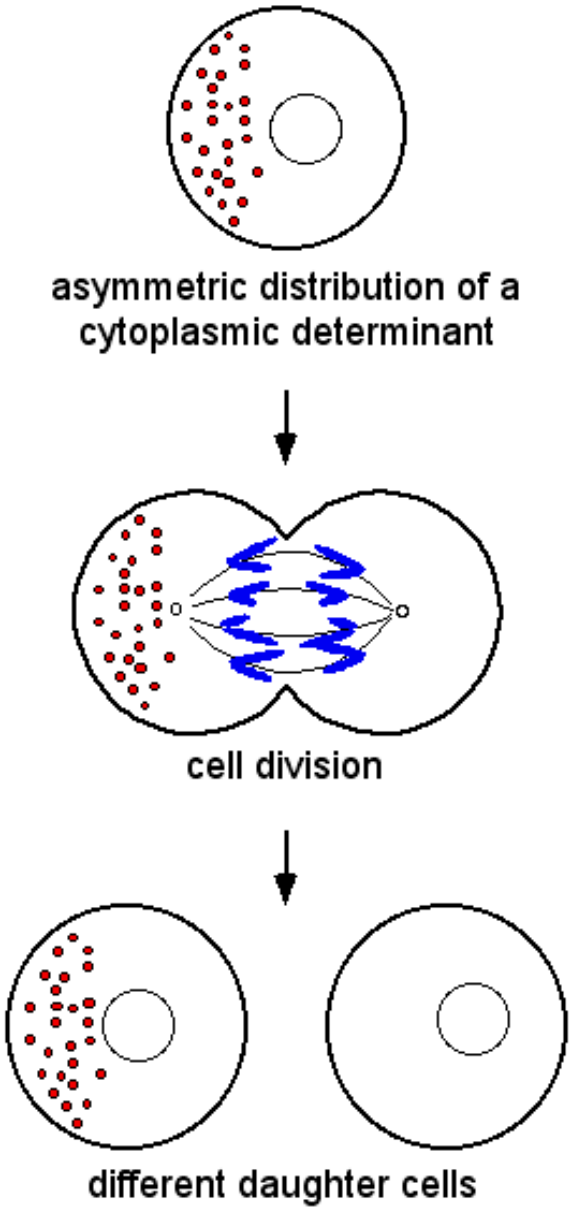
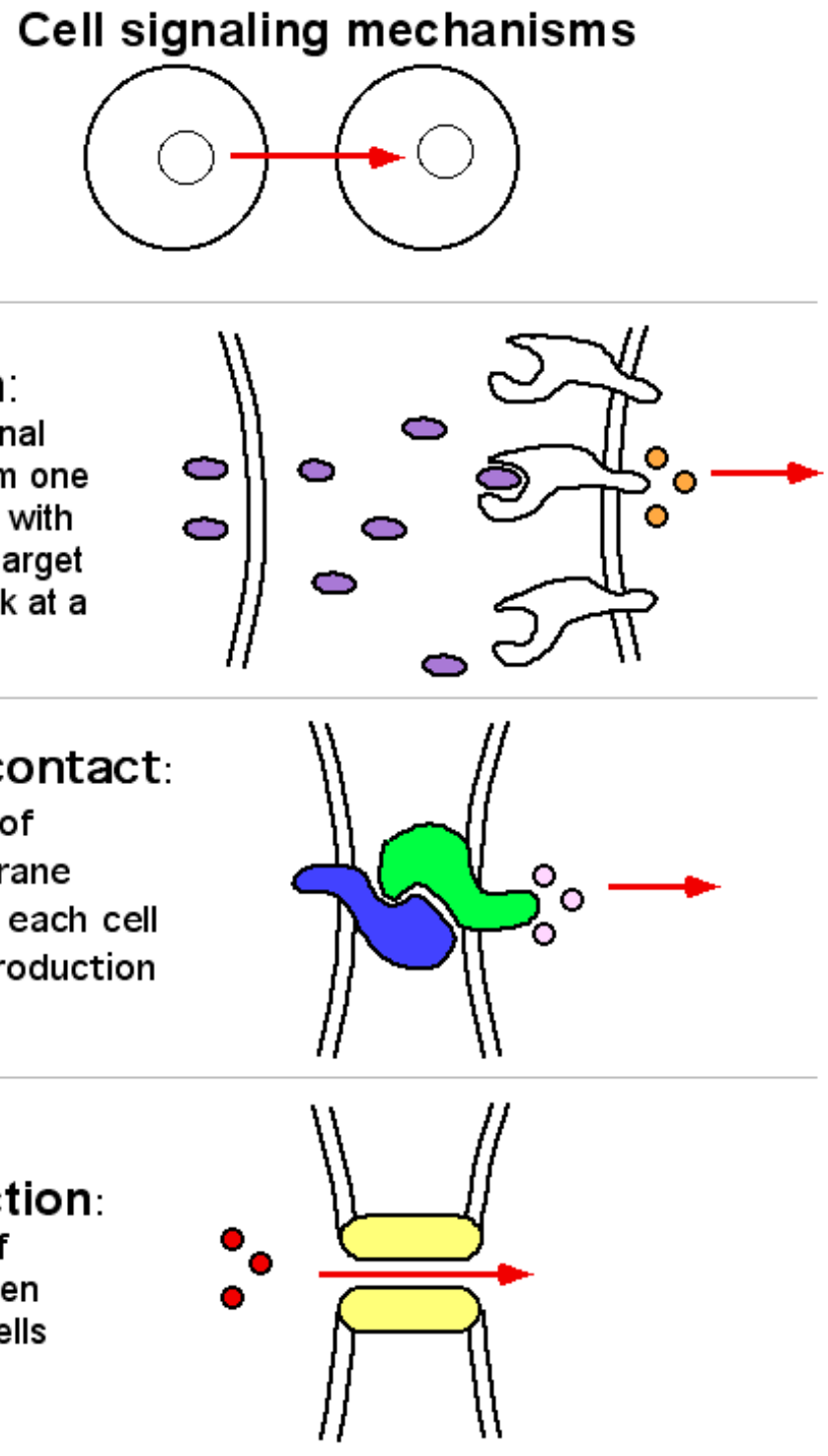
# Cell autonomous



