."

Developing the dominant female sterile technique to identify genes involved in fly development comprised the bulk of Perrimon's thesis work. "This mosaic technique allowed us to identify many new genes, which have very specific effects in early embryonic development," Perrimon says. After he started his own laboratory at Harvard Medical School in 1986, Perrimon would go on to develop other techniques to create mosaics, including one that had a particularly large impact on the fruit fly field.

Influential Technique

In his Inaugural Article, Perrimon reviews different methods that he and his colleagues have generated over the years for creating mosaics (3).

One of the key techniques Perrimon developed, together with Gurdon Institute molecular biologist Andrea Brand, is the Gal4-UAS system to control gene expression in a spatial and temporal way (4). The system consists of the yeast *GAL4* gene, which encodes the transcriptional activator Gal4, and

Norbert Perrimon. Photo courtesy of Norbe
Perrimon.

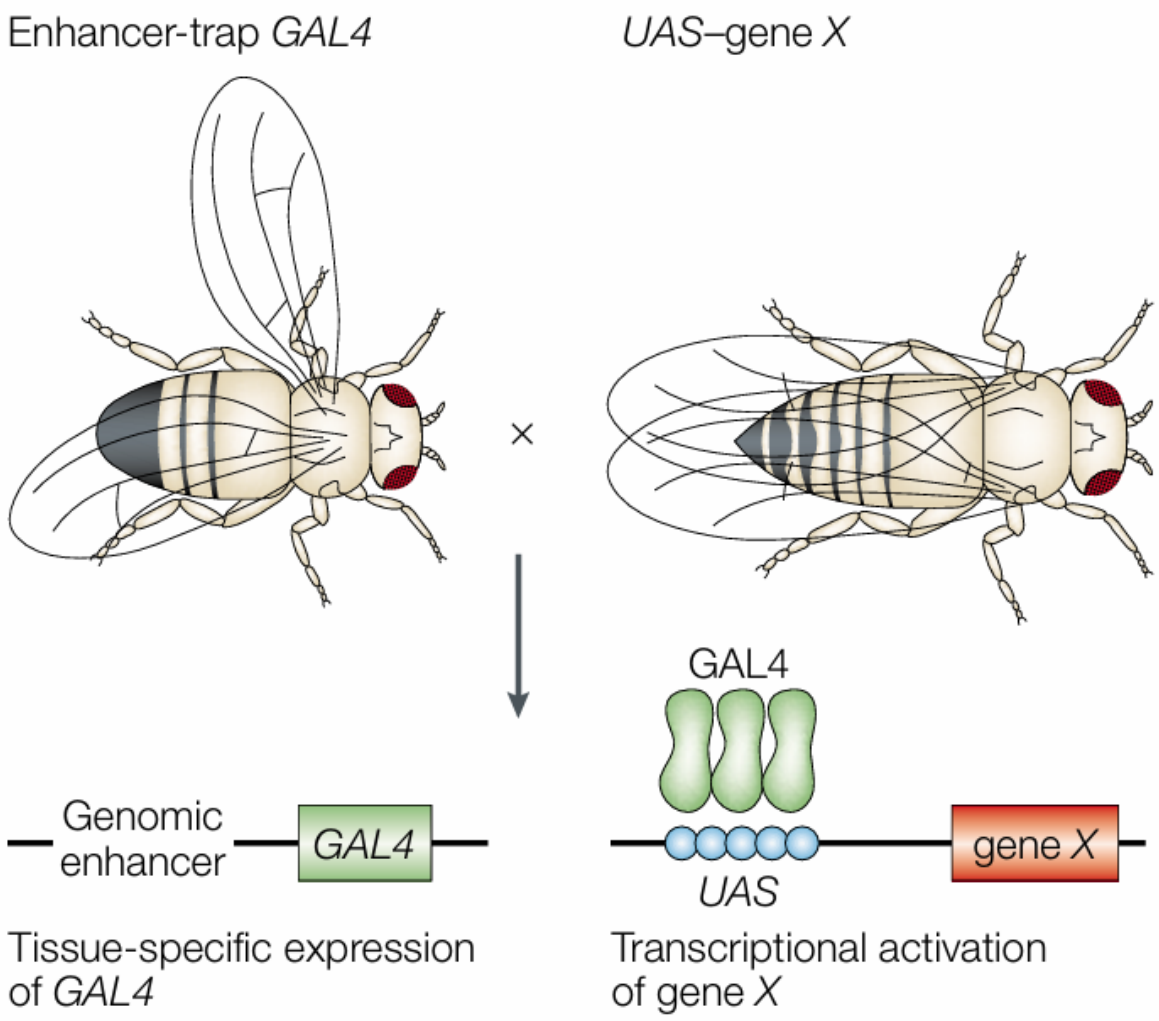
a stretch of DNA called the Upstream Act
vation Sequence (UAS), an enhancer to whi
Gal4 specifically binds to activate transcri
tion. "It allowed us to control where ar
when we could express a specific gene
Perrimon says. "This has very broad applic
tions throughout the field in terms of allowi
conditional tissue-specific gene expression."

Perrimon developed the Gal4-UAS syste
because he wanted to express the activat
form of kinases that were important for
development, he says. "Activated kinases,
you express them ubiquitously in the f
would just kill the fly, so we wanted a syste
where we could restrict their expression
specific body parts," Perrimon says. "That
what led us to develop this bipartite syster
where you have the activated kinase und
UAS control in one set of flies, and then y
have another set of flies which express the
GAL4 gene in different body parts." When both fly strains are bred with each other, the activated kinase is specifically expressed only in the cells where Gal4 is present.

This is a Profile of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 4756 in issue 13 of volume 111.

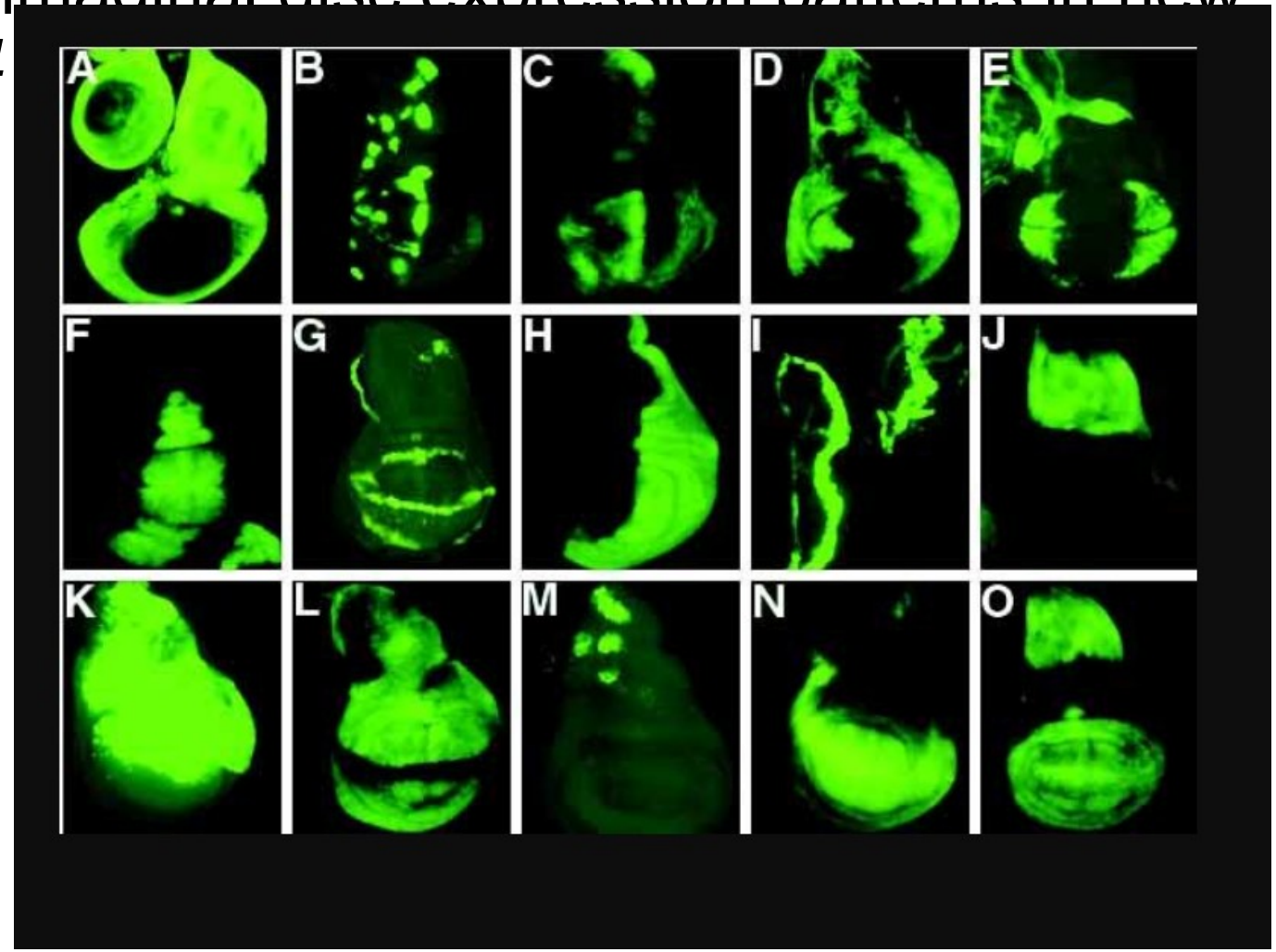
Box 2 | **The *GAL4–UAS* system for directed gene expression**

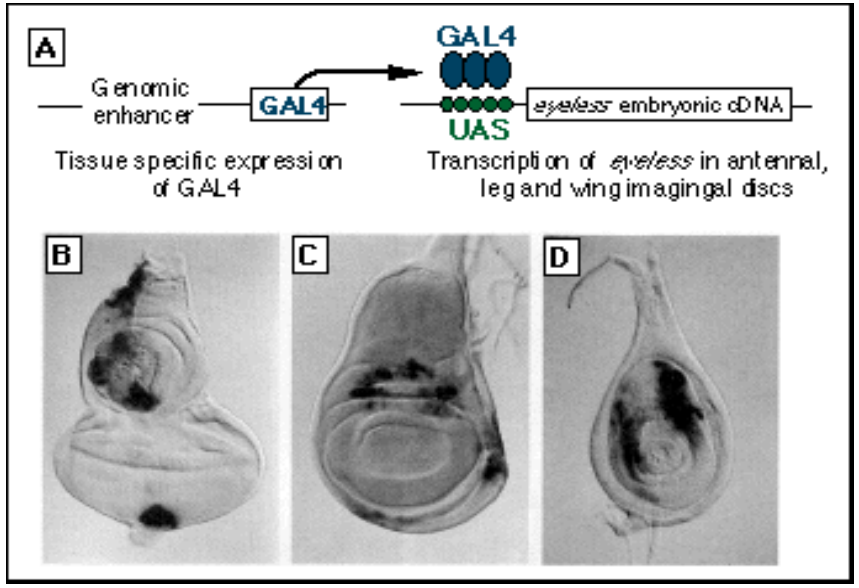
The yeast transcriptional activator Gal4 can be used to regulate gene expression in *Drosophila* by inserting the upstream activating sequence (*UAS*) to which it binds next to a gene of interest (gene *X*)[96]. The *GAL4* gene has been inserted at random positions in the *Drosophila* genome to generate 'enhancer-trap' lines that express *GAL4* under the control of nearby genomic enhancers, and there is now a large collection of lines that express *GAL4* in a huge variety of cell-type and tissue-specific patterns[97]. Therefore, the expression of gene *X* can be driven in any of these patterns by crossing the appropriate *GAL4* enhancer-trap line to flies that carry the *UAS*–gene *X* transgene. This system has been adapted to carry out genetic screens for genes that give phenotypes when misexpressed in a particular tissue (modular misexpression screens)[79].



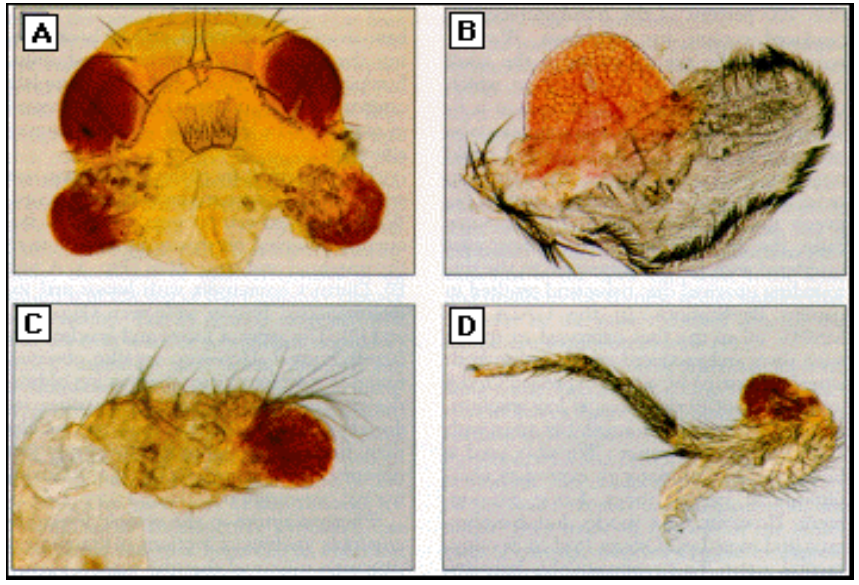
Enhancer trapping. Remobilization of a P-element transgene having promoterless *GAL4,* using cross to Jumpstarter to supply transposase.

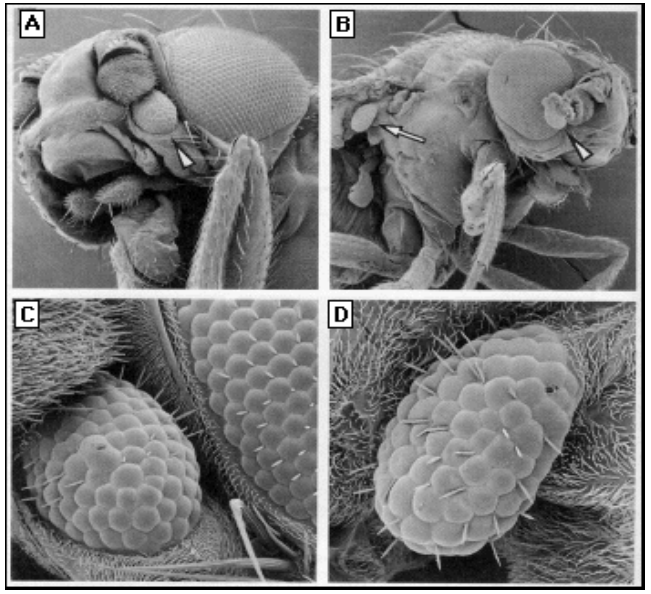
Screening for wing imaginal disc expression patterns in new promoterless *GAL4*





Induction of ectopic eyes by GAL4-targeted expression of the *eyeless* gene in *Drosophila*





GAL4 System in *Drosophila*: A Fly Geneticist's Swiss Army Knife

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Received 30 July 2002; Accepted 2 August 2002

The last decade has seen an enormous expansion in the genetic toolbox of model organisms. This has been particularly apparent in the fruitfly, *Drosophila melanogaster*, in which the development of these tools and their ongoing extension, along with the completion of the genome sequence has allowed for the analysis of most any process. One particularly elegant example of tool development was the creation of the GAL4/UAS system for targeted gene expression in *Drosophila*. In addition to helping make *Drosophila* one of the most genetically tractable metazoans, this system has also helped *Drosophila* attract attention from the biotechnology industry as a viable means to investigate the function of genes implicated in a wide variety of medically and economically important processes. In this article, we review the GAL4/UAS system in *Drosophila* and the numerous extensions that have morphed it into a veritable Swiss army knife for the analysis of gene function.

USER'S MANUAL: GAL4/UAS BASIC TRAINING

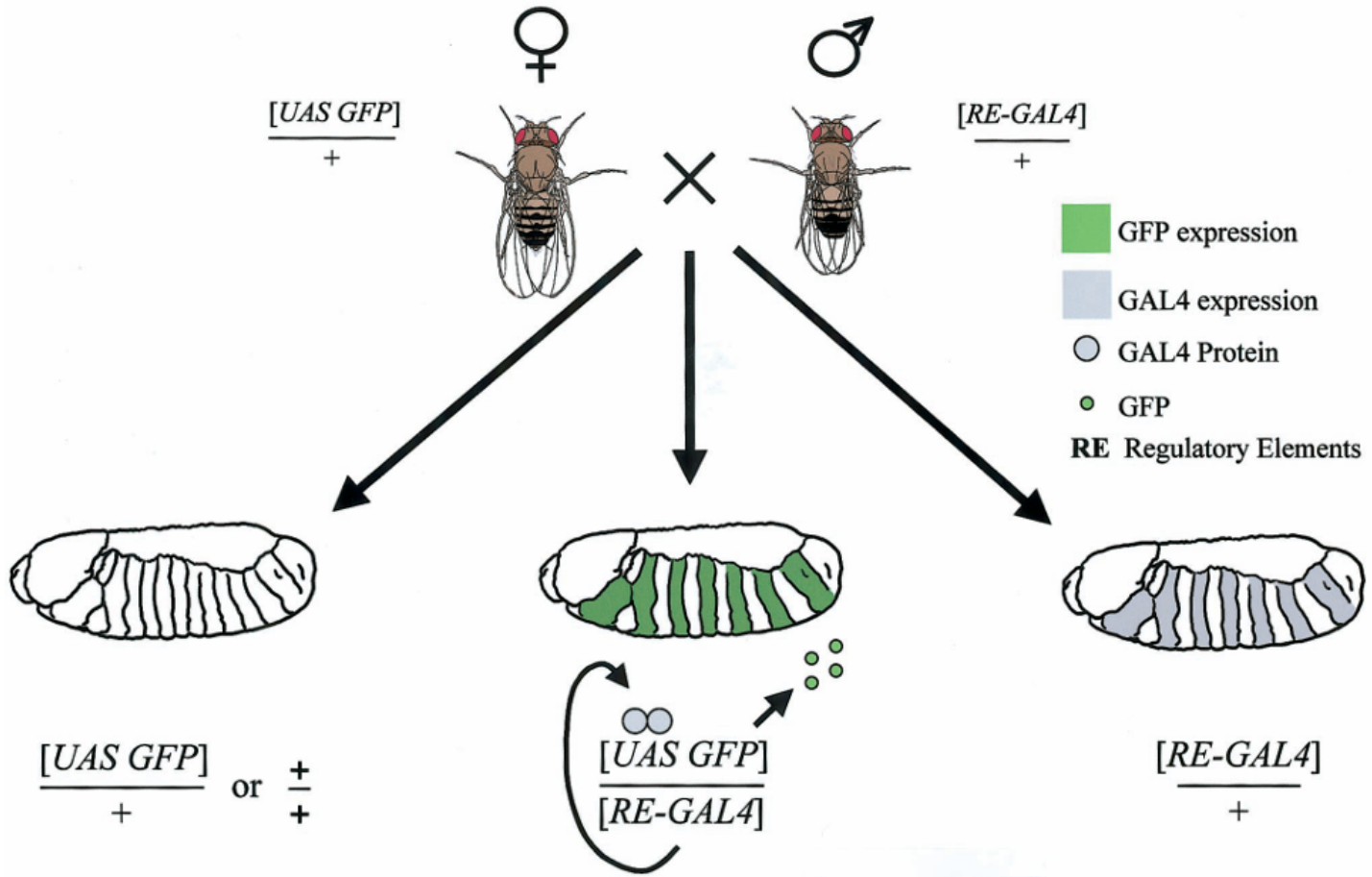


FIG. 1. The bipartite UAS/GAL4 system in *Drosophila*. When females carrying a UAS responder (*UAS-GFP*) are mated to males carrying a GAL4 driver progeny containing both elements of the system are produced. The presence of GAL4 in an alternating segmental pattern in the depicted embryos then drives expression of the UAS responder gene in a corresponding pattern.

Hormone-activated GAL4, GeneSwitch

G

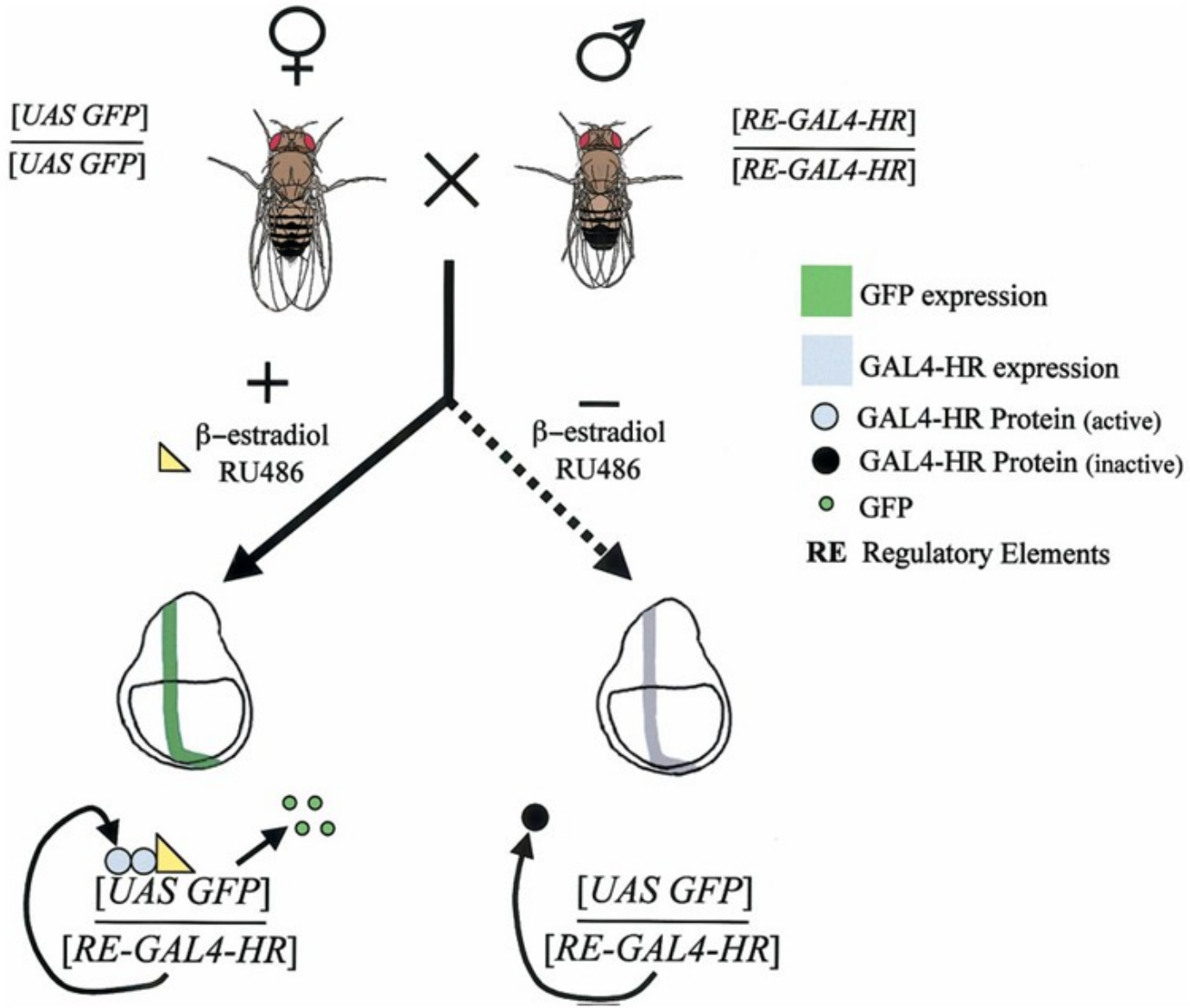


FIG. 3. Hormone-responsive GAL4 induction. Females carrying a UAS responder (*UAS-GFP*) are mated to males carrying a hormone-responsive GAL4 driver (either the GAL4-estrogen receptor chimera or the GAL4-progesterone receptor-human p65 activation domain chimera). In this scheme, the GAL4 driver is expressed in a stripe along the anterior–posterior axis of the wing imaginal disc. In F1 containing both elements of the system, expression of the responder is observed only when these progeny have been exposed to the appropriate hormone.

Tet-off

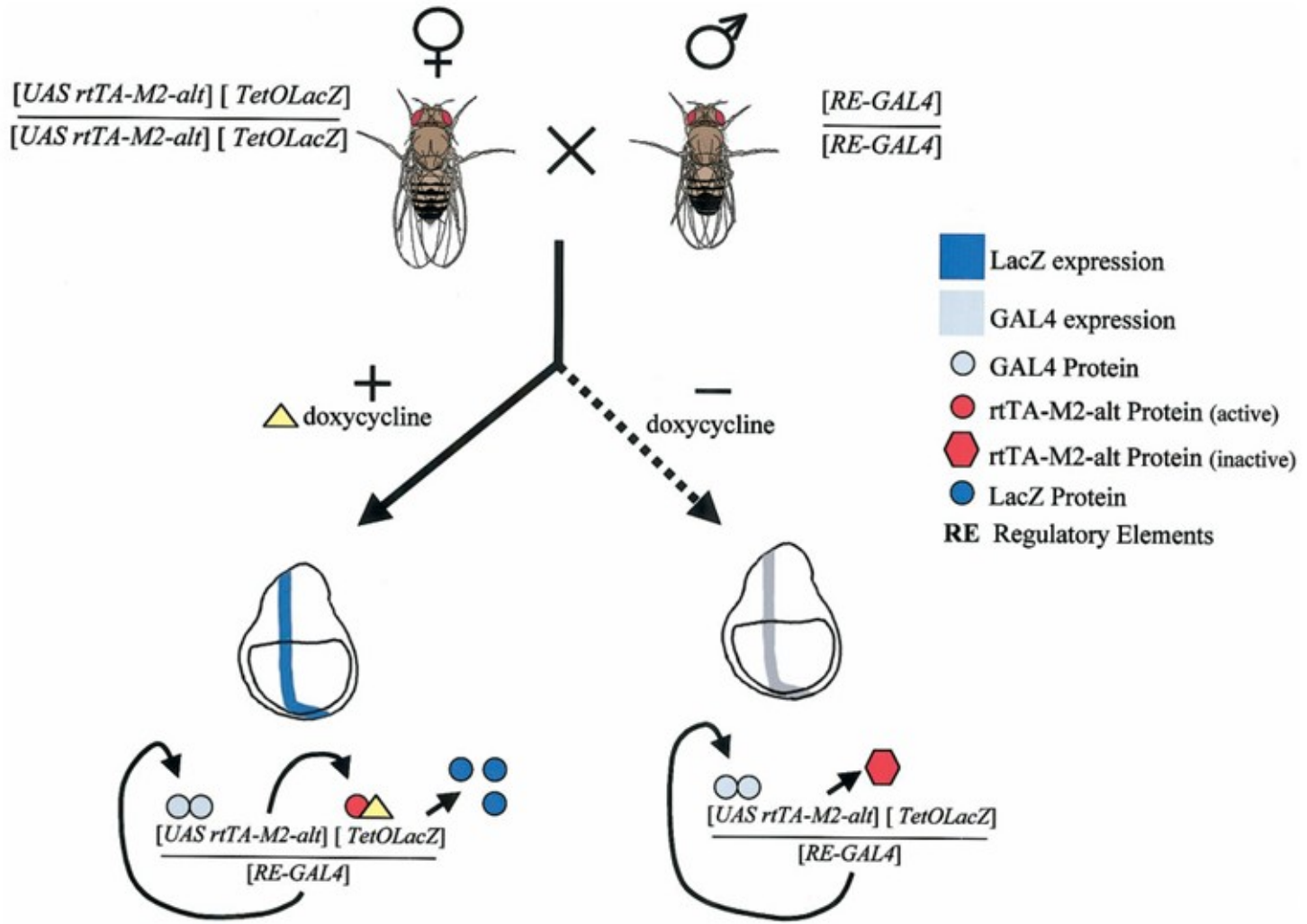


FIG. 4. GAL4 induction with the Tet-On system. In this system, a modified tTA activator, rtTA-M2-alt, is under the control of GAL4, while the *β-galactosidase* (LacZ) gene is under the control of the Tet operator. rtTA-M2-alt activates transcription from the Tet operator only in the presence of tetracycline or its analog doxycycline. As in Figure 3, the GAL4 driver is expressed in a stripe along the anterior–posterior axis of the wing imaginal disc. rtTA-M2-alt is expressed in a corresponding pattern in the wing disc in response to the GAL4 driver, but activates LacZ expression only in the presence of a doxycycline. In this system, the gene of interest is then cloned downstream of the Tet operator to generate an inducible TetO responder.

GAL4-driven tissue-targeted RNAi knockdown of gene expression

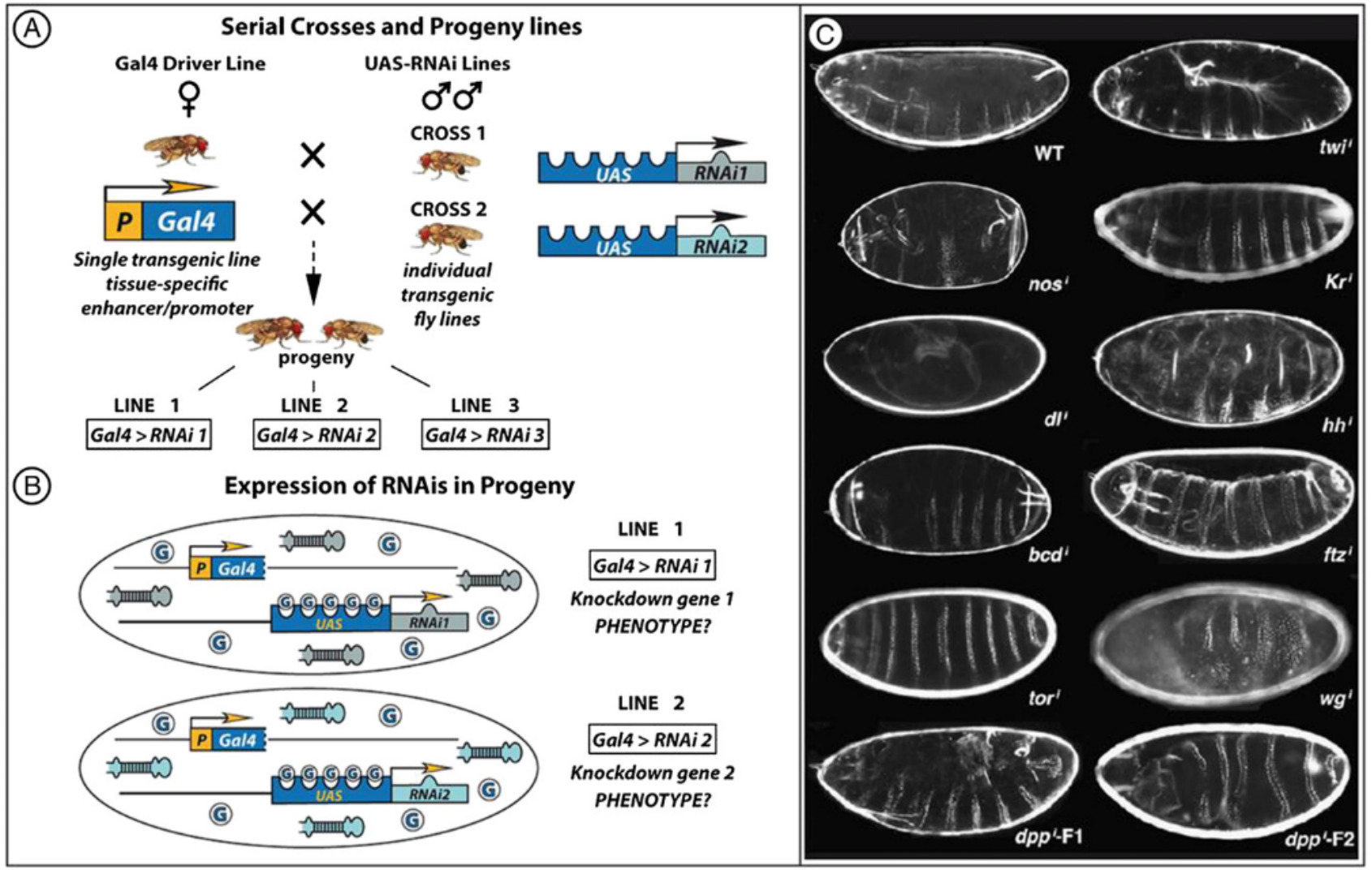


Fig. 4. Somatic and germ-line RNAi screens. (*A* and *B*) Experimental protocol for large-scale in vivo screens in which RNAi induces systematic knockdown of gene expression. (*A*) RNAi screens are initiated by systematically crossing virgin females from a single Gal4 driver line with males from individual UAS target lines, each carrying a specific UAS-RNAi (either dsRNA or shRNA) directed against a known gene. The promoter of Gal4 specifies the time and tissue specificity of Gal4 expression in the driver line, but transactivation of target UAS-RNAi can occur only when both elements are united in the progeny genome. Gal4 > RNAi, Gal4 activation of dsRNA or shRNA transcription. (*B*) Systematic Gal4-driven expression of RNAis induces knockdown of target gene expression to allow systematic phenotypic characterization of the progeny of each cross. (*C*) Examples of embryonic phenotypes detected from germ-line screens using shRNAs (35, 36). Adapted from ref. 35.

Why we need to make genetically mosaic animals

- Probably missing many female sterile mutants here because homozygous mothers are not obtainable.

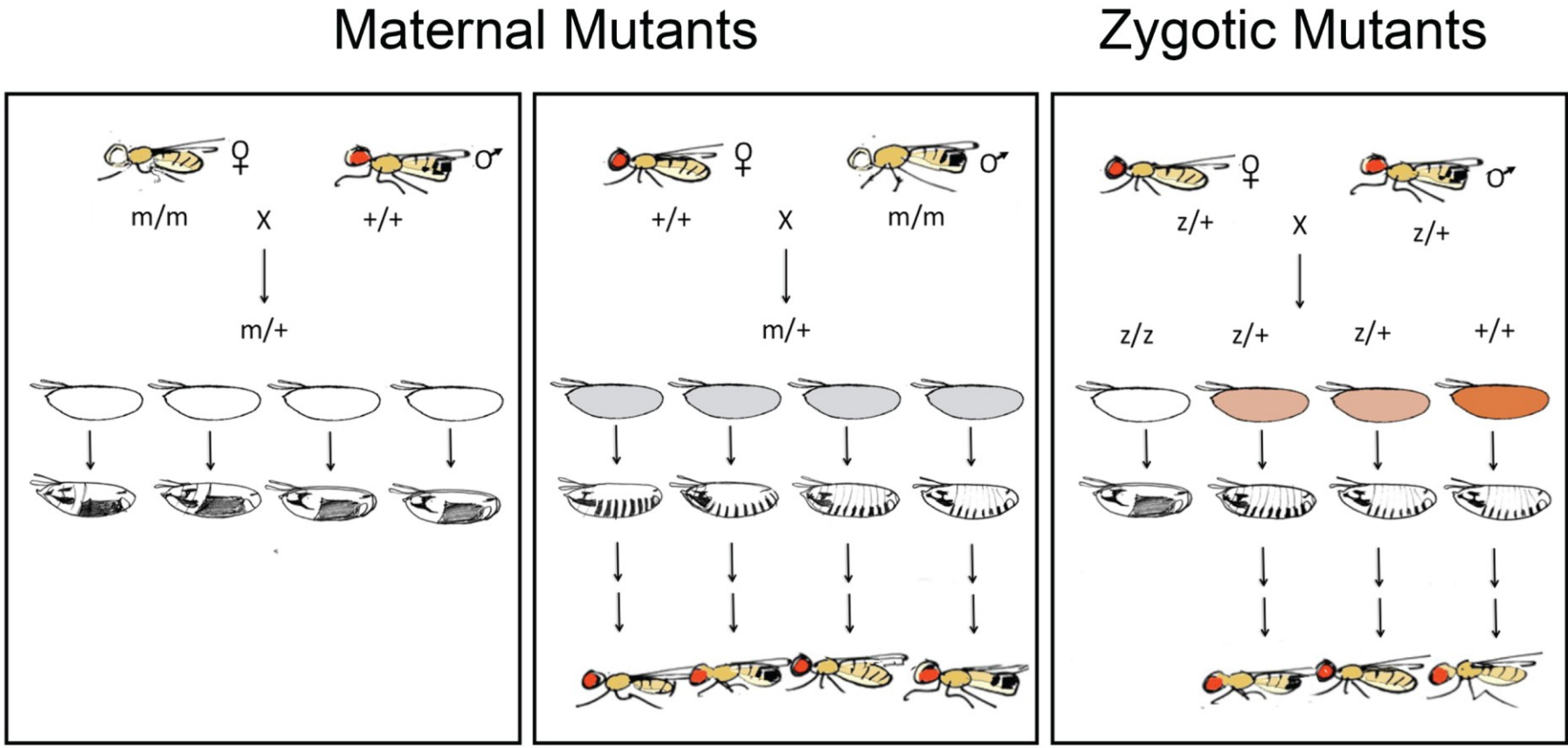


Figure 2

Genetics of embryonic patterning. Maternal and zygotic genes can be distinguished by their genetic behavior. (*Left panel*) All embryos from females that are homozygous mutant for maternally active genes are abnormal, even when crossed with wild-type males. (*Middle panel*) Although the genotype of the resultant embryos is the same as that in the reciprocal cross, in which wild-type females are crossed with mutant males, all embryos are normal. (*Right panel*) For zygotically active genes, only the homozygous one-quarter of the embryos derived from a cross between heterozygotes will be abnormal, even though all embryos develop with identical maternal contributions.

Making mosaics with clones of mutant cells in *Drosophila*

- Many female sterile mutants could have been missed because homozygous mutant mothers cannot be obtained; the homozygous mutant mothers die as embryos or larvae.

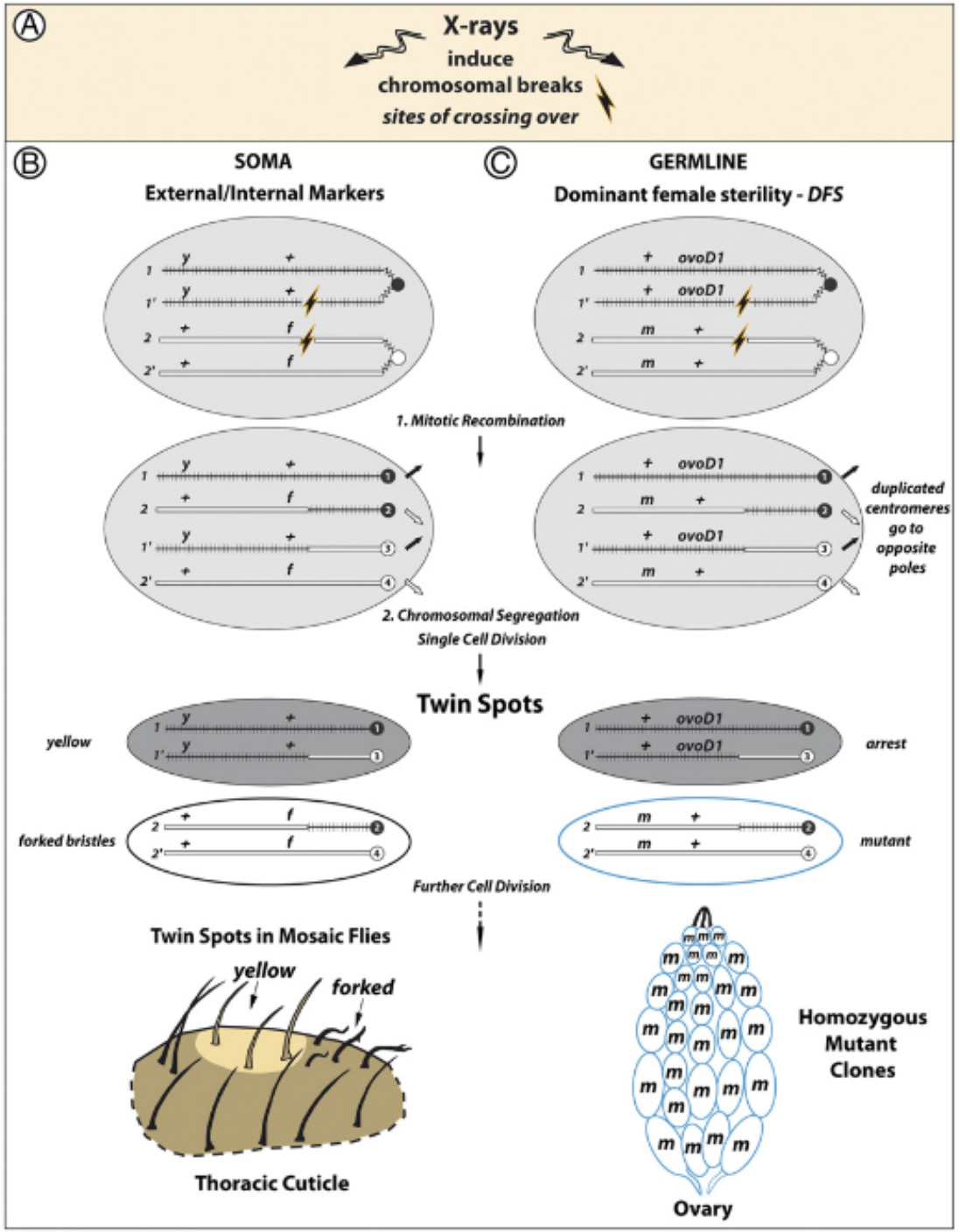
- Such mutants are zygotic lethals. For zygotic lethals in general, studying their effects in detail requires making mosaic animals having clones of homozygous mutant tissue in heterozygous eggs, embryos, larvae or adult flies (somatic clones).