# Cell autonomous (receptor) and cell non-autonomous (ligand)



# Mosaic Analysis

**Generating animals that consist of populations of cells of at least two different genotypes.**

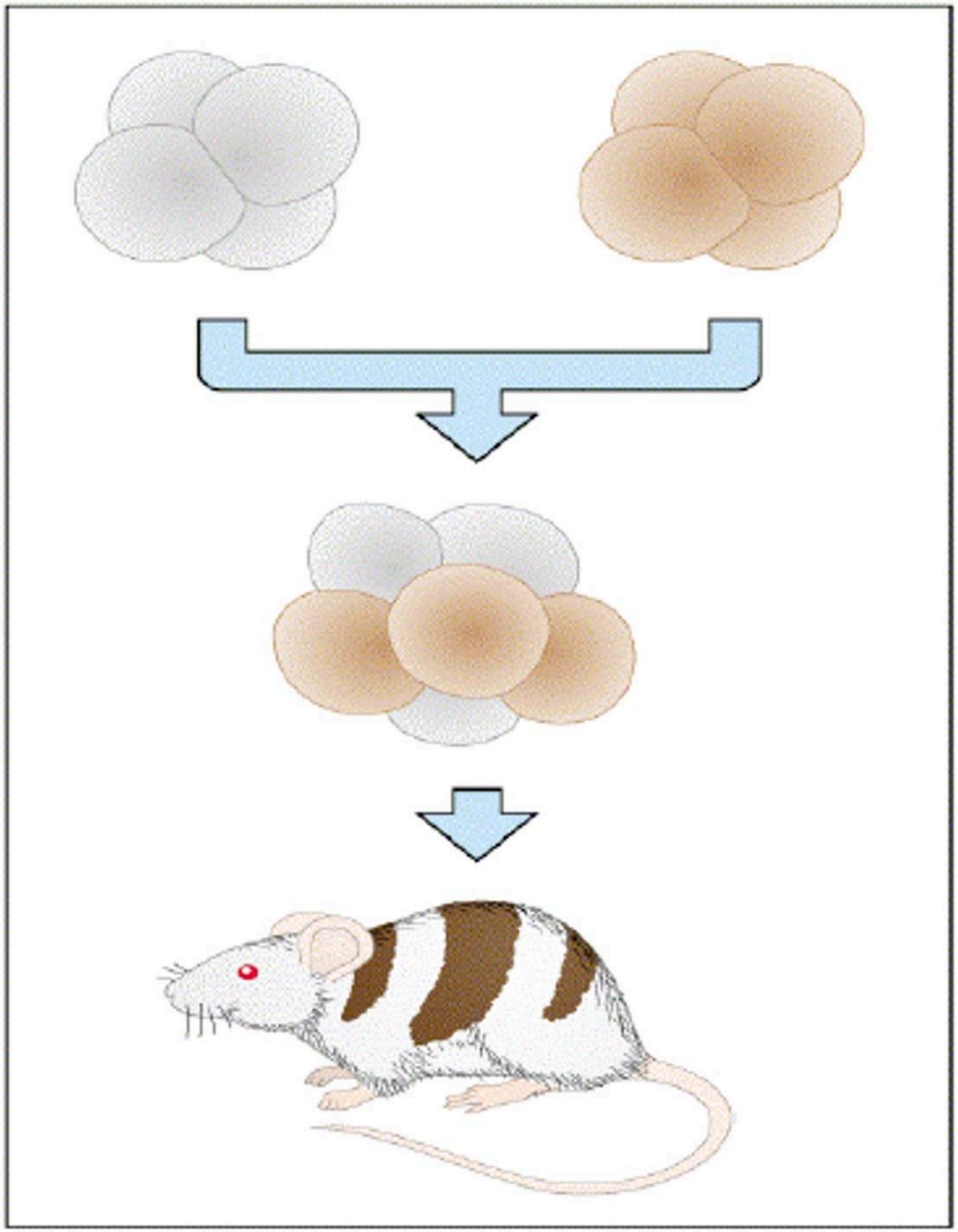
**Determine the function of a gene in specific tissues, even if homozygotes would be dead by this time in development.**

**Cell autonomous versus non-cell autonomous**

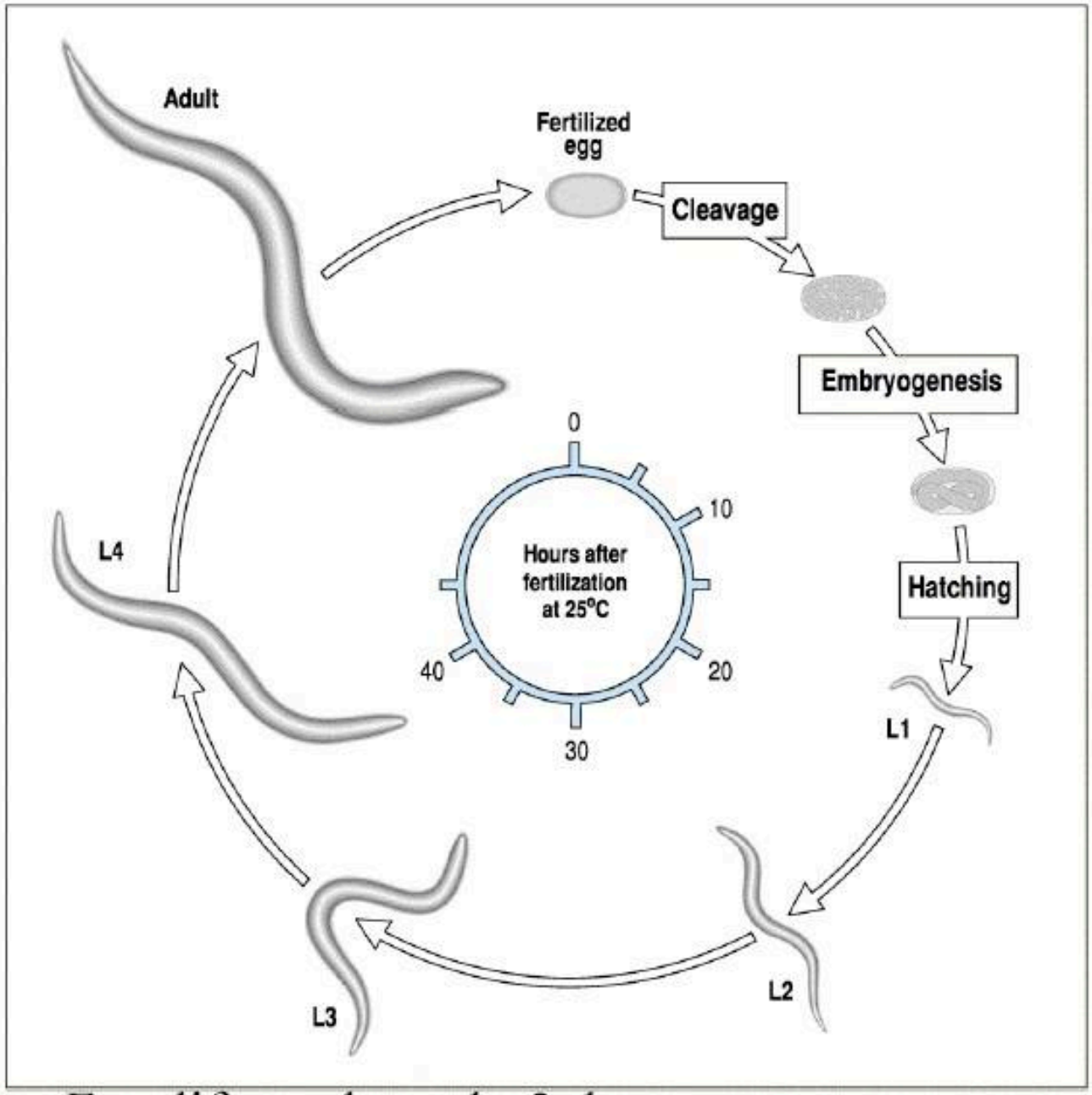
**Determine which cells or tissues your gene is required in.**