- If the lethal mutant has a female sterile defect because the mutation removes something required in nurse cells or oocytes, then it has to be studied using clones made in the germline (germline clones)

Mitotic recombination

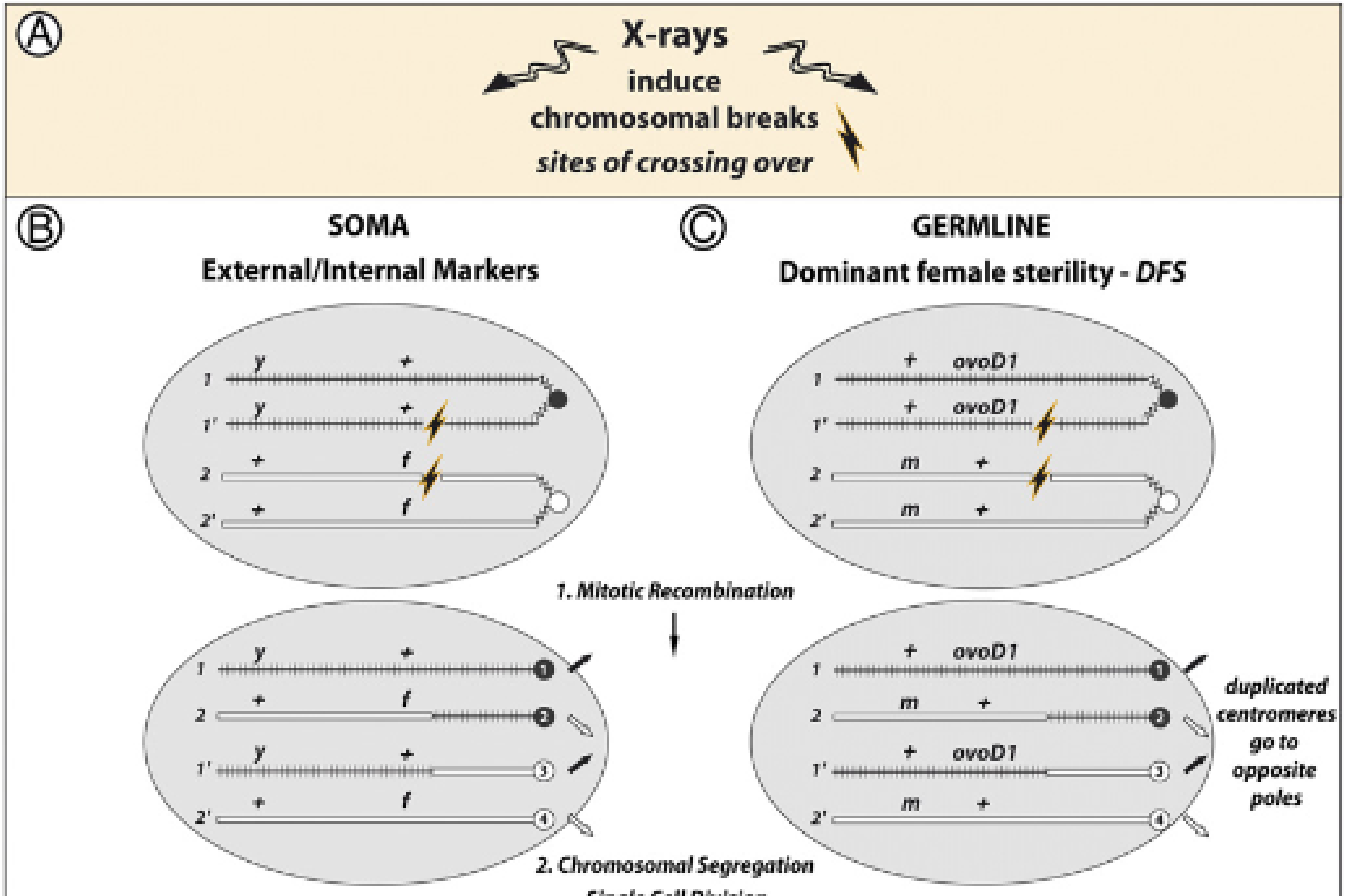
Somatic clones. Twin spots on fly cuticle show recessive mutant markers in side-by side clones arising from a single mitotic recombination event (Curt Stern, 1930).

Germline clones in ovaries using the dominant female sterile (DFS) mutant *OvoD*



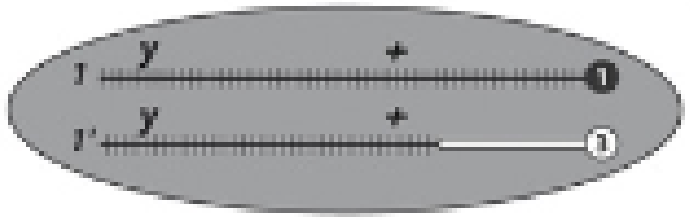
MR = mitotic recombination to form clones of recombinant cells

Fig. 1. Mitotic recombination and generation of twin spots for clonal analyses. (*A*) X-ray irradiation causes chromosomal breaks and induces MR in the G2 phase of the cell cycle. (*B* and *C*) The underlying mechanisms of MR are the same in somatic (*B*) and germ-line (*C*) tissues, but the techniques for visualization of induced twin spots are different. MR causes an exchange of chromosomal arms distal to the site of crossing over. All genes downstream of the chromosomal breakpoint are homozygozed. In a heterozygote, the pattern of chromosomal segregation determines genotype. In G2-X segregation the recombined chromosomes migrate to different poles in a 1:3/2:4 configuration. Cytokinesis generates a mosaic fly with homozygous twin spots: one wild-type (+/+) and the other mutant (–/–); the rest of the cells are heterozygous (+/–). All twin spot genotypes shown in this and following figures arise from this type of segregation pattern. (*B*) In the soma, MR creates within the original heterozygous background multicellular homozygous clones of wild-type and mutant tissue of varying sizes, depending upon the number of further cell divisions. In early clonal analysis studies the homozygous mutant twin spot was identified by external phenotypes such as cuticle color or forked thoracic bristles; later, with the development of fluorescent protein markers, clones were identified within internal organs. (*C*) The DFS technique. Flies heterozygous and homozygous for *DFS* do not lay eggs; however, following MR in a heterozygote, double-positive germ cells will develop and produce normal eggs. If a mutation is positioned *in trans* to *DFS*, *m*/*m* homozygous clones will be generated in the germ line. *OvoD1* ovaries degenerate very early, and wild-type eggs are lost. Thus, the only eggs that develop, if *m* does not interfere with germ-line development, will be homozygous mutants.

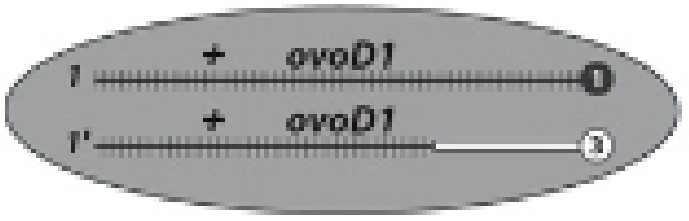

A
X-rays
induce
chromosomal breaks
sites of crossing over
B
SOMA
External/Internal Markers
C
GERMLINE
Dominant female sterility - DFS
y
+
f
ovoD1
m
1. Mitotic Recombination
2. Chromosomal Segregation
duplicated centromeres go to opposite poles

Twin Spots

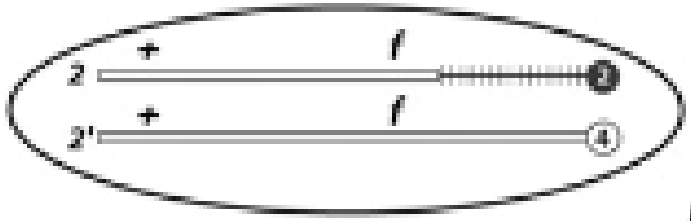
yellow



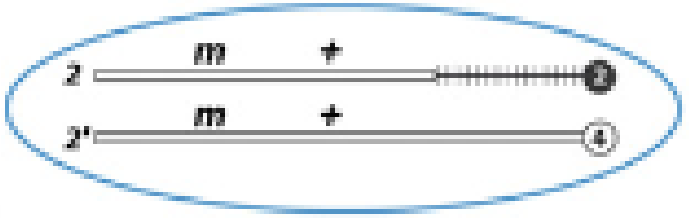
arrest



forked bristles

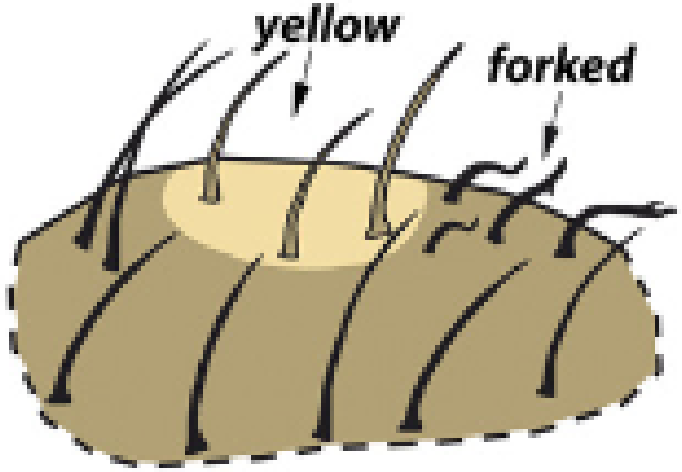


mutant

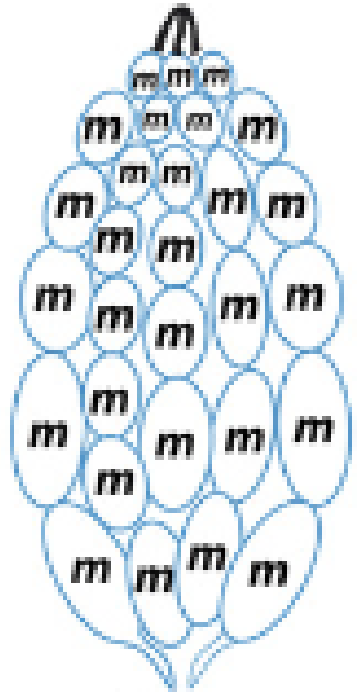


Further Cell Division

Twin Spots in Mosaic Flies



Thoracic Cuticle



Ovary

Homozygous Mutant Clones

THE ART AND DESIGN OF GENETIC SCREENS: *DROSOPHILA MELANOGASTER*

Daniel St Johnston

The success of *Drosophila melanogaster* as a model organism is largely due to the power of forward genetic screens to identify the genes that are involved in a biological process. Traditional screens, such as the Nobel-prize-winning screen for embryonic-patterning mutants, can only identify the earliest phenotype of a mutation. This review describes the ingenious approaches that have been devised to circumvent this problem: modifier screens, for example, have been invaluable for elucidating signal-transduction pathways, whereas clonal screens now make it possible to screen for almost any phenotype in any cell at any stage of development.

POLYTENE CHROMOSOME
A giant chromosome that is formed by many rounds of replication of the DNA. The replicated DNA molecules tightly align side-by-side in parallel register, which creates a non-mitotic chromosome that is visible by light microscopy.

PROTOSTOME–DEUTEROSTOME
The two principal divisions of animal phyla, based on how the mouth forms in the embryo.

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DOI: 10.1038/nrg751

The fruitfly *Drosophila melanogaster* has been one of the favourite model organisms of geneticists, since Thomas Hunt Morgan decided to use it to investigate the chromosomal theory of inheritance at the beginning of the last century[1]. Morgan chose *Drosophila* because it is easy and cheap to rear in the laboratory, has a ten-day generation time and produces many progeny. However, he soon discovered that it has several other advantages for genetic analyses. For example, there is no meiotic recombination in males, and there are only four chromosomes, which can be directly visualized in the giant POLYTENE CHROMOSOMES of the larval salivary gland. Furthermore, its exoskeleton provides a wealth of external features, such as bristles, wing veins and compound eyes, which can be affected by mutations, and for which the resulting mutant phenotypes can be scored simply by looking down the stereomicroscope. This early start has been built on by succeeding generations of drosophilists, who have developed an ever-increasing repertoire of techniques that make *Drosophila* one of the most tractable multicellular organisms for genetic analysis[2]. In fact, *Drosophila* has only one main drawback, which is that the stocks have to be continuously maintained in the laboratory because it is not possible to freeze them (and successfully revive them afterwards).

An unfortunate feature of genetic model organisms is that the easier they are to work with, the worse they are as models for the animal that most funding agencies find most interesting, namely ourselves. In this respect, however, *Drosophila* provides a very happy compromise. A surprisingly large number of developmental processes seem to be conserved between flies and vertebrates, even though they diverged at the PROTOSTOME–DEUTEROSTOME split ~700 million years ago. To cite two of the more famous examples: the dorsoventral (D/V) axes of the *Drosophila* and vertebrate embryo are patterned by opposing gradients of Decapentaplegic (Dpp; BMP4 (bone morphogenetic protein 4) in vertebrates) and Short gastrulation (Sog; CHRD (chordin) in vertebrates), even though the orientation of the axes is reversed; whereas Hedgehog (Hh) and its vertebrate counterpart, sonic hedgehog (SHH), have remarkably similar roles in limb patterning in both systems[3,4]. The sequencing of the *Drosophila* genome has now revealed the true extent of these similarities[5]. *Drosophila* has only ~15,000 genes, which is fewer than has *Caenorhabditis elegans*, but twice as many of these have clear homologues in humans (*E*-value $<10^{-50}$)[6]. Furthermore, 197 out of 287 known human disease genes have *Drosophila* homologues, and even those that do not can produce very similar symptoms when expressed in flies[7,8]. So,

Making somatic clones with Flp/FRT

Examples of somatic FRT clones and screens

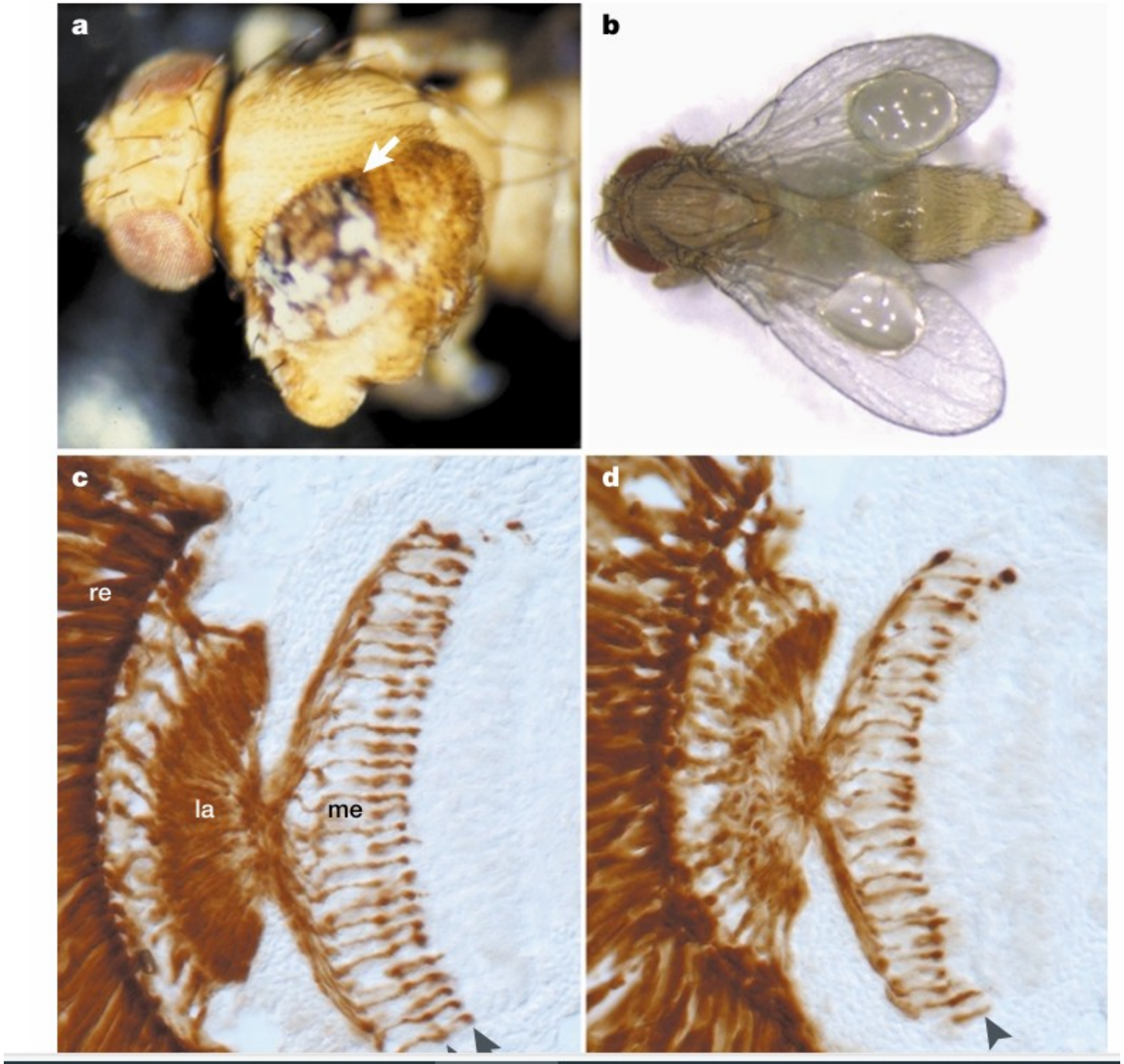
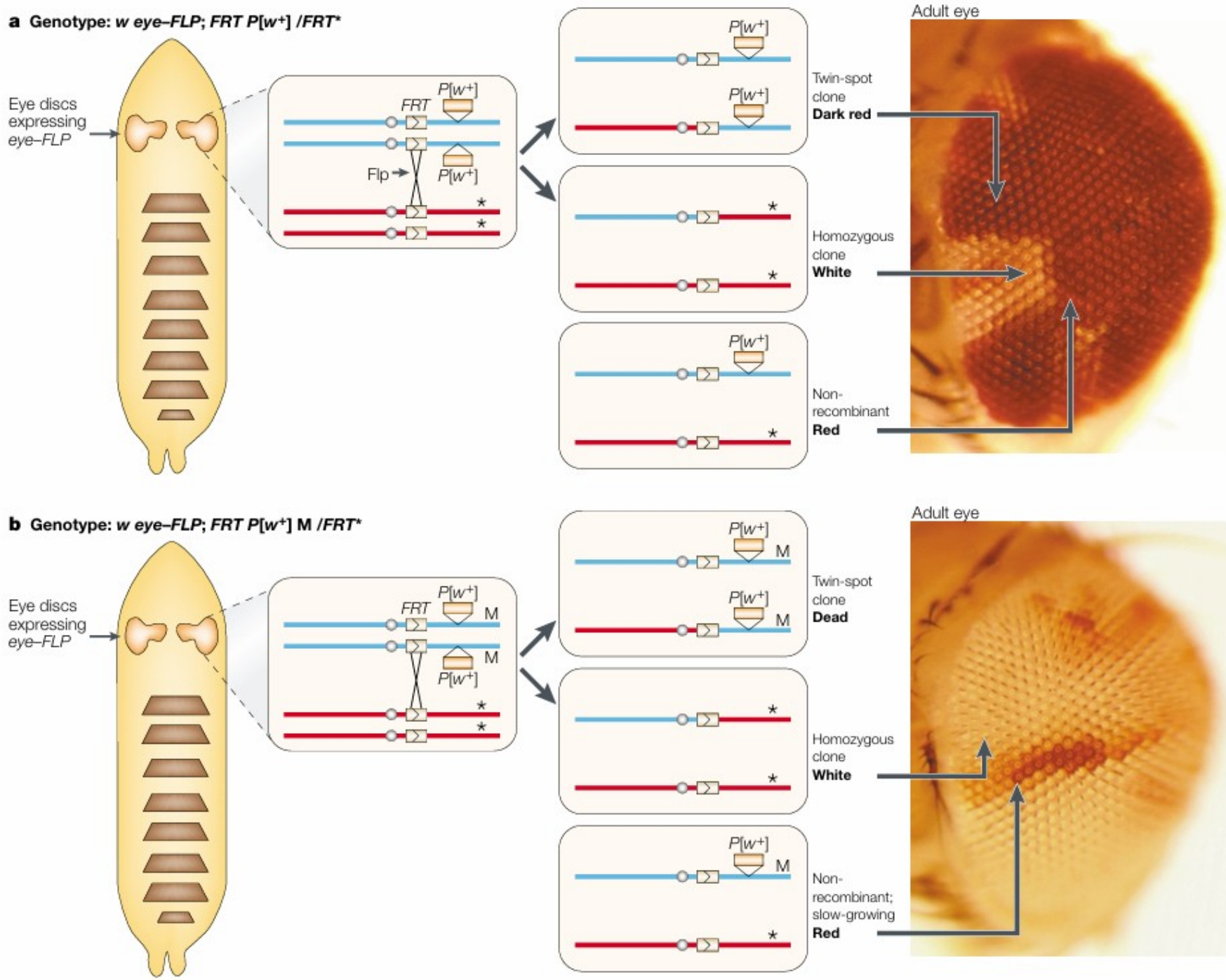