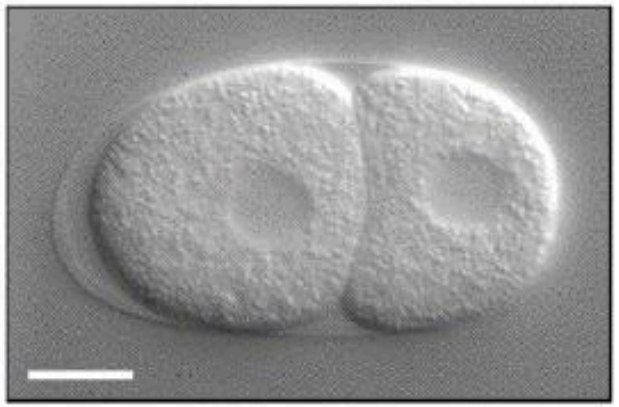
# Fusion of mouse embryos gives rise to a chimera/mosaic

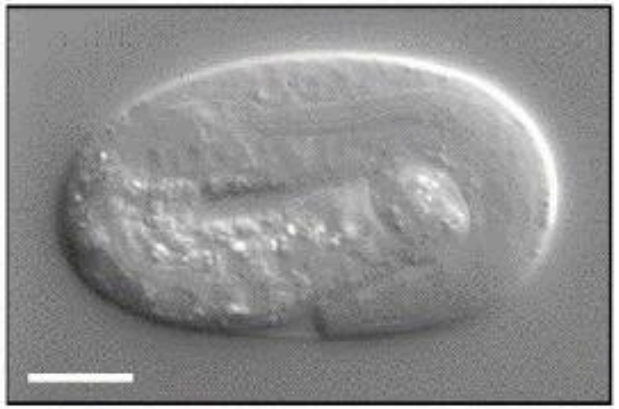
**If cells from the brown mouse are homozygous mutant and mixed with cells from the wildtype white mouse, a chimeric mouse, consisting of cells from both embryos will develop. Tissues that never develop from brown mouse cells (or that develop abnormally) are likely to require the product of the knocked-out gene.**

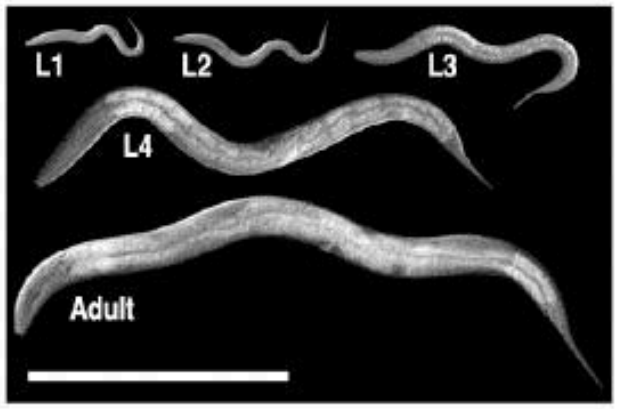


Don't confuse chimera with hybrid








Fast life cycle: only 3 days

# Nobel Lecture

## *C. elegans*: The Cell Lineage and Beyond

John E. Sulston held his Nobel Lecture December 8, 2002, at Sal Adam, Berzeliuslaboratoriet, Karolinska Institutet. He was presented by Professor Jan-Åke Gustafsson.

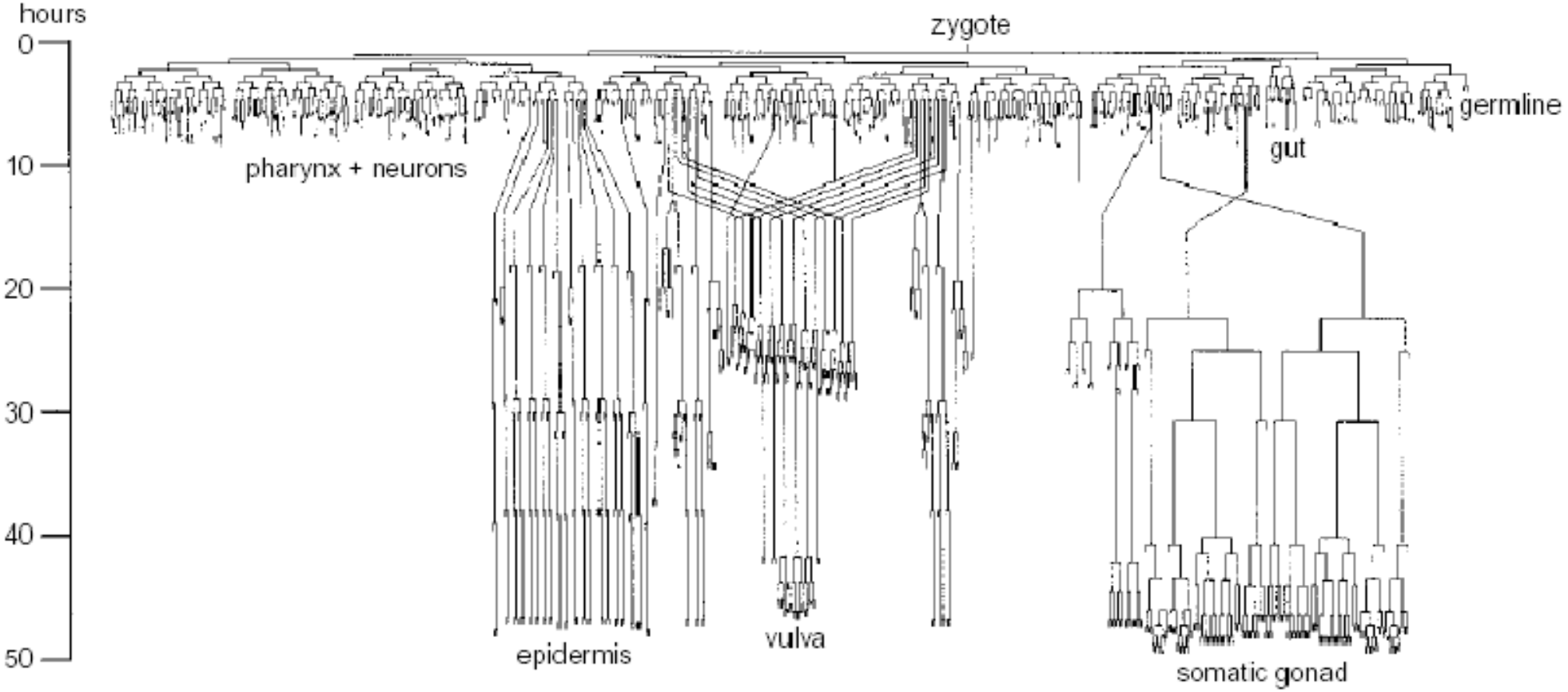
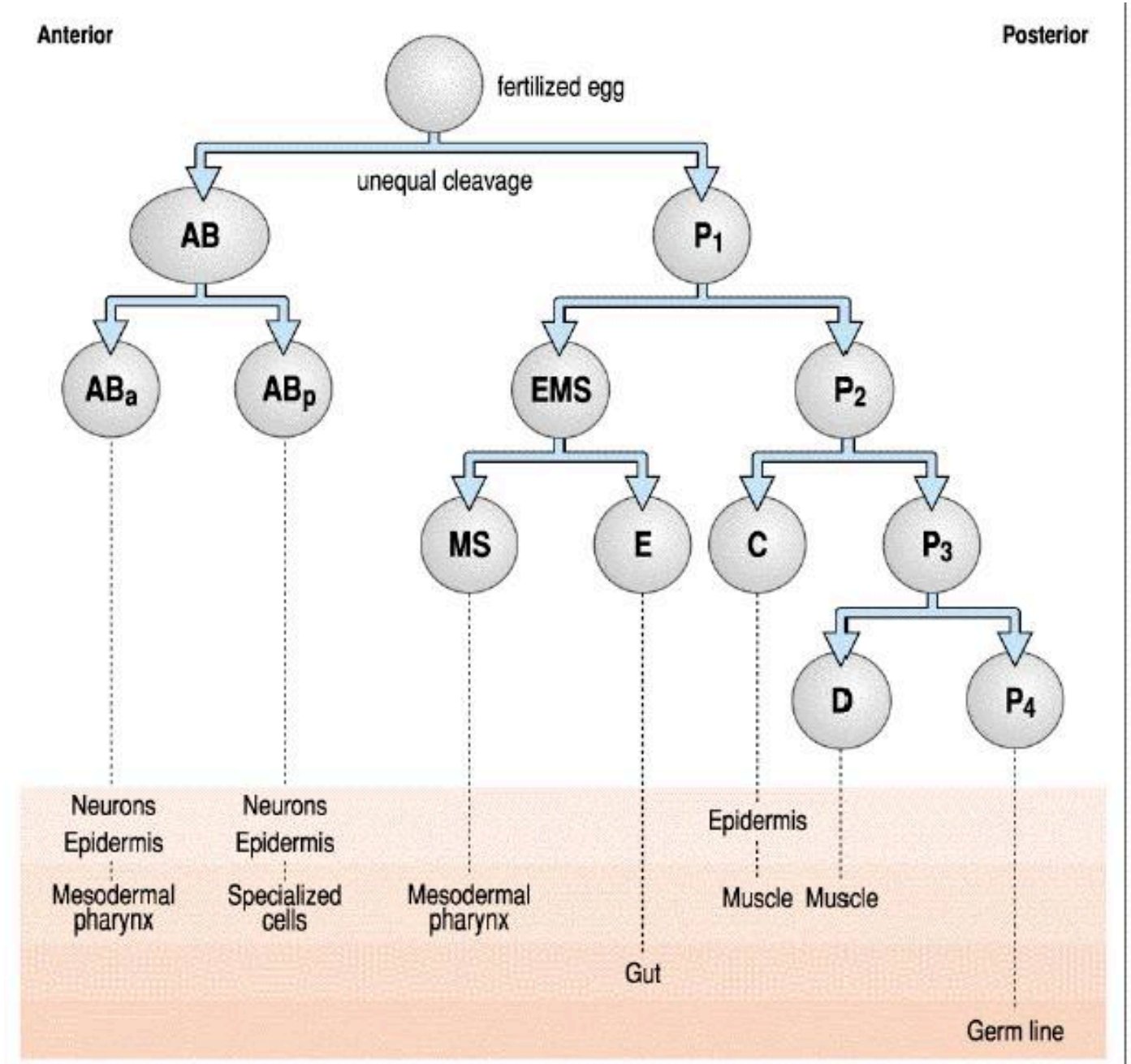
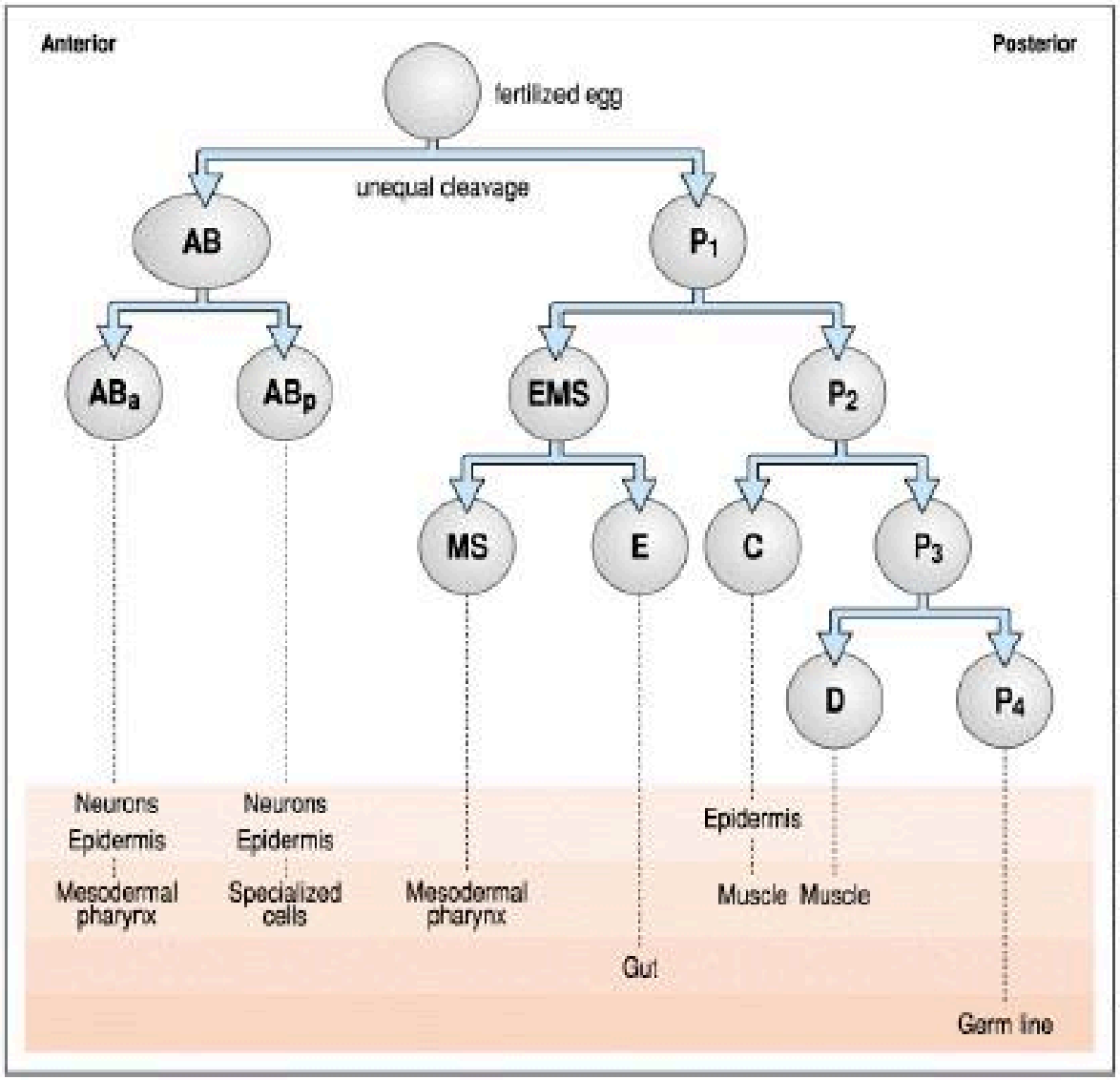