Figure 3 | **Examples of mutant phenotypes from Flp/*FRT* screens. a** | A NOTUM containing a homozygous *lats/warts* mutant clone, which has overgrown to form a large tumerous outgrowth (arrow). **b** | An adult fly with bubbles in both wings produced by clones of a mutant in *piopio* (*pio*), which disrupts adhesion between the dorsal and ventral surfaces of the wing. **c**,**d** | Section through an adult head showing the projections of retinal axons into the lamina (la) and medulla (me) of the optic lobe. 're' marks the position of the retina. **c** | Wild type. The R7 and R8 axons project to two distinct layers in the medulla. **d** | The R7 and R8 axons terminate in the same region of the medulla in *Leukocyte-antigen-related-like* (*Lar*) mutant clones, generated using *eye–FLP* with the *Minute* technique. In panels **a**–**d**, anterior is to the left. **e** | Scanning electron micrographs of the head and thorax of a wild-type fly (centre), and flies from the 'pinhead' screen with either a smaller (left) or larger (right) than normal head. (Panel **a** courtesy of Tain Xu, Yale University, USA, and reproduced with permission from REF. 50 © (1995) The Company of Biologists, Ltd; panel **b** courtesy of Nick Brown and Christian Boekel, Wellcome/CRC Institute, Cambridge, UK; panels **c**, **d** courtesy of Barry Dickson, Institute for Molecular Pathology, Vienna, and reproduced with permission from REF. 72 © (2001) Elsevier Science; panel **e** courtesy of Ernst Hafen, University of Zürich, Switzerland.)


a Genotype: w eye–FLP; FRT P[w+] /FRT*
Eye discs expressing eye–FLP
FRT
P[w+]
Flp
Twin-spot clone
Dark red
Homozygous clone
White
Non-recombinant
Red
Adult eye
b Genotype: w eye–FLP; FRT P[w+] M /FRT*
M
Twin-spot clone
Dead
Homozygous clone
White
Non-recombinant; slow-growing
Red
Adult eye

Mosaic Analysis with a Repressible Marker (MARCM) combines Flp/FRT with GAL4/UAS

GAL80 removal by Flp away

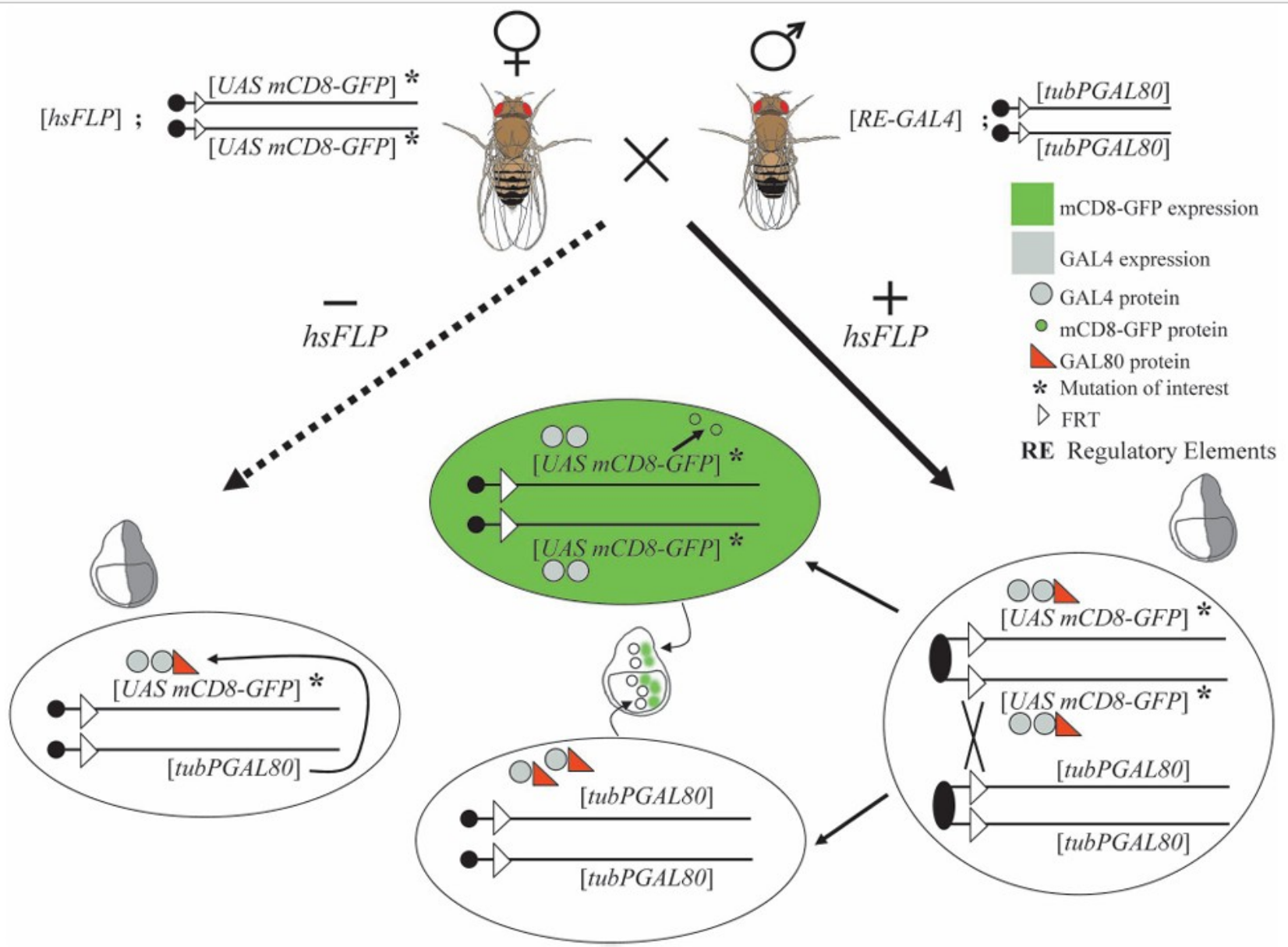
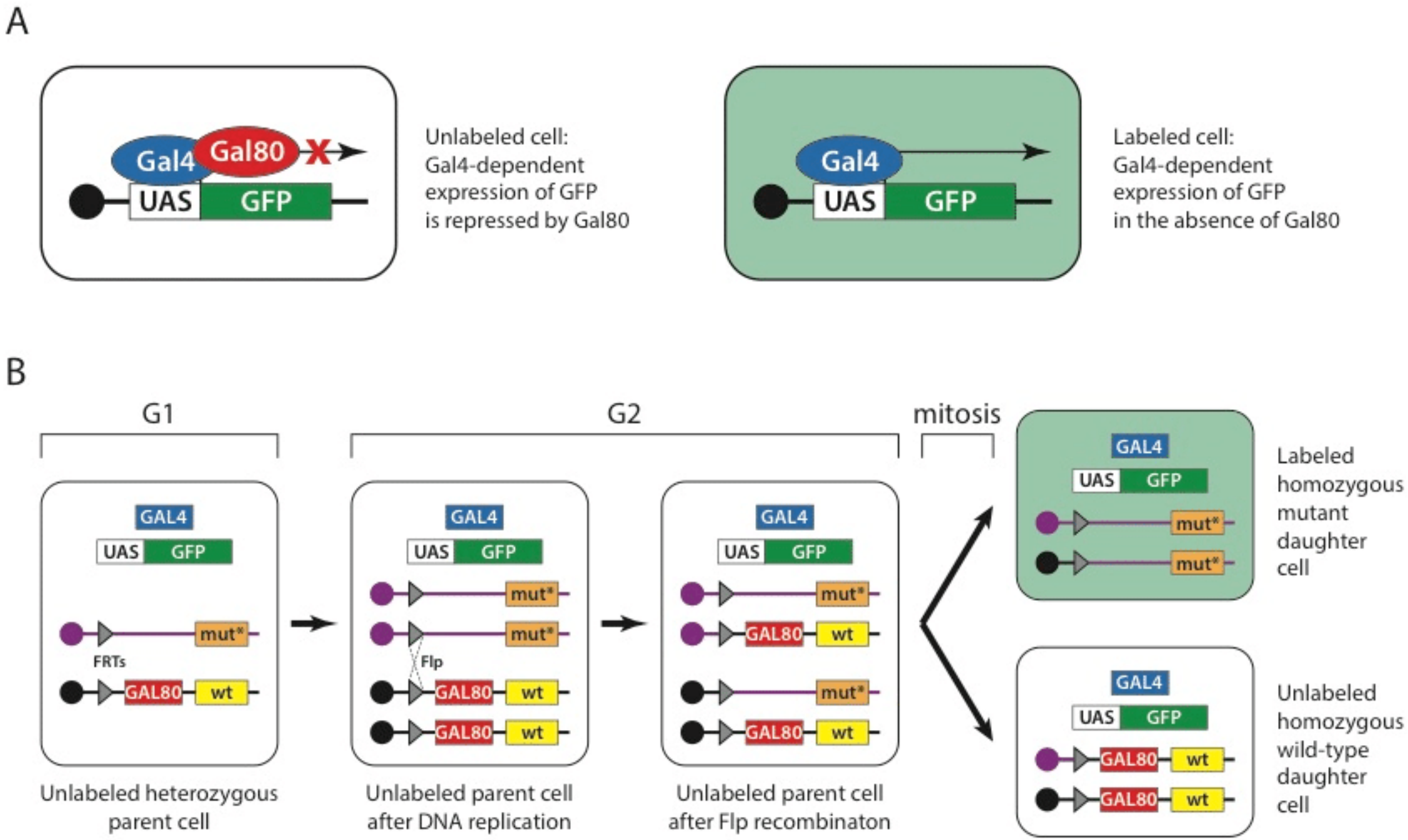


FIG. 7. A positive marking scheme for clonal analysis. In this figure, GAL80 prevents expression of mCD8-GFP in all cells heterozygous for the mutation of interest (*). Mitotic recombination is induced using a heat-shock inducible *FLP* recombinase. During mitosis FLP mediated recombination at the proximally located FRT sequences leads to the production of two daughter cells, each homozygous for the corresponding chromosomes. This results in the production of wing discs that are mosaic for the mutation under study (*). After mitotic recombination, cells that are homozygous for the mutation of interest (*) also now lack GAL80. Consequently, these cells have GAL4 activity and are marked by the presence of the mCD8-GFP, which is under the control of GAL4.


A
Gal4
Gal80
X
UAS
GFP
Unlabeled cell:
Gal4-dependent
expression of GFP
is repressed by Gal80
Gal4
UAS
GFP
Labeled cell:
Gal4-dependent
expression of GFP
in the absence of Gal80
B
G1
G2
mitosis
GAL4
UAS
GFP
mut*
FRTs
GAL80
wt
Flp
Unlabeled heterozygous
parent cell
Unlabeled parent cell
after DNA replication
Unlabeled parent cell
after Flp recombinaton
Labeled
homozygous
mutant
daughter
cell
Unlabeled
homozygous
wild-type
daughter
cell

Making germline clones efficiently with Flp/FRT

- Probably missing many female sterile mutants here because homozygous mothers are not obtainable.

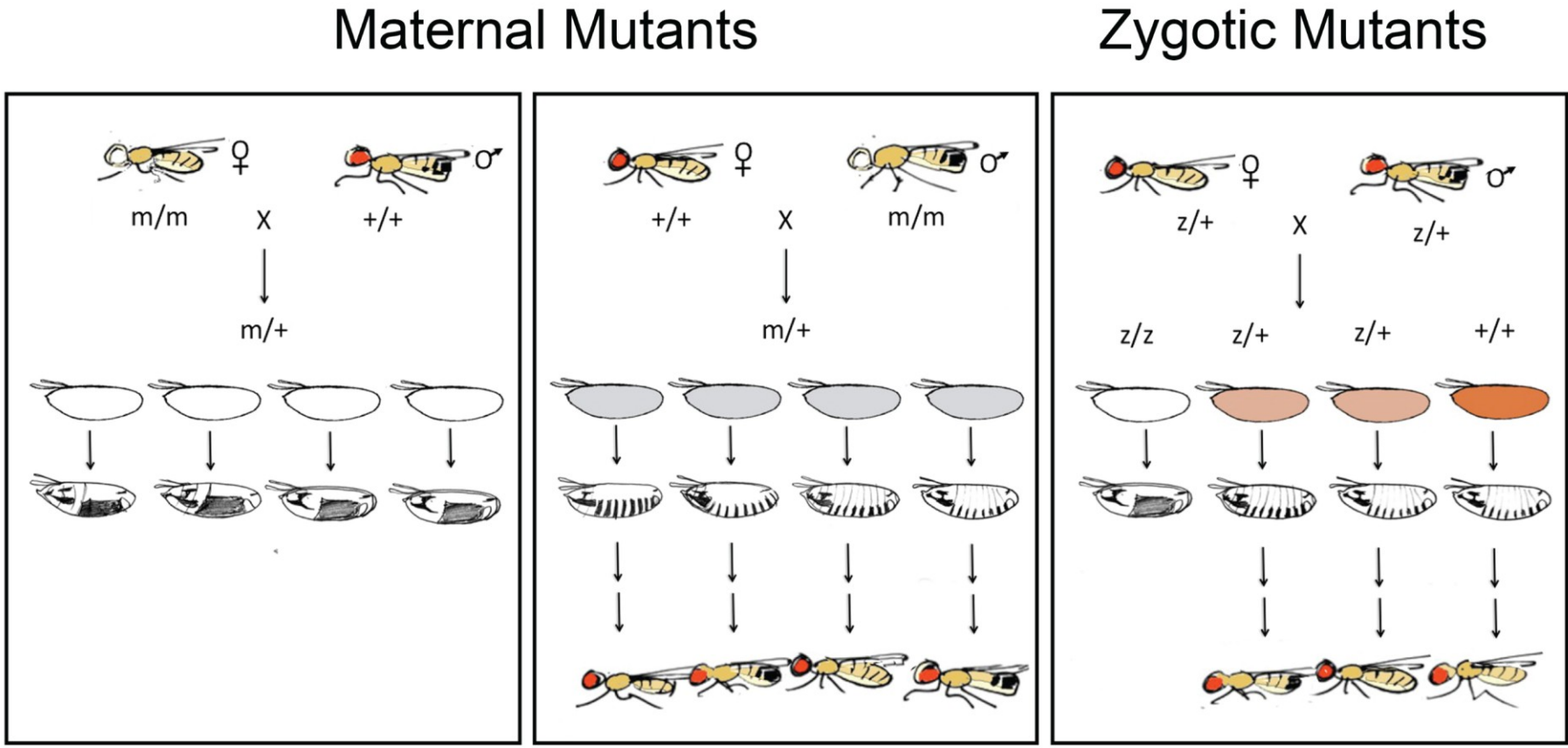


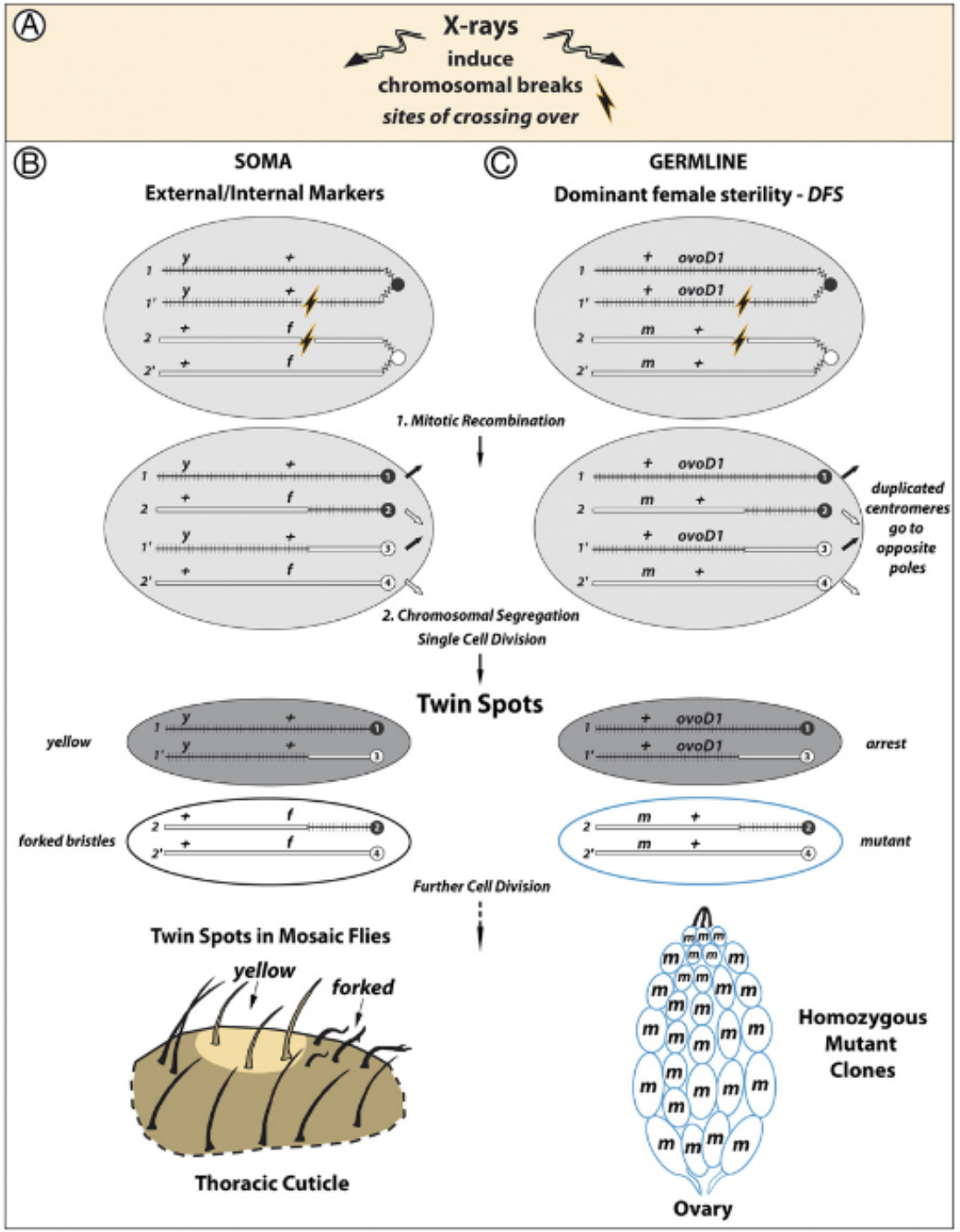
Figure 2

Genetics of embryonic patterning. Maternal and zygotic genes can be distinguished by their genetic behavior. (*Left panel*) All embryos from females that are homozygous mutant for maternally active genes are abnormal, even when crossed with wild-type males. (*Middle panel*) Although the genotype of the resultant embryos is the same as that in the reciprocal cross, in which wild-type females are crossed with mutant males, all embryos are normal. (*Right panel*) For zygotically active genes, only the homozygous one-quarter of the embryos derived from a cross between heterozygotes will be abnormal, even though all embryos develop with identical maternal contributions.

Mitotic recombination

Somatic clones. Twin spots on fly cuticle show recessive mutant markers in side-by side clones arising from a single mitotic recombination event (Curt Stern, 1930).

Germline clones in ovaries using the dominant female sterile (DFS) mutant *OvoD*



Perrimon came for Madeleine Gans lab in Paris.

Screen for female sterile mutants on the X chromosome gave OvoD mutant.

Mutagenized X chromosomes were crossed to Compound X, a balancer with fused copies of the X, used to keep stocks of female-lethal or female-sterile mutants.

Female sterile mutants on the X chromosome included a dominant female sterile that is now called *OvoD*

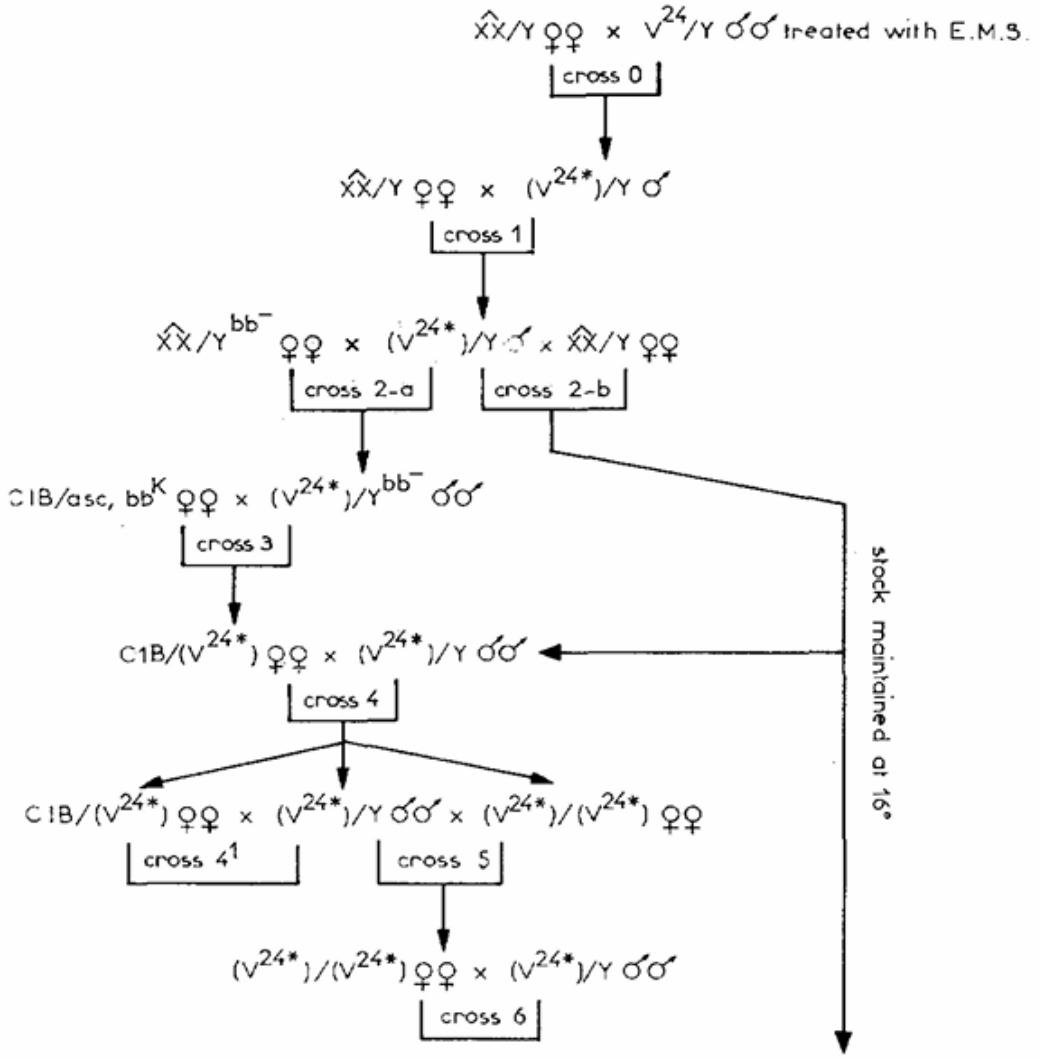
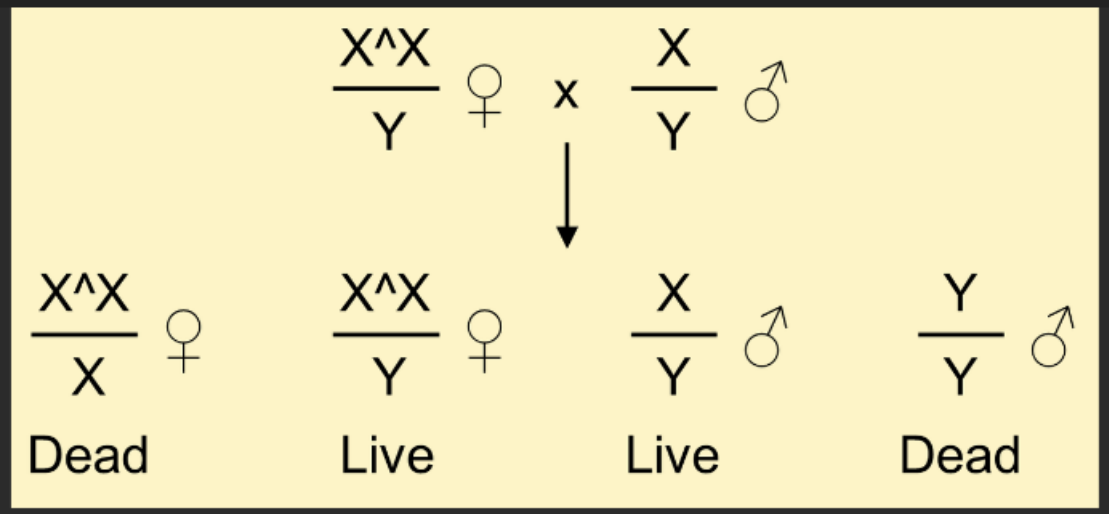


FIGURE 1.—Sequence of crosses used to screen for female-sterile mutants. Cross 1, 2a and 2b were performed with single males; cross 0, 1 and 2a were carried out at 23° ± 1° in the first experiment, at 28.5° ± 1° in the second one; cross 3, 4, 4¹, 5 and 6 were carried out at 16° ± 1° and 28.5° ± 1° in the two experiments. Chromosomes treated by mutagen are described as (v^{24*}) and attached-*X* chromosomes as $\widehat{XX}$.



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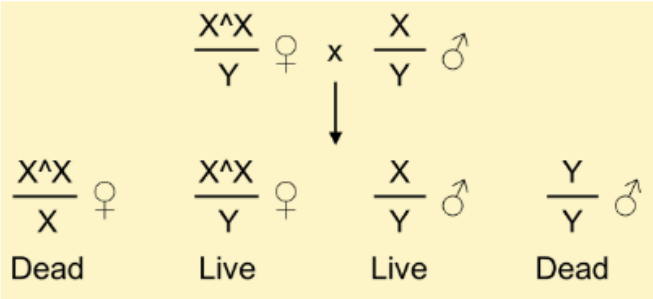
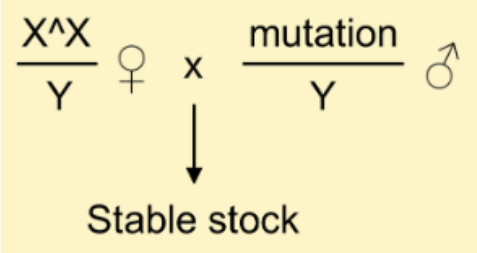
An introduction to attached-X chromosomes

Compound or attached-X chromosomes (denoted X^X) consist of two full-length X chromosomes sharing a common centromere so that they are always inherited together.

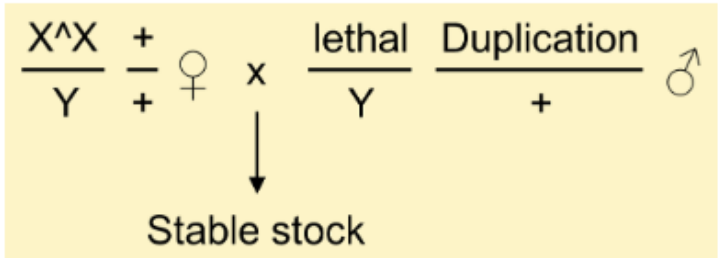


In crosses of normal males to females carrying an attached X, male progeny inherit their X from their father and their Y from their mother.

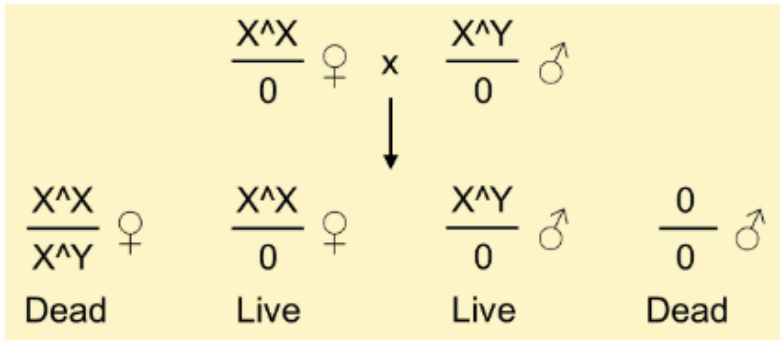
Stable stocks of X-linked visible and female-sterile mutations can be established "instantly" by crossing mutant males to females carrying an attached X.



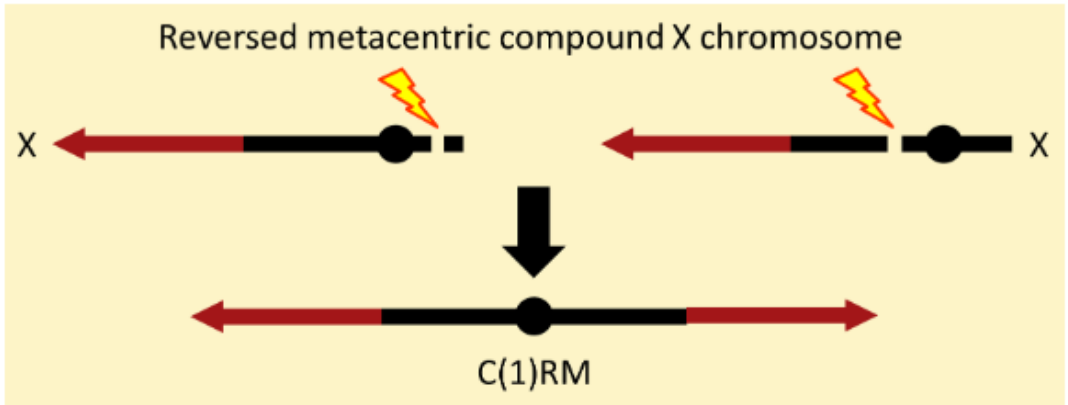
It is possible to maintain X-linked lethal mutations in stock without accumulating additional lethals on the chromosome if you include a rescuing transgene or chromosomal duplication.



Attached-X stocks are also useful for maintaining attached-XY chromosomes in the absence of a free Y chromosome.



The simplest compound-X chromosome is the "reversed metacentric", denoted C(1)RM, which is formed by a reciprocal translocation with one break in centric heterochromatin to the left of the centromere and the other break in heterochromatin to the right of the centromere.

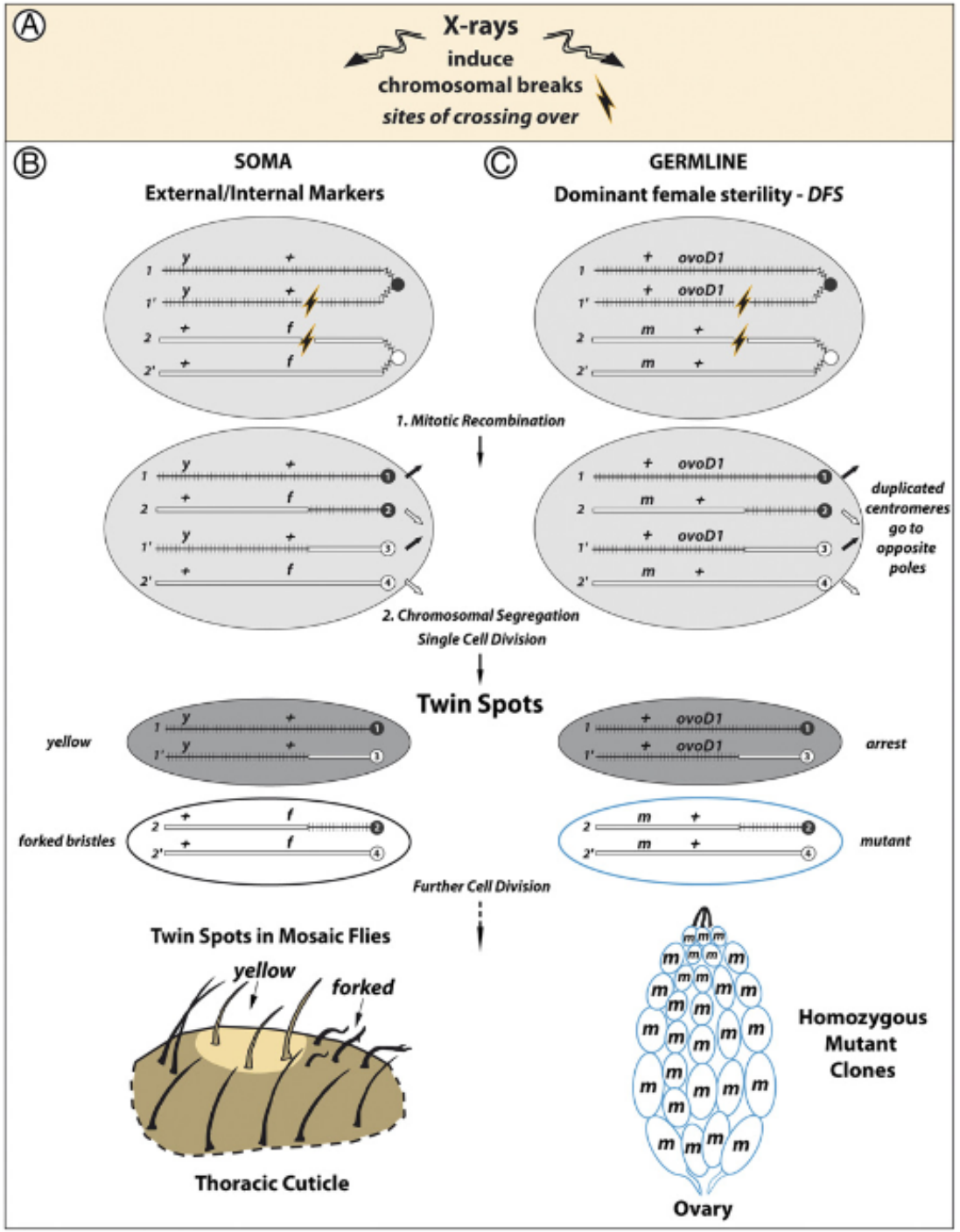


Flp/FRT clones are used to study stem cell maintenance in germline stem cells and in somatic stem cells

Mitotic recombination

Somatic clones. Twin spots on fly cuticle show recessive mutant markers in side-by side clones arising from a single mitotic recombination event (Curt Stern, 1930).

Germline clones in ovaries using the dominant female sterile (DFS) mutant *OvoD*



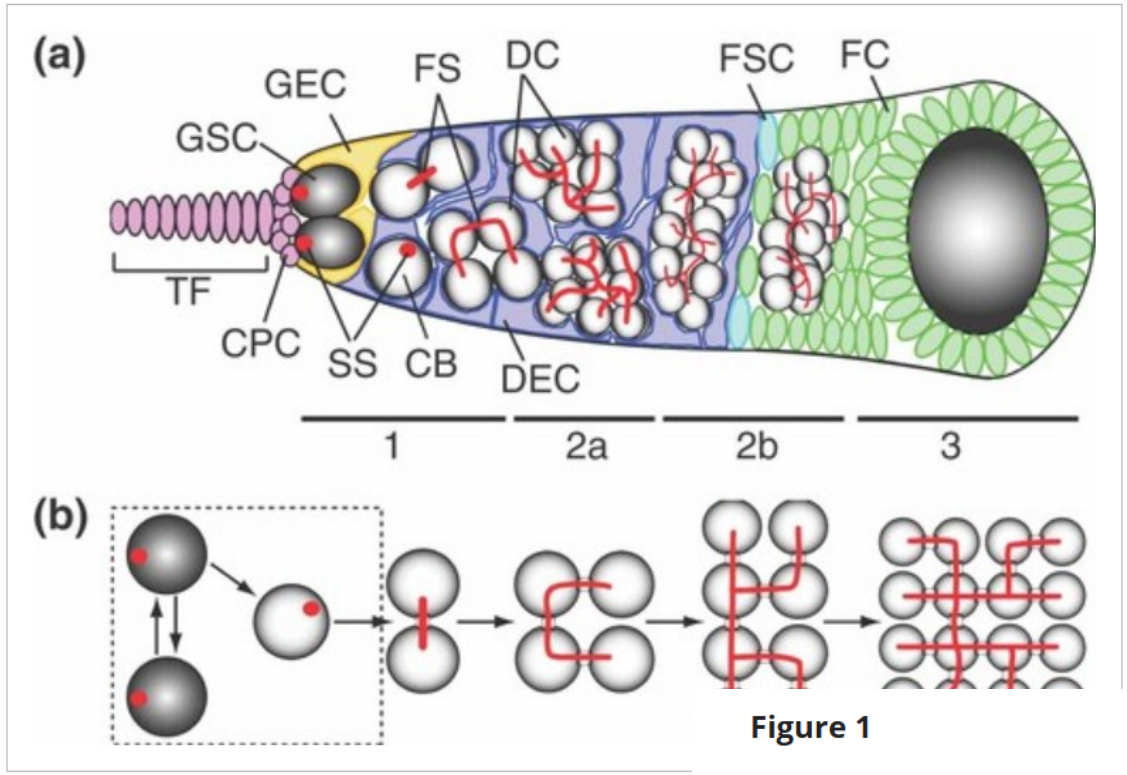


Figure 1

Open in figure viewer | PowerPoint

The *Drosophila* ovarian germline stem cell (GSC) system. (a) A schematic diagram of a *Drosophila* germarium, which is divided into three regions based on germ cell developmental stages. Region 1 contains mitotic germ cells including GSCs, cystoblasts (CBs) and mitotic cysts (2-cell, 4-cell, and 8-cell cysts). Region 2 contains ball-like 16-cell cysts wrapped by escort cells (2a) and lens-shaped 16-cell cysts surrounded by follicle cells (2b); Region 3 contains a stage 1 egg chamber. Abbreviations: TF, terminal filament; CPC, cap cell; GEC, GSC-contacting escort cell; DEC, differentiated germ cell-contacting escort cell; SS, spectrosome; FS, fusome; DC, differentiated cyst; FSC, follicular stem cell; and FC, follicle cells. (b) A GSC (harboring an anteriorly anchored SS) undergoes self-renewing division to generate a GSC and a CB (carrying a SS) (highlighted by broken lines). The CB divides four times without cytokinesis to form a 16-cell cyst. 2-cell, 4-cell, 8-cell, and 16-cell cysts can be easily identified by their branched fusome morphology.

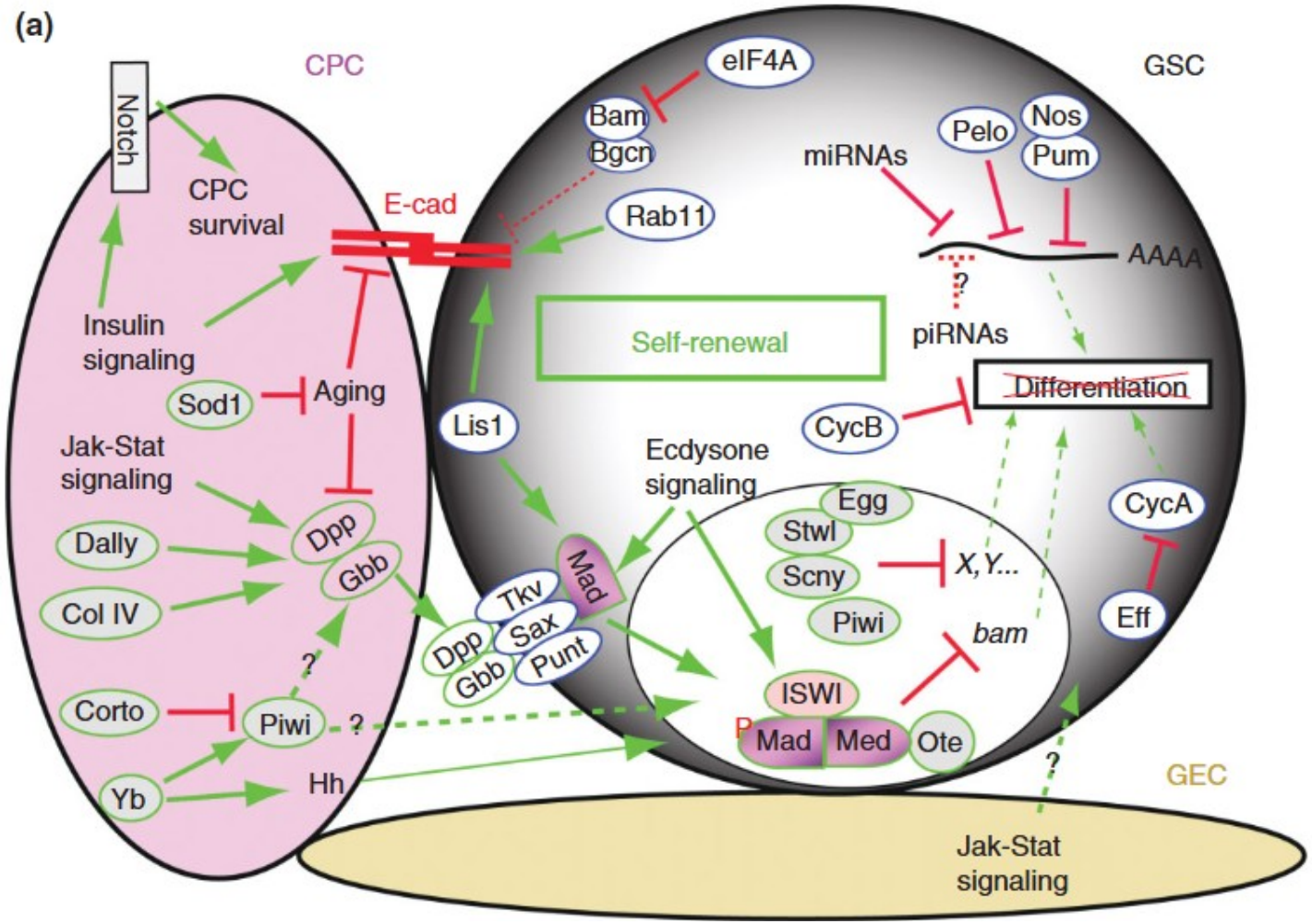


FIGURE 2 | Extrinsic and intrinsic factors work concertedly to control germline stem cell (GSC) self-renewal. (a) A schematic diagram shows how known intrinsic factors in GSCs and niche signaling work together to control GSC self-renewal by repressing Bam-dependent and Bam-independent differentiation pathways. Solid green arrows indicate known positive regulations, while solid red inhibition signs denote known inhibitory relationships. The broken green arrows indicate that the regulations have been inhibited, while the broken lines with a question mark show speculative relationships. (b) A summary on relationships among systemic factors, niche signaling and intrinsic factors in the regulation of GSC self-renewal.