**Figure 2** The *Caenorhabditis elegans* cell lineage. Time axis is vertical; each cell division is a horizontal line. The origin of some cell types is indicated.




*C. elegans* lineage analysis is the ultimate fate map. The fate of each individual cell is known.

Mosaic animals in *C. elegans* can be isolated by random losses of extrachromosomal arrays.




Where is my gene required?

Inject a wt copy of gene and a cell marker (i.e. GFP) into a mutant animal.

This should rescue the phenotype.

We know the cell lineage of *C. elegans*.

Look for losses of the array in certain lineages and ask whether you see the phenotype.

| Genotype | Phenotype |
| --- | --- |
| ***unc-x (0)/unc-x(0)*** | Uncoordinated |
| ***unc-x (0)/unc-x (0); Ex unc-x (+) pGFP*** | Wild type movement<br>+ Green Fluorescence |

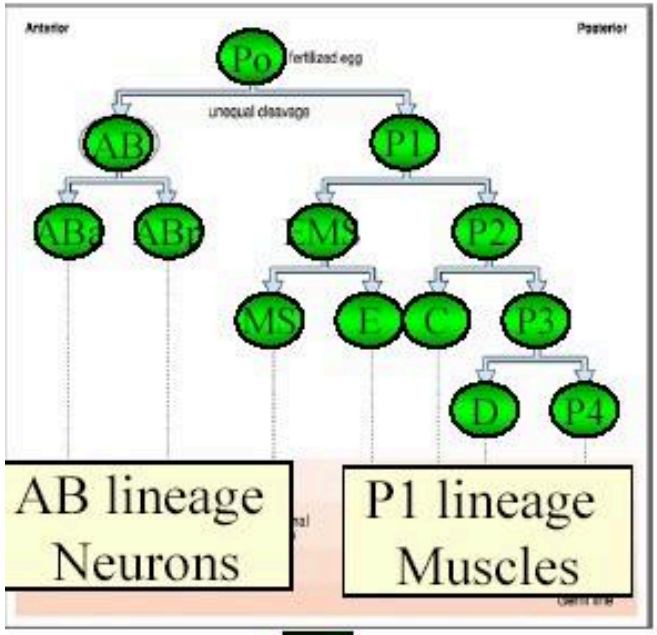
Transgenic animal with wild type copy of *unc-x* plus a cell marker *pGFP (*green fluorescent protein) on an extrachromosomal array

**Ex unc-x (+) is an extrachromosomal array containing a wildtype copy of the unc gene, and GFP controlled by a ubiquitous promoter**

Transgenic animal genotype: *unc-x (0); Ex unc-x (+) pGFP*

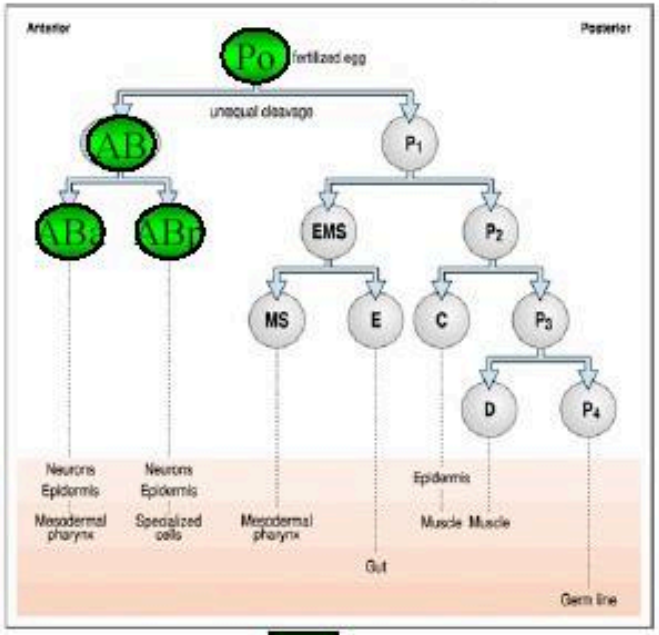
green (GFP) cells indicate the extrachromosomal *(Ex)* array is present

## No loss of array




Wild-type

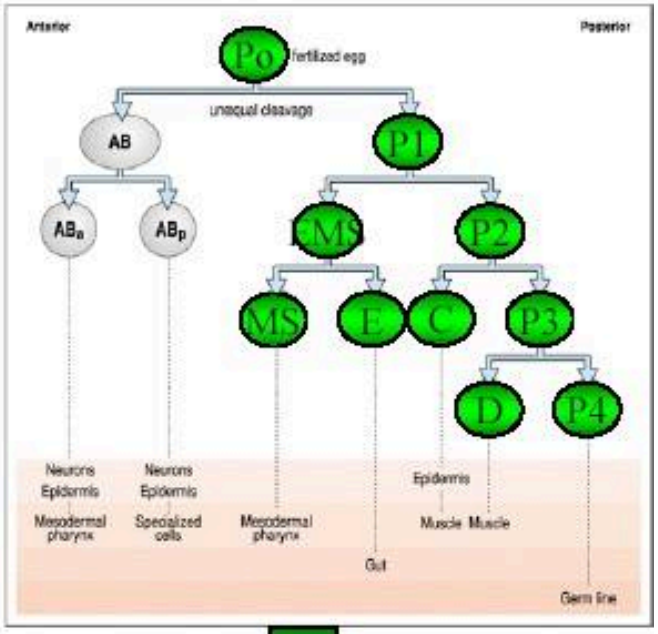
## Loss in P1 lineage




?

Uncoordinated

## Loss in AB lineage

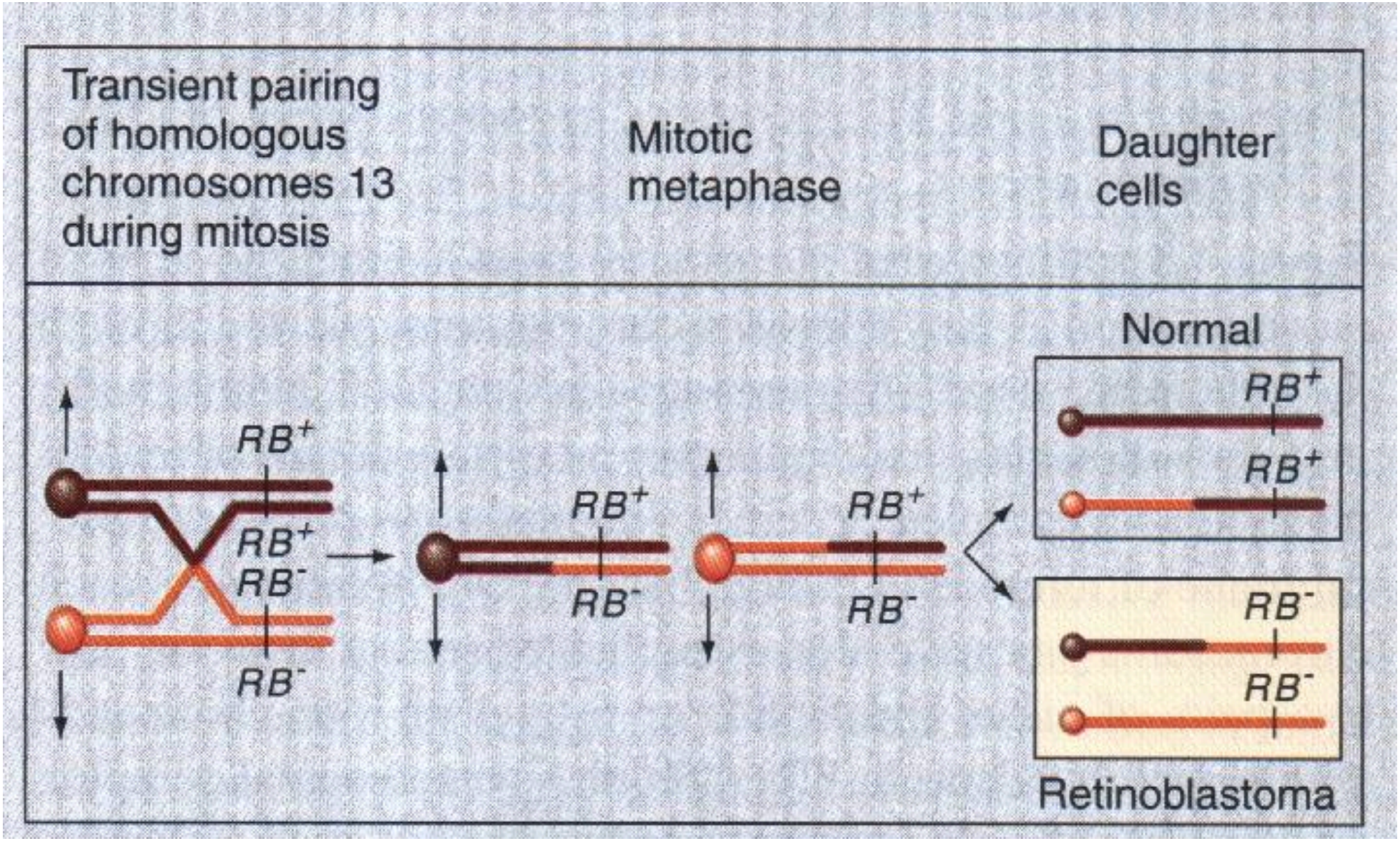



?

Wild-type

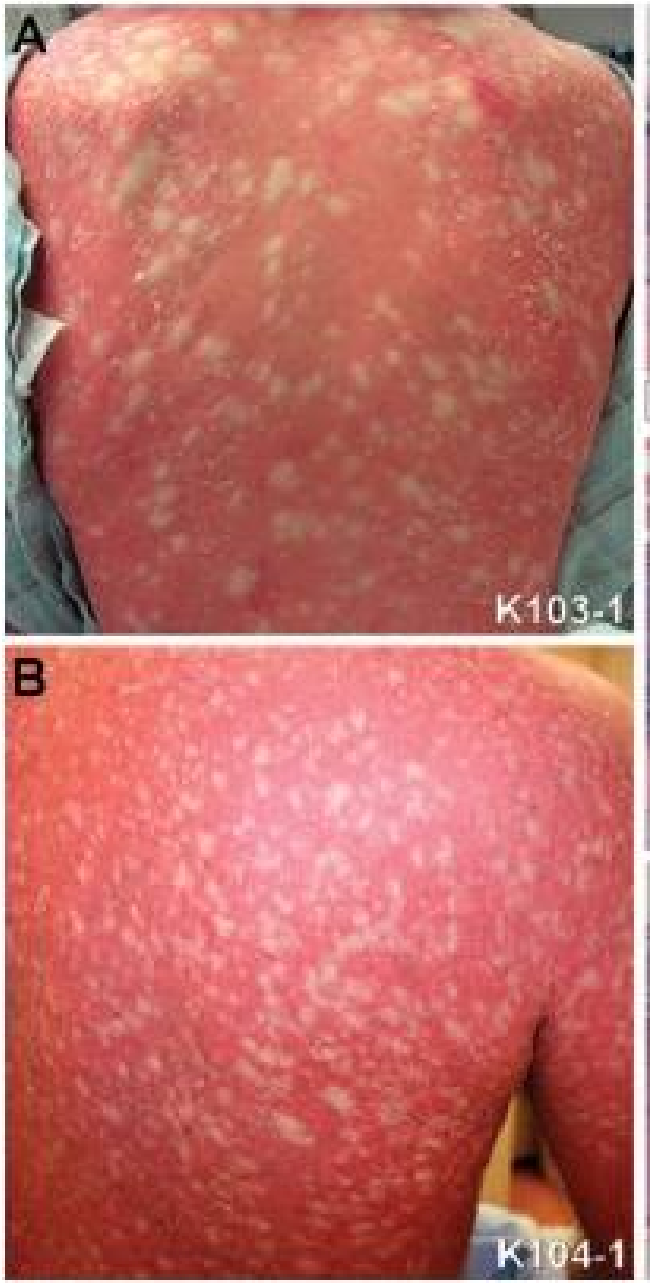
In this example we conclude that the *unc-x* gene is required in muscle tissues and not in neurons