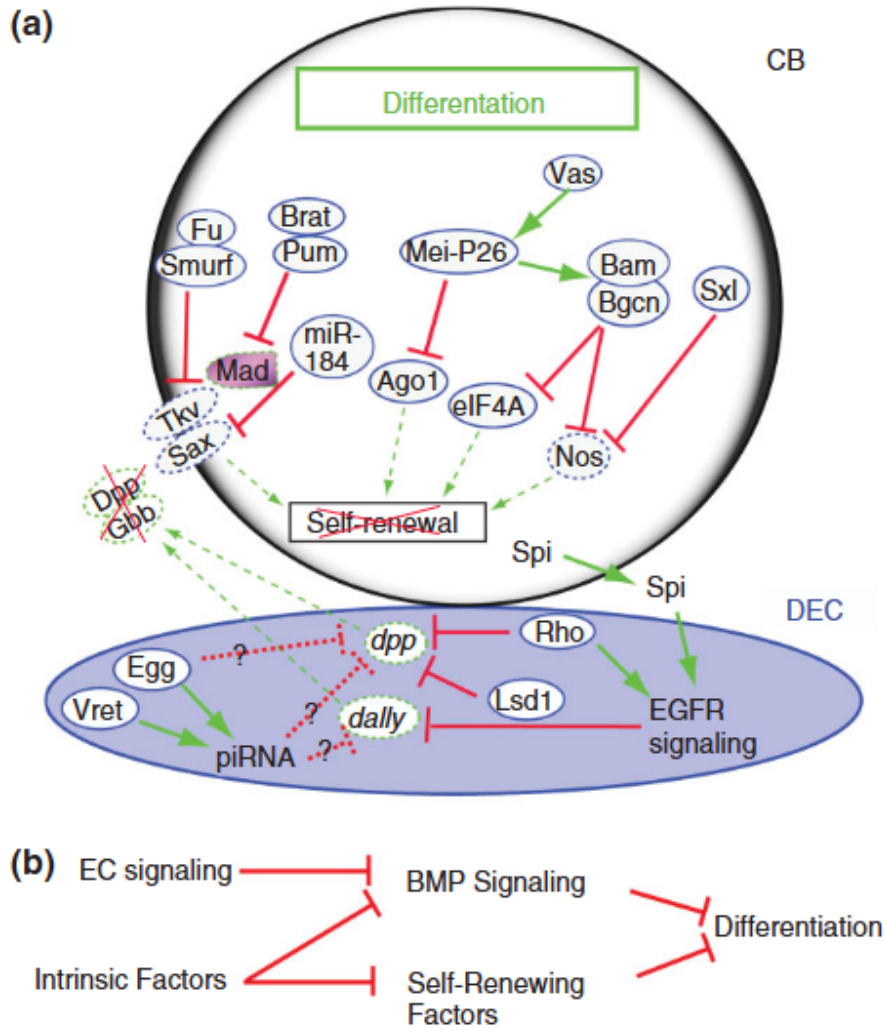


FIGURE 3 | Escort cell (EC) signaling and intrinsic factors control cystoblast (CB) differentiation. (a) A schematic diagram shows that known intrinsic factors in CBs promote differentiation by repressing self-renewal factors or work with EC signaling to block bone morphogenetic protein (BMP) signaling. (b) A summary of the relationships among EC signaling and intrinsic factors in the regulation of CB differentiation.

Reading

- The **art** and **design** of genetic screens: ***Drosophila*** *melanogaster*
- D St Johnston - Nature reviews genetics, 2002 - nature.com

- **[PDF]** Creating mosaics in **Drosophila**
- N Perrimon - International Journal of Developmental …, 1998 - genepath.med.harvard.edu

- **[PDF]** GAL4 system in ***drosophila***: A fly geneticist's **swiss army knife**
- JB Duffy - genesis, 2002 - fenix.ciencias.ulisboa.pt

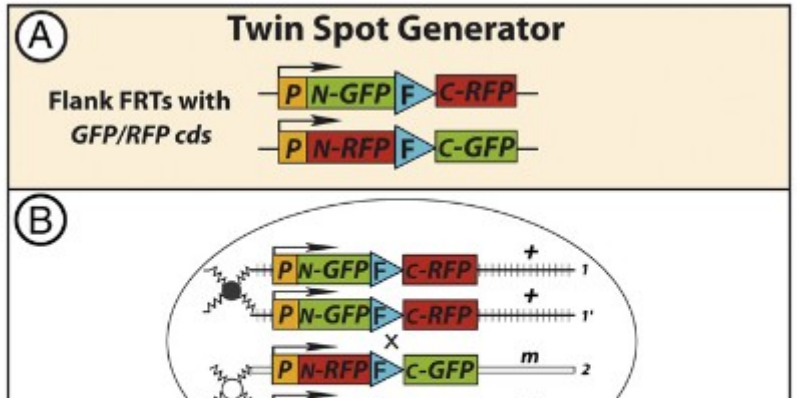


Fig. 5. TSG differential labeling of twin spots. (*A*) TSG is a Flp-FRT–based MR strategy, as in Fig. 2, except that the FRT site, located in an intron, is flanked with complementary *GFP* and *RFP* sequences to create TSG hybrid cassettes (*Materials and Methods*). (*B*) Heat shock-induced MR generates full-length but interrupted *cds* for GFP on one recombined chromosome and for RFP on the other. Splicing removes the FRT site along with the rest of the intron to reconstruct continuous full-length GFP or RFP *cds*. In a heterozygote, placing the wild-type allele distal to the 3′ RFP and the mutant allele distal to the 3′ GFP enables direct identification of both the red wild-type control and green mutant twins. (*C*) TSG provides a direct readout of toxicity. In control experiments in which both twin spots are homozygous wild type, statistical analysis showed equal numbers of cells present in the red and green twin spots, as expected and confirming that ectopic expression of GFP or RFP had no apparent toxic effects (compare with Fig. 6*C*). Adapted from ref. 39.

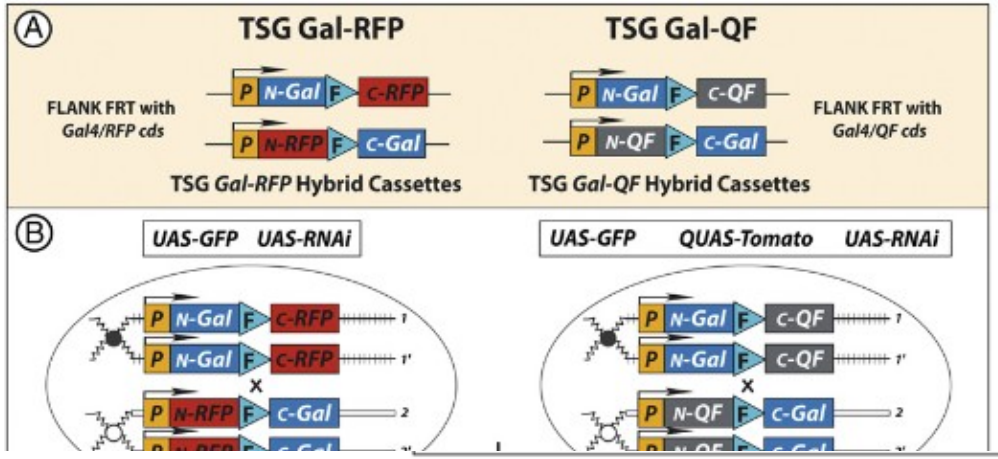
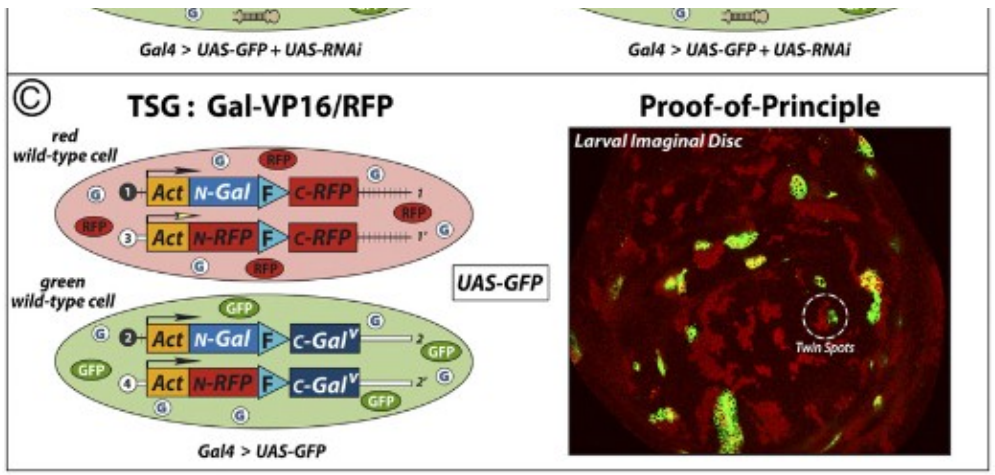


Fig. 6. Extension of the TSG method to control differential gene expression in each twin spot cell. (*A*) As in Fig. 5, except that we replaced the *GFP* sequences with those of *Gal4* to create TSG *Gal-RFP* hybrid cassettes (*Left*) and additionally replaced the *RFP* sequences with those of the *Neurospora crassa QF* transactivator to create TSG *Gal-QF* hybrid cassettes (*Right*). (*B*) Boxes at the top of the panel show the UAS target sequences that were inserted into one or the other of the TSG parental lines before TSG fly crosses. MR proceeds as in Fig. 1, except that both the RNAi-induced mutation and green marker fluorescence are generated indirectly by Gal4 activation of the *UAS-RNAi* and *UAS-GFP* sequences, respectively (*Left*), and the red marker fluorescence of the internal wild-type control twin is generated indirectly by QF activation of the *QUAS-mTomato*, a strong red-fluorescing marker (*Right*). (*C*) Proof-of-principle and toxicity assay. Only *UAS-GFP* is present in the background. Both red and green clones are visible, demonstrating that the Gal4-UAS binary expression system works in TSG. However, Gal4-VP16 expression affects clone size, presumably because of its toxicity.



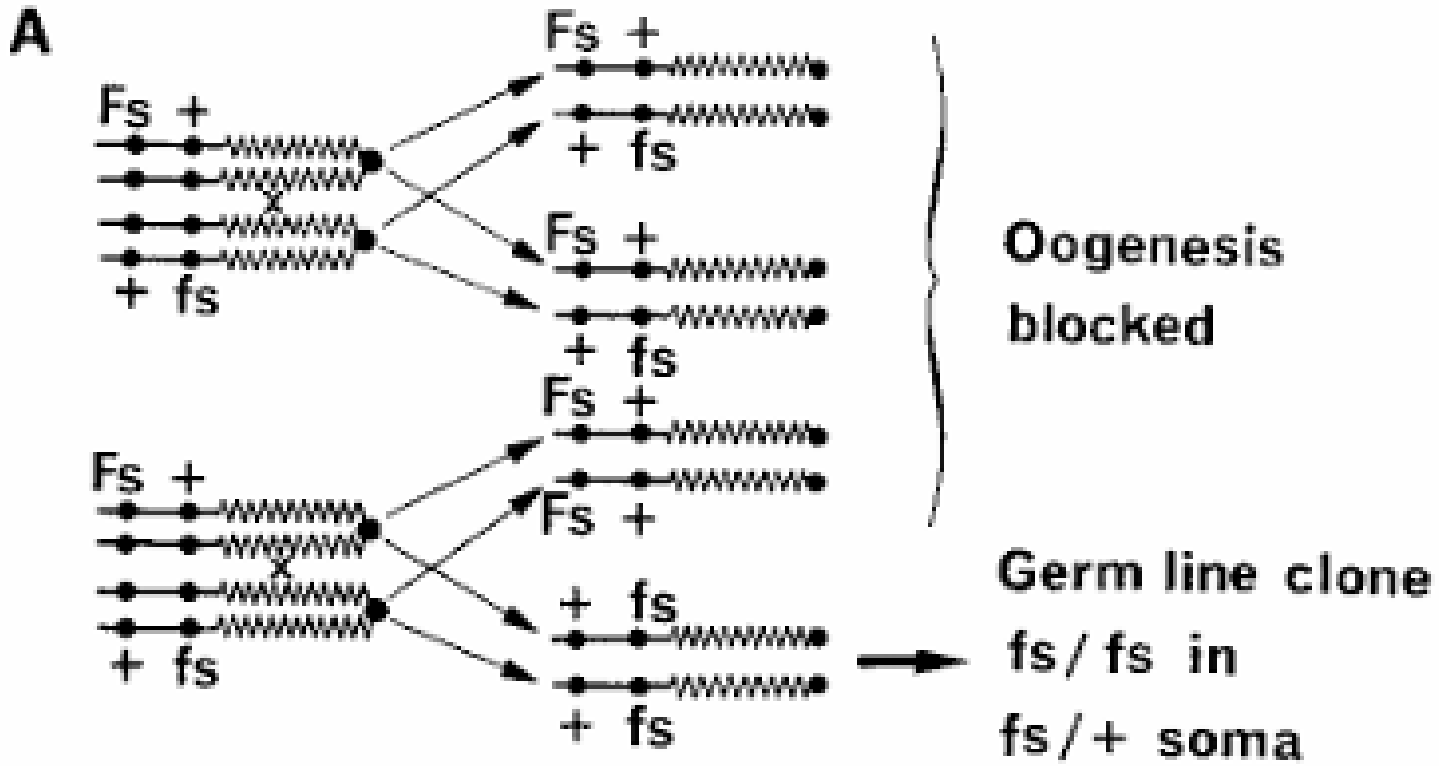


FIG. 2. Principle of the test of tissue specificity of recessive female-sterile mutations using *Fs(1)K1287*. (A) Mitotic recombination in heterochromatin (proximal to *fs and Fs*) leads to an *fs(1)/fs(1)* germ line clone in an *fs(1)/+* soma. If the *fs(1)* affects only a function required in the soma, females with this proximal clone should be fertiles. Otherwise, if the *fs(1)* affects a germ line function, the clone should have the same characteristic as the *fs(1)* mutant itself. (B) Use of mor-

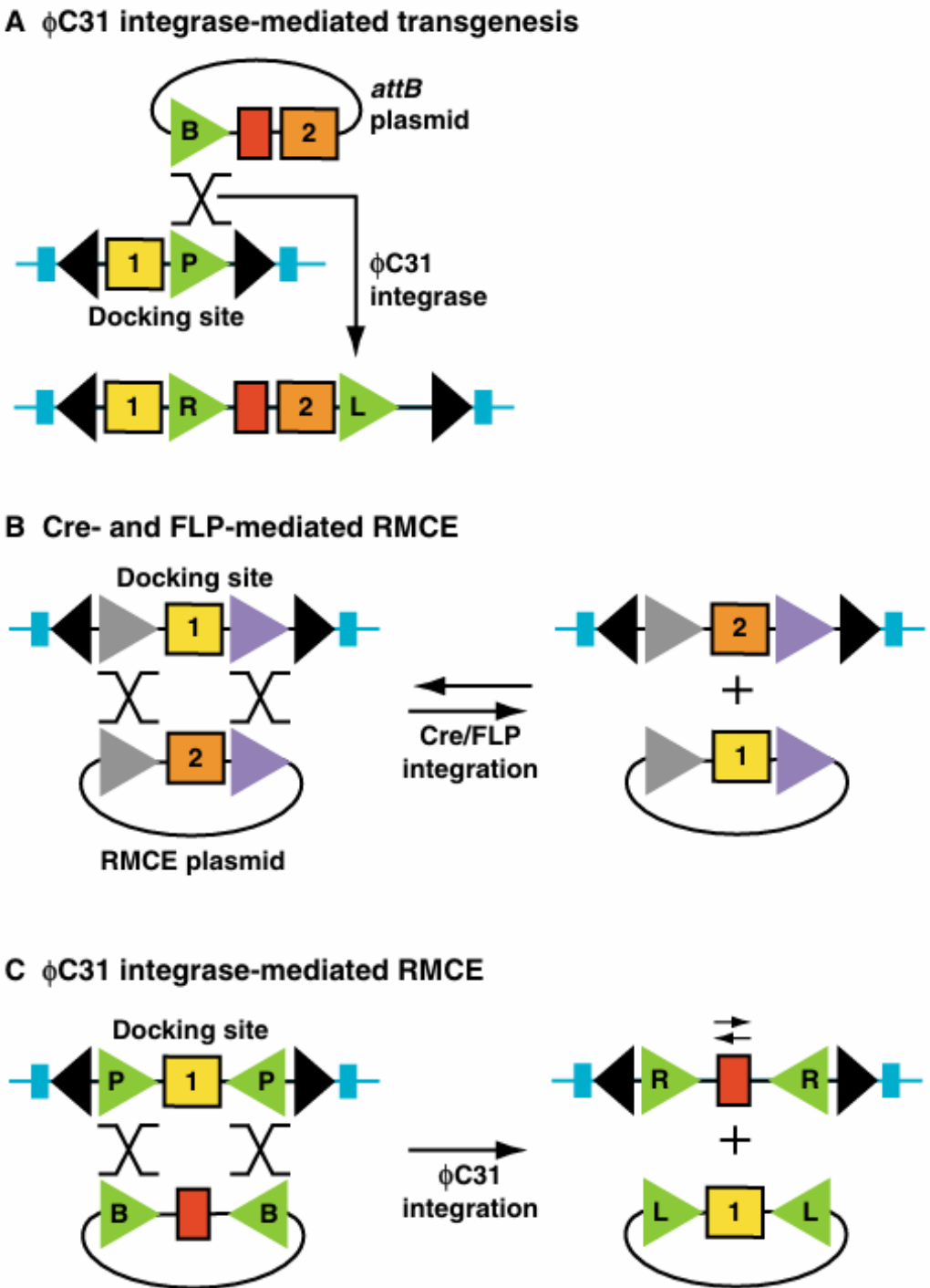
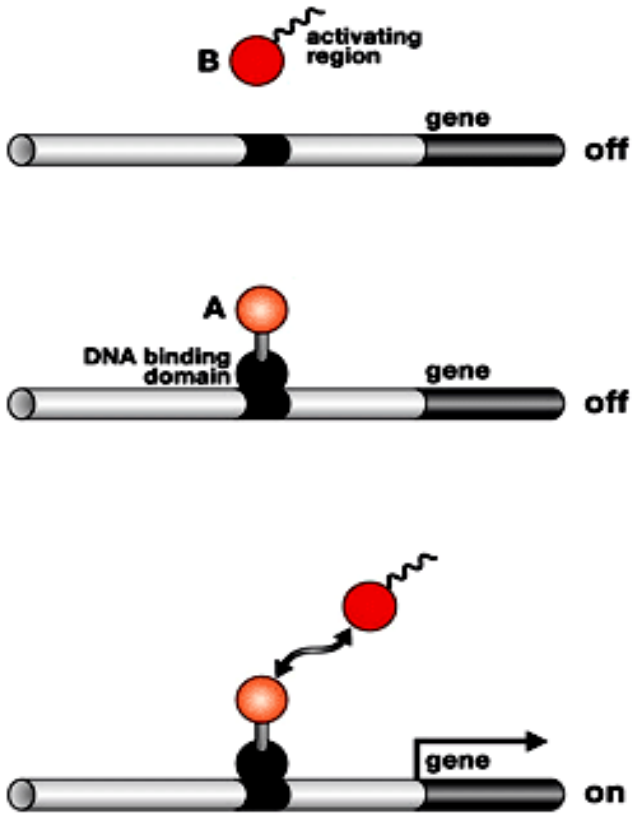


Fig. 7. Site-specific integration in *Drosophila*. (**A**) ΦC31 integrase-mediated transgenesis using single *attP* docking sites. Docking sites are transposons, such as *P* elements (Groth et al., 2004), *piggyBac* (Venken et al., 2006) or *Mariner* (Bischof et al., 2007), that contain a single *attP* recombination site and a marker 1, and that are integrated into the genome. A plasmid containing an insert, marker 2 and an *attB* recombination site, can then integrate into the docking site when ΦC31 integrase is provided. Correct recombination events between *attP* and *attB* are identified using marker 2. They result in two hybrid sites, *attL* and *attR*, that are no longer a substrate for ΦC31 integrase – the reaction is therefore irreversible. (**B**) Cre- and FLP-mediated RMCE. Docking site transposons (with *5′* and *3′* transposon termini), such as *P* (Oberstein et al., 2005) or *piggyBac* (Horn and Handler, 2005) elements, contain marker 1 flanked by heterotypic direct-oriented recombination sites (RS) 'RS1' (*loxP* or *FRT*, gray) and 'RS2' (such as *lox2272* or *F3*, purple). The RMCE plasmid, containing marker 2 flanked by a similar configuration of heterotypic recombination sites, can integrate when Cre or FLP is provided. Correct recombination events are identified by the absence of marker 1 and presence of marker 2. Recombination can be partial (single integration events are not shown) and is reversible. (**C**) ΦC31 integrase-mediated RMCE. A docking site *P* element transposon (*5′P* and *3′P* element termini) (Bateman et al., 2006) contains a marker 1 flanked by inverted *attP* recombination sites. The RMCE plasmid, containing insert flanked by inverted *attB* recombination sites, can integrate when ΦC31 integrase is provided. Correct recombination events, between both *attP* and *attB* sites, are identified through absence of marker 1 and result in hybrid sites, *attL* and *attR*, that are no longer substrates for ΦC31 integrase. The integrated DNA can be in either orientation (arrows).