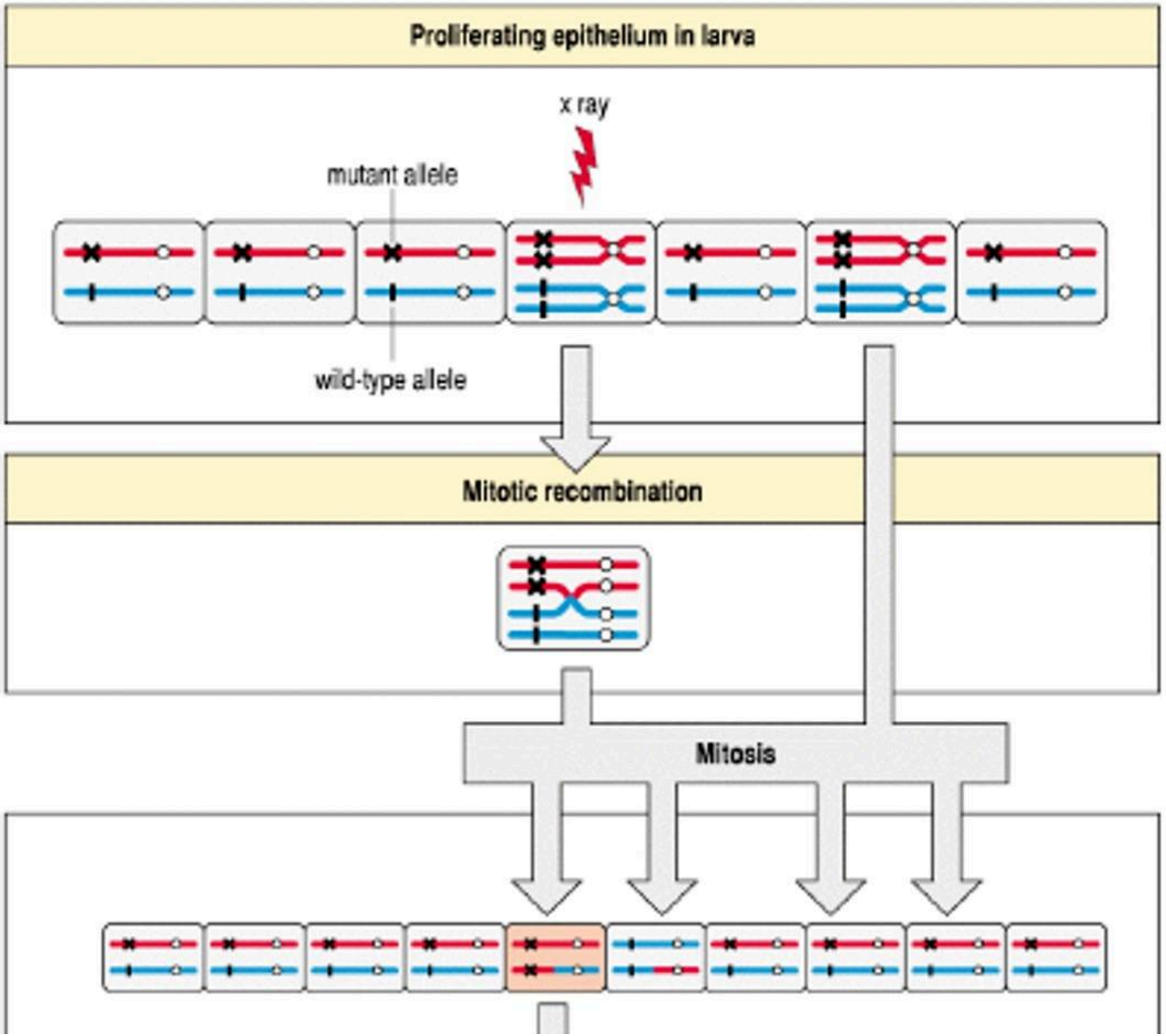
# Loss of heterozygosity for a tumor suppressor through mitotic recombination