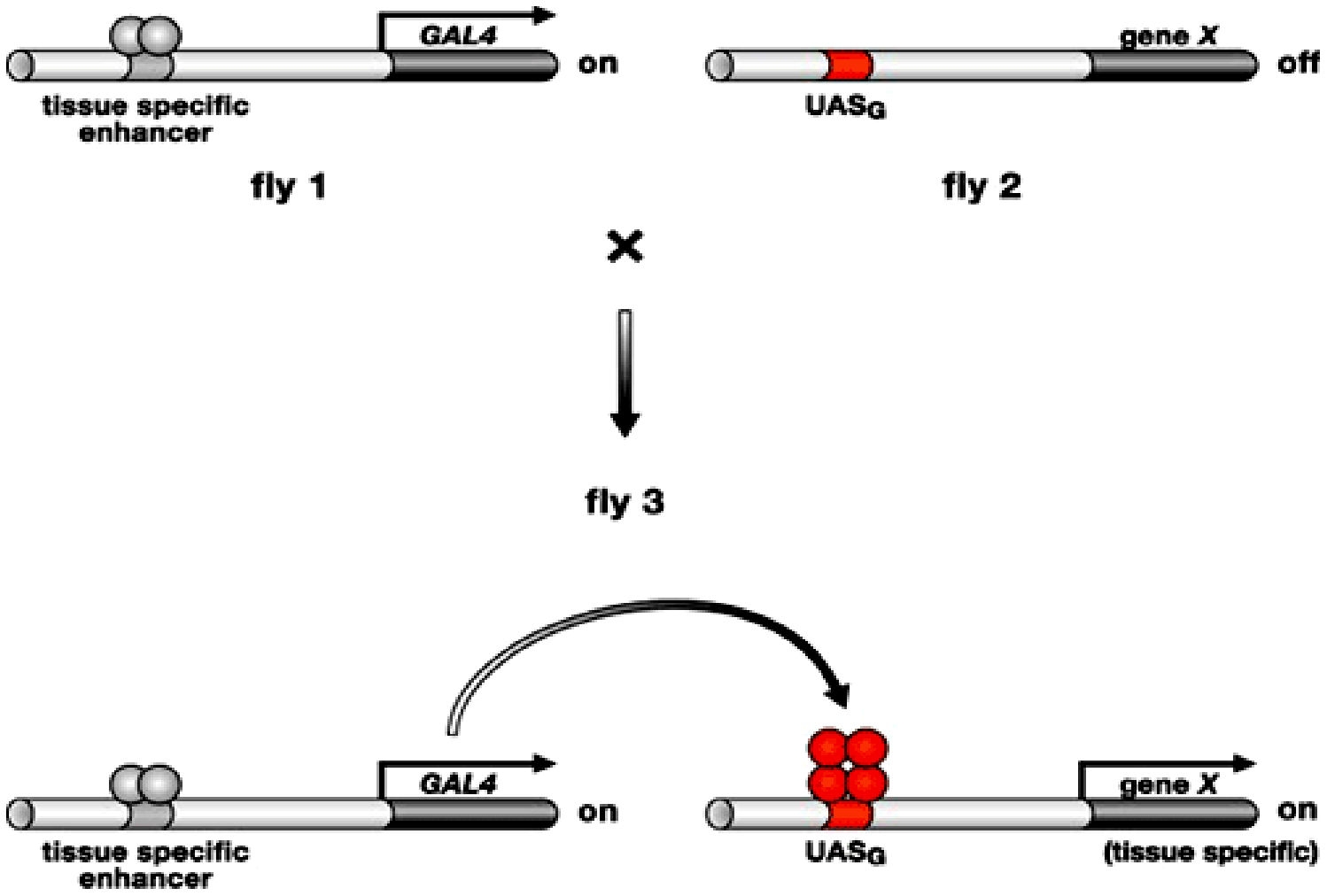
Uses of GAL4 Number 1: The yeast two hybrid system for identification of interacting proteins.



Can be used on a genomic scale to test all proteins against all others. Target protein fused to DNA-binding domain in cells of one mating type mated to a library of activating region fusion proteins.

Uses of GAL4: GAL4 activates transcription from UAS_G in *Drosophila*.

Widely used for targeted protein expression in *Drosophila* (GAL4-UAS two-component system).



Genetic odyssey to generate marked clones in *Drosophila* mosaics

Ruth Griffin[a,b,c], Richard Binari[d,e], and Norbert Perrimon[d,e,1]

[a]Commissariat à l'Energie Atomique, Laboratoire de Biologie à Grande Echelle, F-38054 Grenoble, France; [b]Centre National de la Recherche Scientifique, Unité FR3425, Université Grenoble-Alpes, F-38000 Grenoble, France; [c]Institut National de la Santé et de la Recherche Médicale, U1038, F-38054 Grenoble, France; [d]Department of Genetics and [e]Howard Hughes Medical Institute, Harvard Medical School, Boston, MA 02115

This contribution is part of the special series of Inaugural Articles by members of the National Academy of Sciences elected in 2013.

Contributed by Norbert Perrimon, February 21, 2014 (sent for review January 31, 2014)

Chimaeras, fanciful beasts that drew their force from being composed of parts of disparate animals, have stimulated our collective imagination for centuries. In modern terms, chimaeras are composite animals consisting of genetically distinct cell populations and are called "mosaics" if the different cell types have emerged from the same zygote. Phenotypic studies of chimeric animals formed from invertebrates, amphibians, birds, and mammals have provided many fundamental insights into biological processes, most notably in developmental biology. Many methods for generating both chimaeras and a range of markers for tracing their lineages have been developed over the years. Our laboratory has been intimately involved in the development of methods that facilitate the creation of genetic mosaics in *Drosophila*. Here, we review our contributions to the development of this field and discuss a number of approaches that will improve further the tool kit for generating mosaic animals.

clonal analysis | twin spot | Gal4-UAS | Flp-FRT

Mitotic Recombination to Create Mosaics in the Soma and Germ Line

In 1936 Stern (3) first coined the term "twin spots" to refer to the two homozygous daughter cells generated by mitotic recombination (MR) in a heterozygous animal (Fig. 1). MR events are very rare in wild-type animals, but their frequency can be increased significantly by X-ray irradiation that generates DNA double-stranded breaks (Fig. 1*A*). Subsequent DNA repair enzyme activity is not always strand specific, leading to crossover exchange of genetic material between homologous chromosomes. Importantly, because the timing of irradiation can be controlled, MR events can be induced at different time points to generate clones of different sizes (the earlier the irradiation, the larger the clone size). Further, because the frequency of events depends upon the number of cells present, analysis of the resultant clone sizes can be used to estimate progenitor numbers and their division rates (e.g., see refs. 4 and 5).

MR provides a powerful tool to mark all of the descendants of

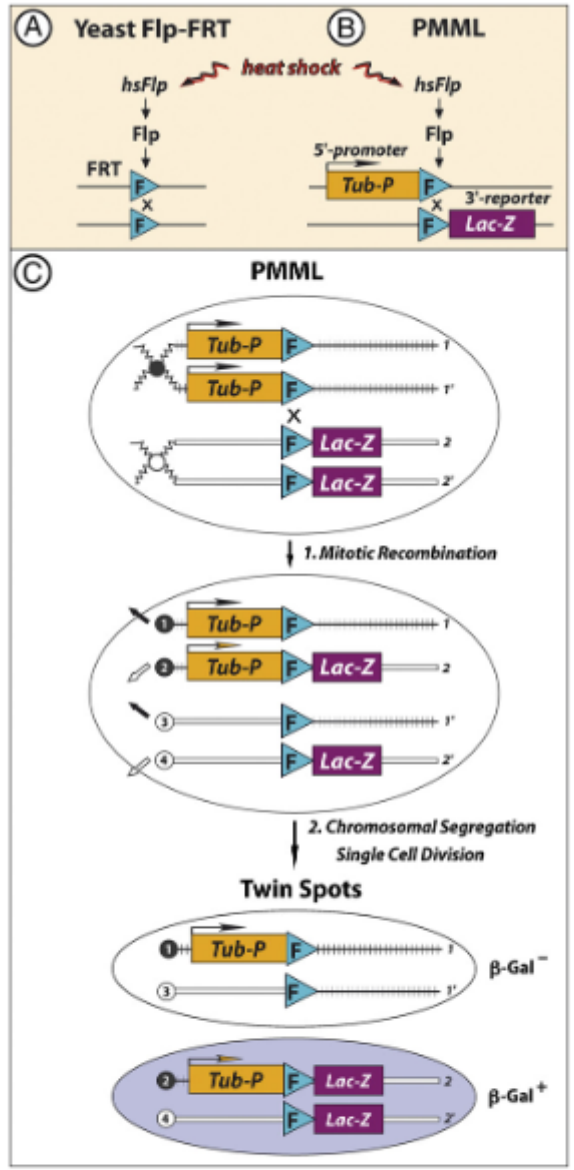


Fig. 2. The Flp-FRT method and the PMML strategy. (*A*) The yeast recombinase Flippase is under the control of the *hsp70* promoter in *hs-Flp*. Heat shock induces Flp expression, which activates recombination specifically at target FRT sites previously inserted into the genome. (*B*) In the PMML strategy, FRT sites are differentially flanked by a 5′-*tubulin* promoter on one homologous chromosome and a 3′-*Lac-Z* on the other. (*C*) Heat-shock induces MR, creating a mosaic animal with one unmarked twin spot and one twin spot in which a functional fused *tubulin promoter-LacZ cds* leads to expression of β-galactosidase.

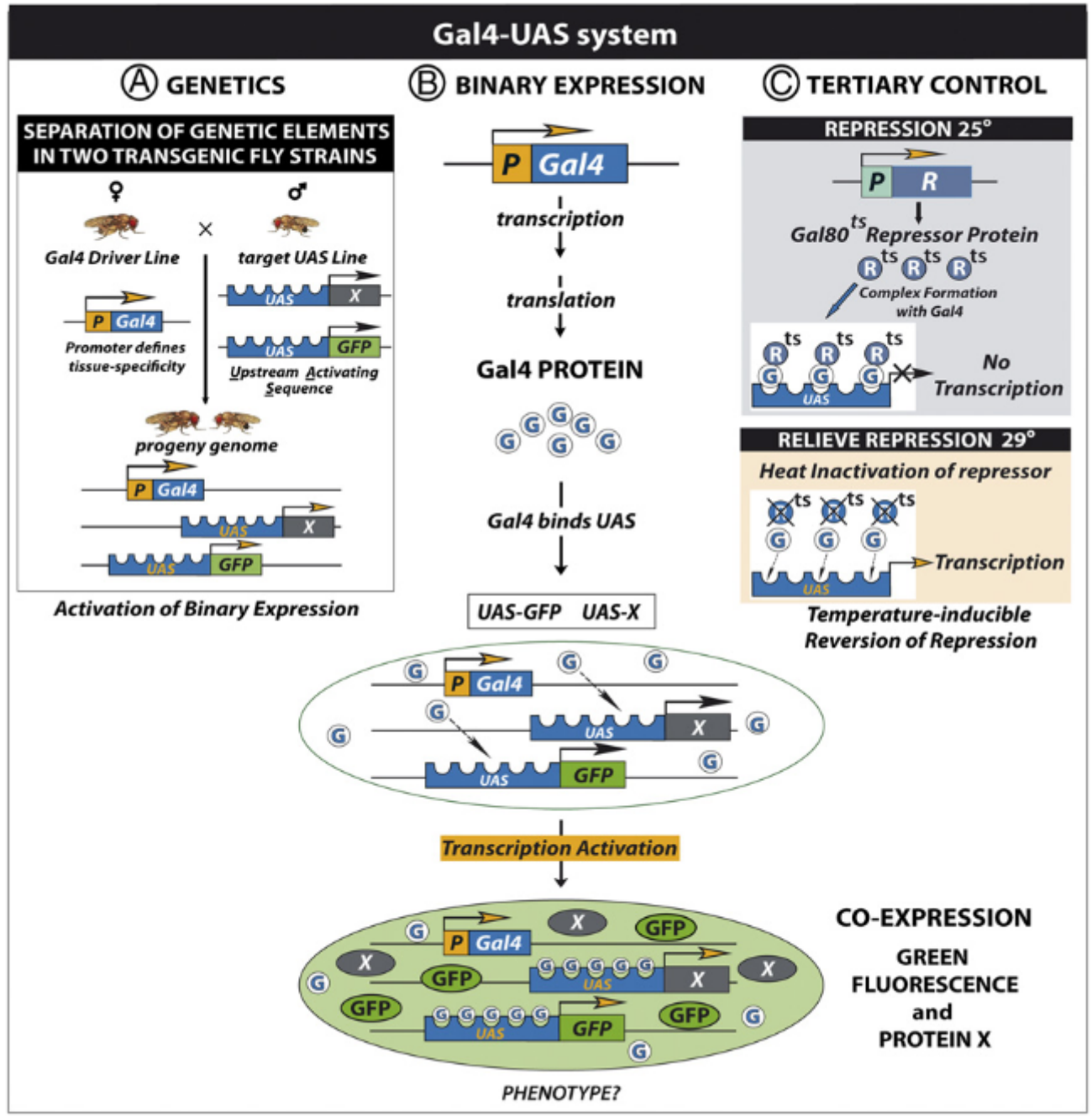

Gal4-UAS system
Ⓐ GENETICS
Ⓑ BINARY EXPRESSION
Ⓒ TERTIARY CONTROL
SEPARATION OF GENETIC ELEMENTS IN TWO TRANSGENIC FLY STRAINS
Gal4 Driver Line
target UAS Line
P Gal4
Promoter defines tissue-specificity
UAS X
UAS GFP
Upstream Activating Sequence
progeny genome
Activation of Binary Expression
P Gal4
transcription
translation
Gal4 PROTEIN
Gal4 binds UAS
UAS-GFP UAS-X
Transcription Activation
PHENOTYPE?
REPRESSION 25°
P R
Gal80ts Repressor Protein
Complex Formation with Gal4
No Transcription
RELIEVE REPRESSION 29°
Heat Inactivation of repressor
Transcription
Temperature-inducible Reversion of Repression
CO-EXPRESSION
GREEN FLUORESCENCE and PROTEIN X

Fig. 3. The Gal4 binary expression system expands levels of control. (*A*) The genetic components of the Gal4-UAS system are present in two distinct transgenic fly lines so that leaky expression and potential genetic selection are avoided. The driver and target UAS fly lines are crossed, and the full complement of genetic elements comes together exclusively in the progeny genome. (*B*) In the first phase of binary expression, the Gal4 promoter determines the time and cell specificity of transcription. After translation, Gal4 protein binds to UAS sequences placed upstream of any and all *cds*, causing their coordinate expression, so a single UAS-marker reveals the specific expression pattern of all UAS-linked sequences. The box above the colorless cell shows UAS-target sequences inserted into the background. (*C*) A tertiary level of control is possible through the use of a specific Gal4 repressor, $Gal80^{TS}$, which is temperature sensitive. At lower temperatures, the repressor is active, and there is no Gal4 transcription; at higher temperatures, the repressor cannot function, and the active repressor pool becomes depleted. Gal4 transcription progressively resumes, and binary expression is reactivated.

Flp-out, GAL4-on

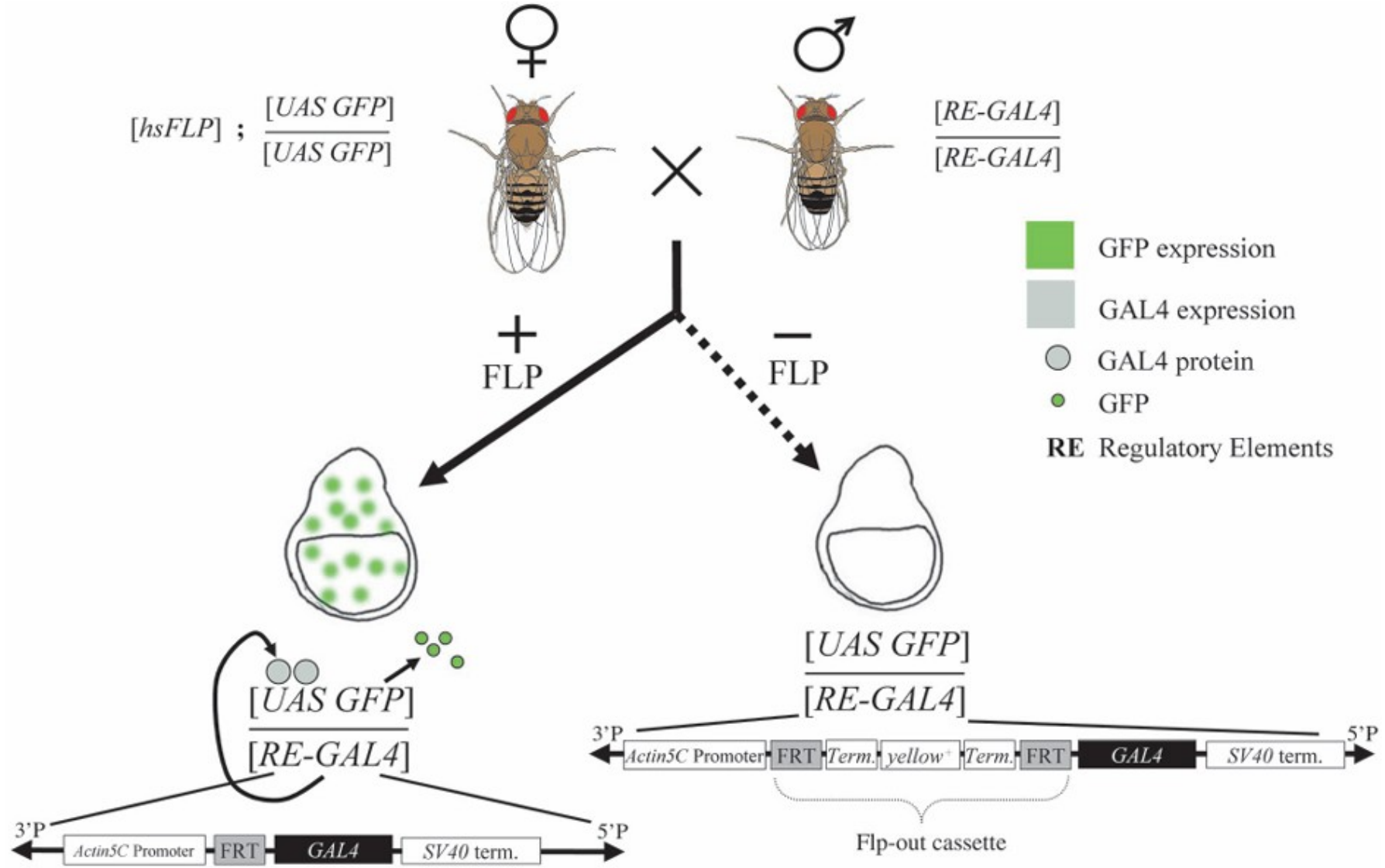


FIG. 5. GAL4 induction with the FLP/FRT system. In this modification of the UAS/GAL4 system, the presence of a FLP-out cassette prevents the *Act5C* promoter from triggering GAL4 expression. Upon induction of FLP expression (via a heat-shock FLP recombinase) the intervening FLP-out cassette is excised. This juxtaposes the *Act5C* promoter and the *GAL4* gene leading to *GAL4* transcription and subsequently expression of the *UAS-GFP* responder only in the cells in which the FLP-out cassette has been excised (depicted here as green patches).

UAS Flp-on

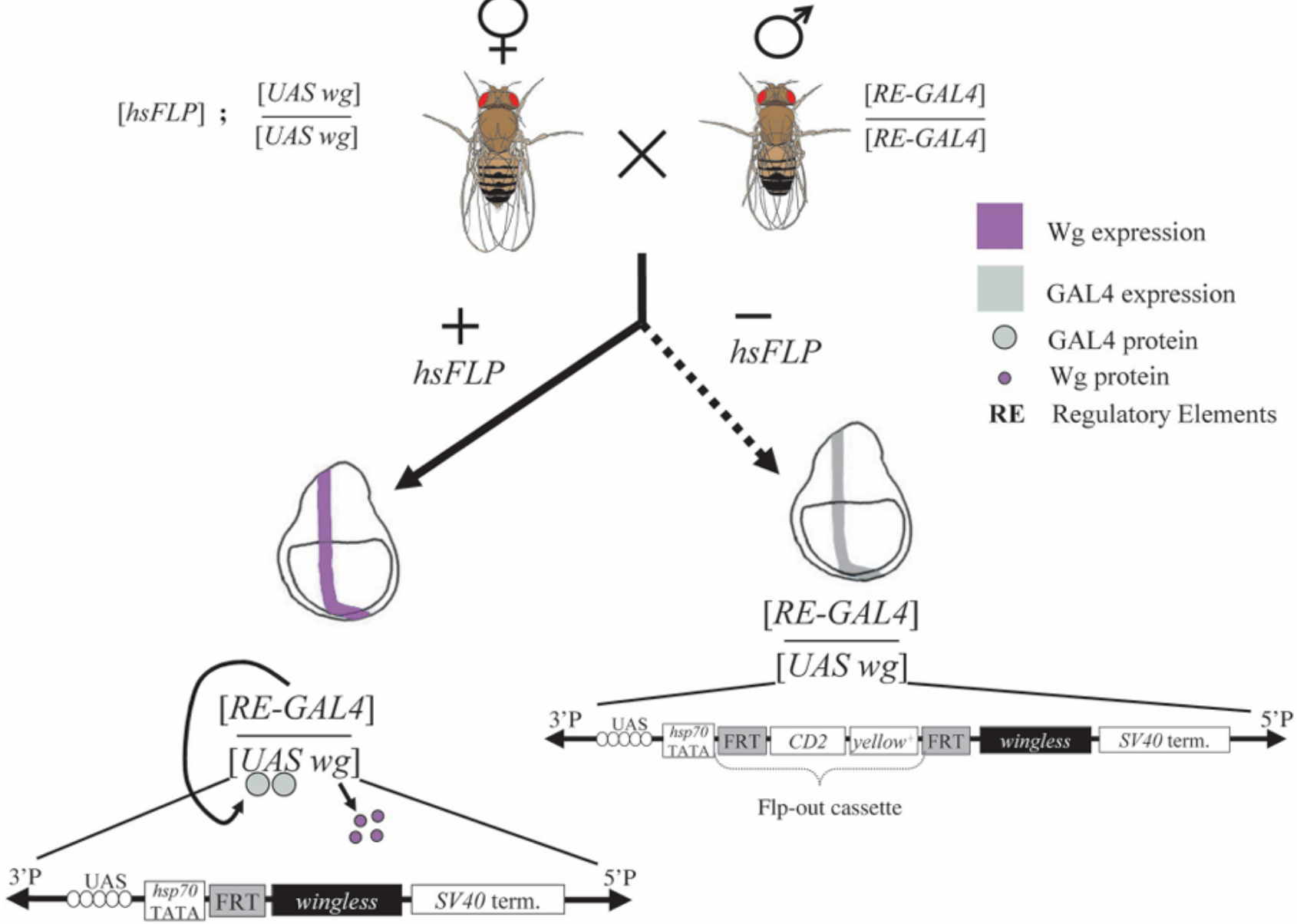


FIG. 6. UAS responder induction with the FLP/FRT system. In a slight variation on the system depicted in Figure 5, the presence of a FLP-out cassette now separates the UAS element from the responder gene (in this case, *wingless (wg)*). This prevents transcription of the responder in response to GAL4 binding to the UAS element. Upon induction of FLP expression (via a heat-shock FLP recombinase) the intervening FLP-out cassette is excised and juxtaposes the UAS element and the responder gene leading to its transcription and subsequent expression. In this figure, the GAL4 driver is expressed in a stripe along the anterior–posterior axis of the wing imaginal disc.

GAL80 removal by Flp away

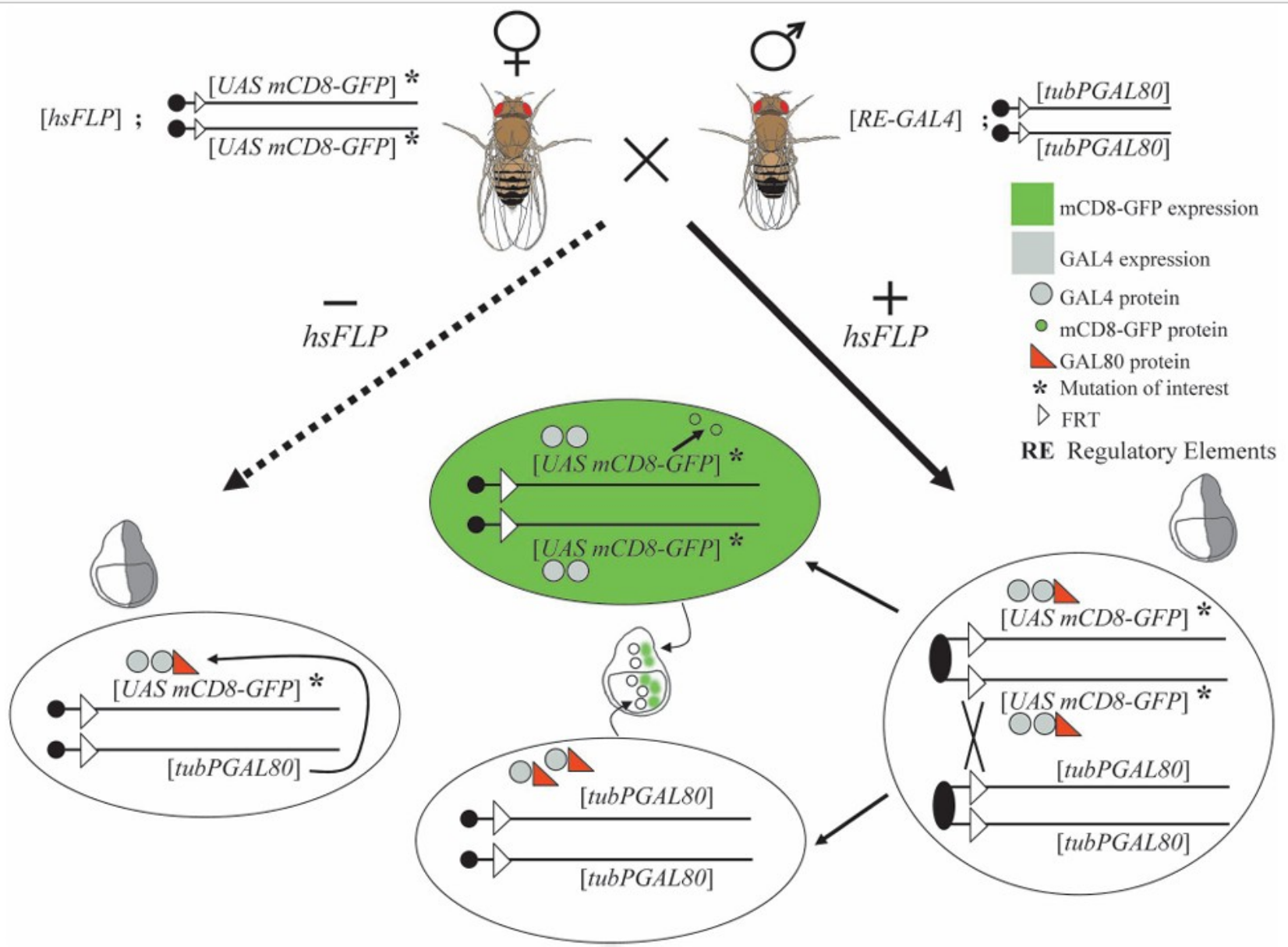
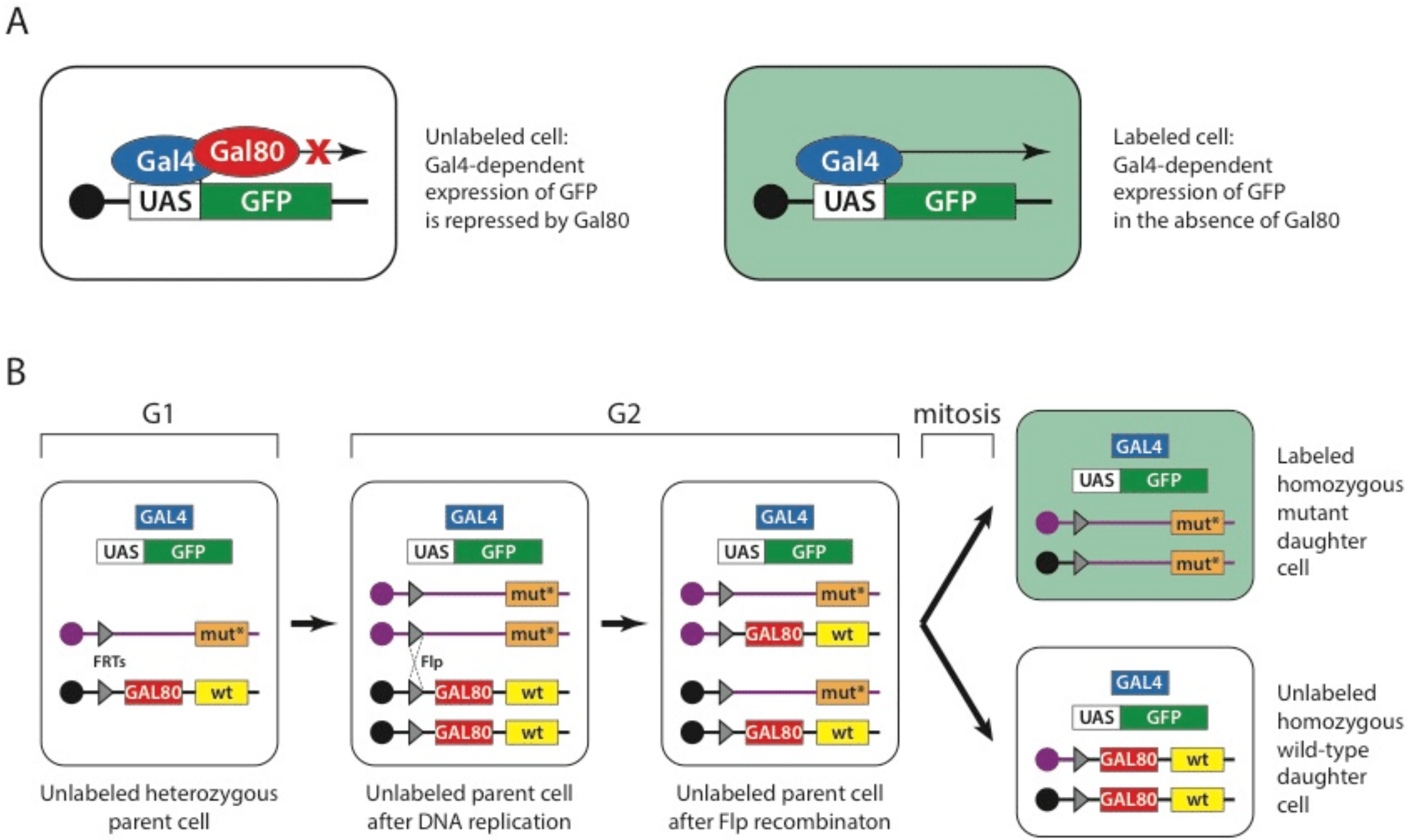


FIG. 7. A positive marking scheme for clonal analysis. In this figure, GAL80 prevents expression of mCD8-GFP in all cells heterozygous for the mutation of interest (*). Mitotic recombination is induced using a heat-shock inducible *FLP* recombinase. During mitosis FLP mediated recombination at the proximally located FRT sequences leads to the production of two daughter cells, each homozygous for the corresponding chromosomes. This results in the production of wing discs that are mosaic for the mutation under study (*). After mitotic recombination, cells that are homozygous for the mutation of interest (*) also now lack GAL80. Consequently, these cells have GAL4 activity and are marked by the presence of the mCD8-GFP, which is under the control of GAL4.


A
Gal4
Gal80
X
UAS
GFP
Unlabeled cell:
Gal4-dependent
expression of GFP
is repressed by Gal80
Gal4
UAS
GFP
Labeled cell:
Gal4-dependent
expression of GFP
in the absence of Gal80
B
G1
G2
mitosis
GAL4
UAS
GFP
mut*
FRTs
GAL80
wt
Flp
Unlabeled heterozygous
parent cell
Unlabeled parent cell
after DNA replication
Unlabeled parent cell
after Flp recombinaton
Labeled
homozygous
mutant
daughter
cell
Unlabeled
homozygous
wild-type
daughter
cell

Ey-Flp, directed mosaics

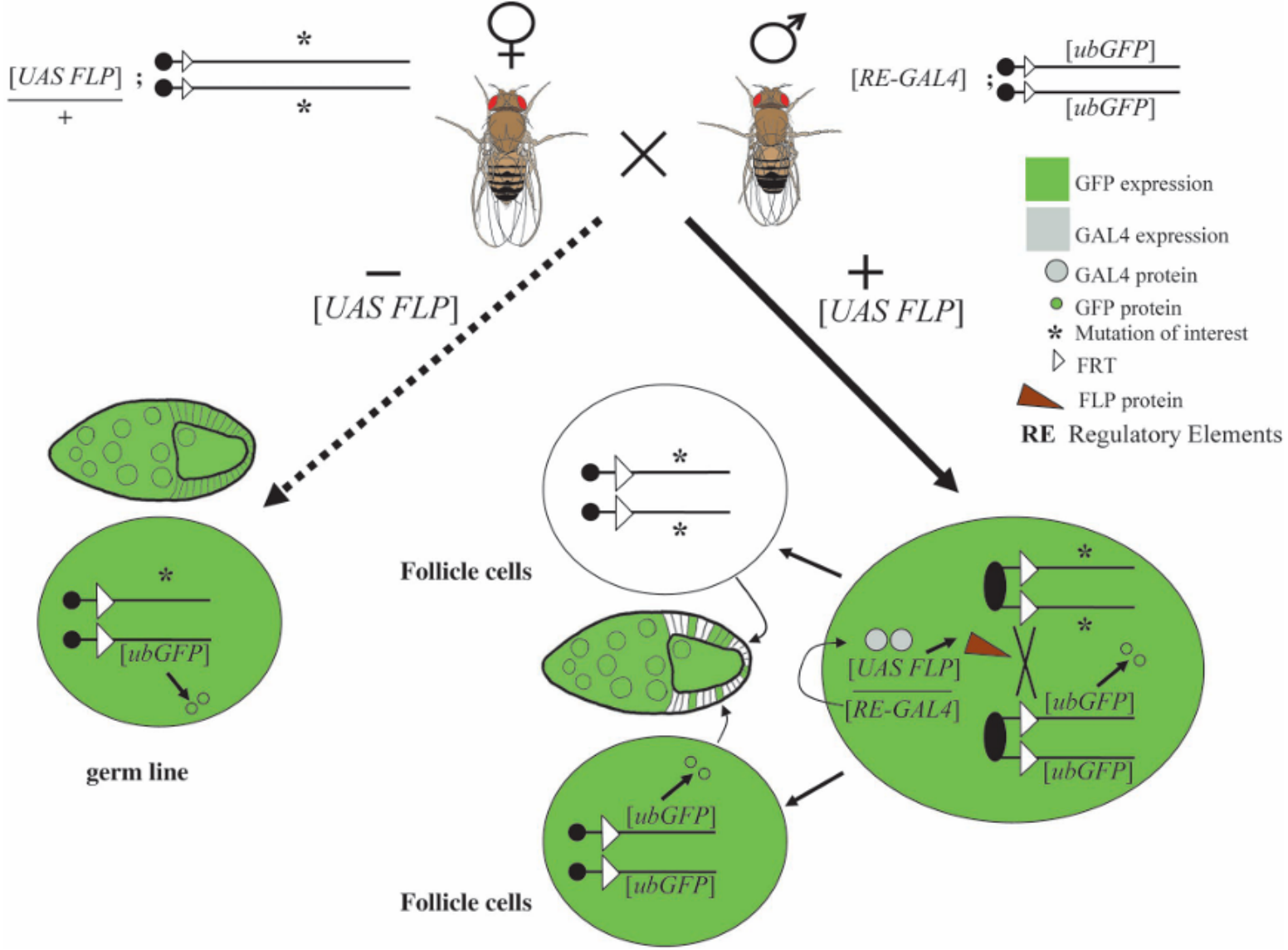


FIG. 8. Generation of directed mosaics. By constructing a *UAS-FLP* responder, mitotic recombination can be driven in a GAL4 dependent fashion. In this figure, GAL4 drives expression of FLP in the stem cells for the somatic follicle cells. Wild-type cells are marked by the presence of GFP, which is expressed under the control of the *ubiquitin* promoter. During mitosis, FLP-mediated recombination in the soma at the proximally located FRT sequences leads to the production of two daughter cells, each homozygous for the corresponding chromosomes. This results in the production of egg chambers that are mosaic within the follicle cell epithelium for the mutation under study (*). These cells, homozygous for this mutation, are then identified by the absence of the GFP marker. In contrast to the soma, FLP activity is absent from the germ line and is therefore composed solely of cells heterozygous for the GFP marker and the mutation under study.

Modifier screen in eyes

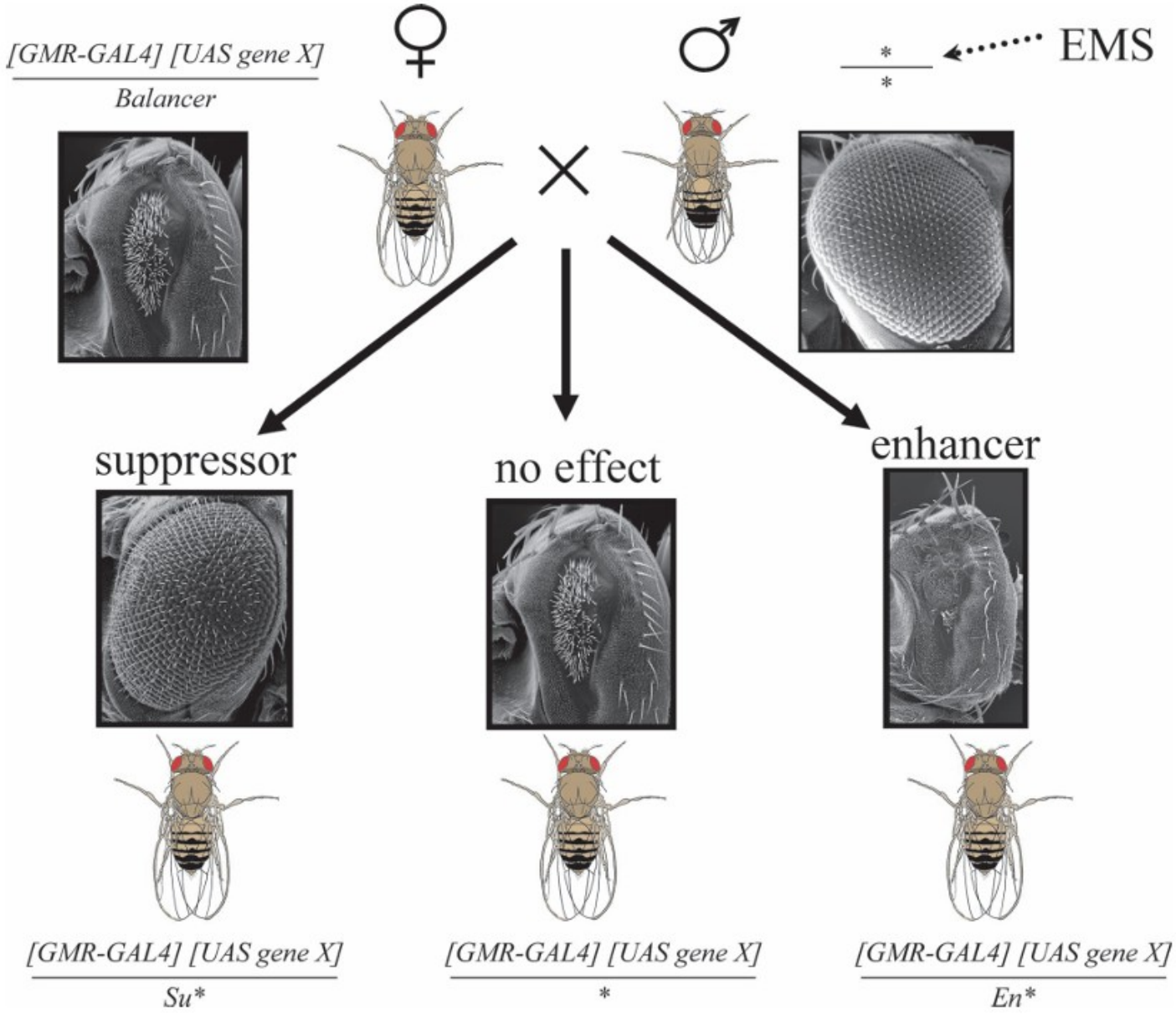


FIG. 10. Screening for modifiers with the GAL4/UAS system. Depicted is an example of screen for modifiers of *gene X* in which GMRGAL4 (*P{GAL4-ninaE.GMR}*) driven expression of *gene X* results in a rough eye phenotype. A recombinant line containing both the GMRGAL4 driver and the UAS *gene X* responder is then crossed to a wild-type strain, which has been mutagenized. The F1 progeny are then screened for modifiers that suppress or enhance the corresponding rough eye phenotype.