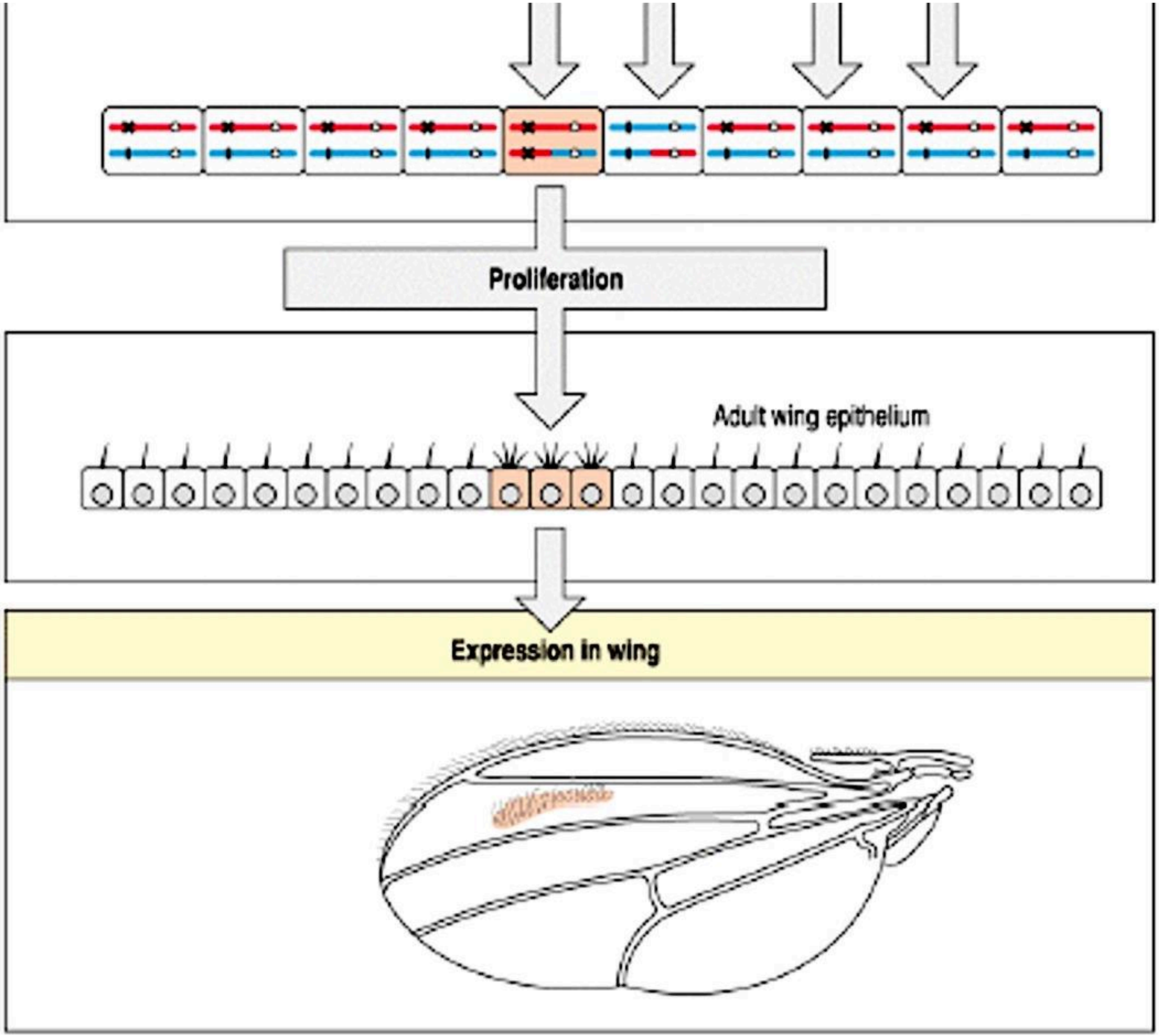


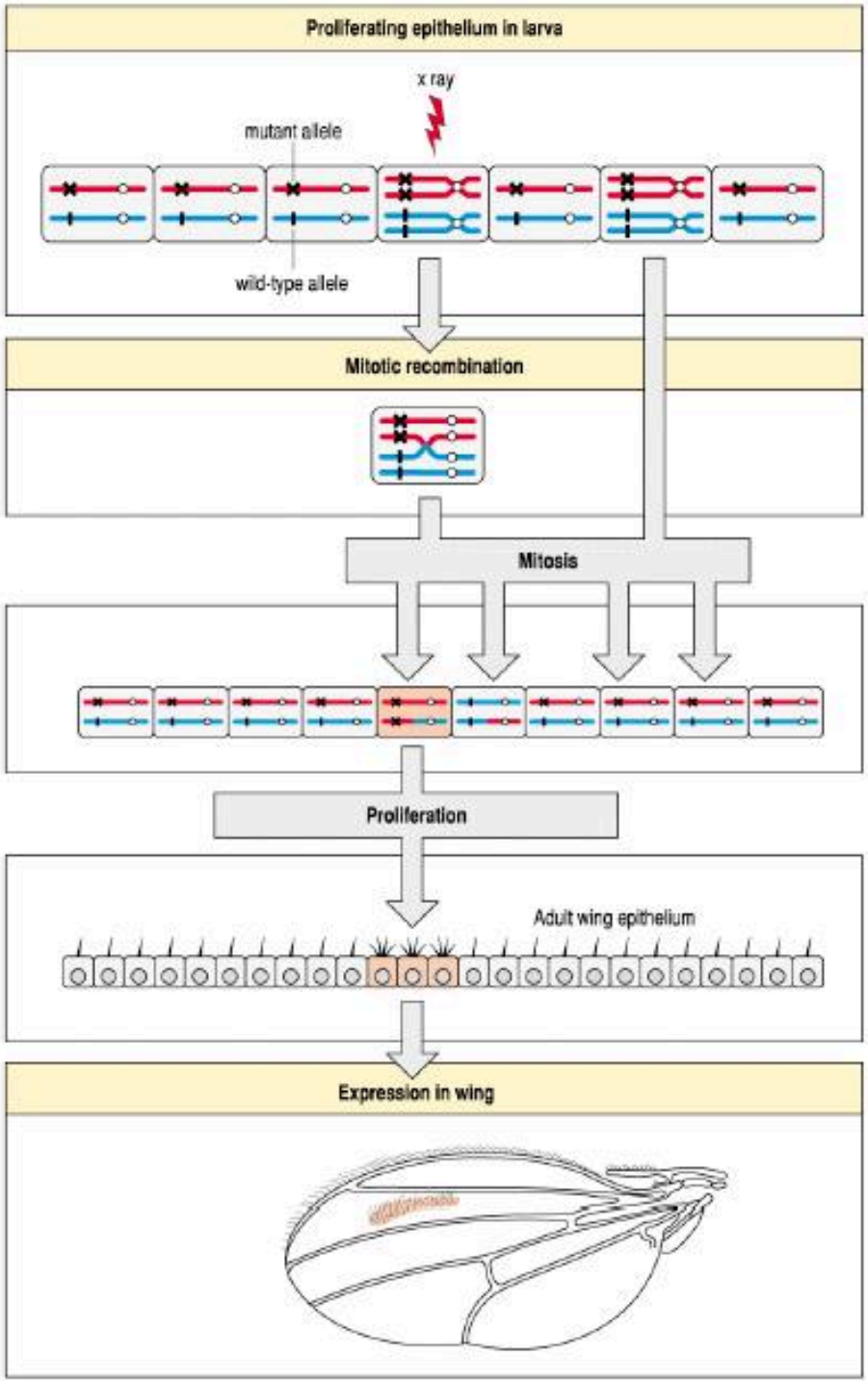
# A dominant condition reverting back to wildtype through mitotic recombination and loss of heterozygosity

**Fig. 1.** Frequent revertants in ichthyosis with confetti. (**A** and **B**) The backs of an 18-year-old female subject (103-1) and 42-year-old male (104-1) show background redness and scaling with hundreds of white, normal-appearing "confetti" spots. (**C**) Histology of normal human skin showing basal layer (labeled B), stratum spinosum (S), granular layer (G), and stratum corneum (SC). (**D**) Affected skin shows loss of differentiation of all layers above the basal layer and hypercellularity with increased epidermal thickness. There is no granular layer, and




Proliferating epithelium in larva
x ray
mutant allele
wild-type allele
Mitotic recombination
Mitosis


Proliferation
Adult wing epithelium
Expression in wing




# Mosaic analysis in *Drosophila*

Heterozygous animal

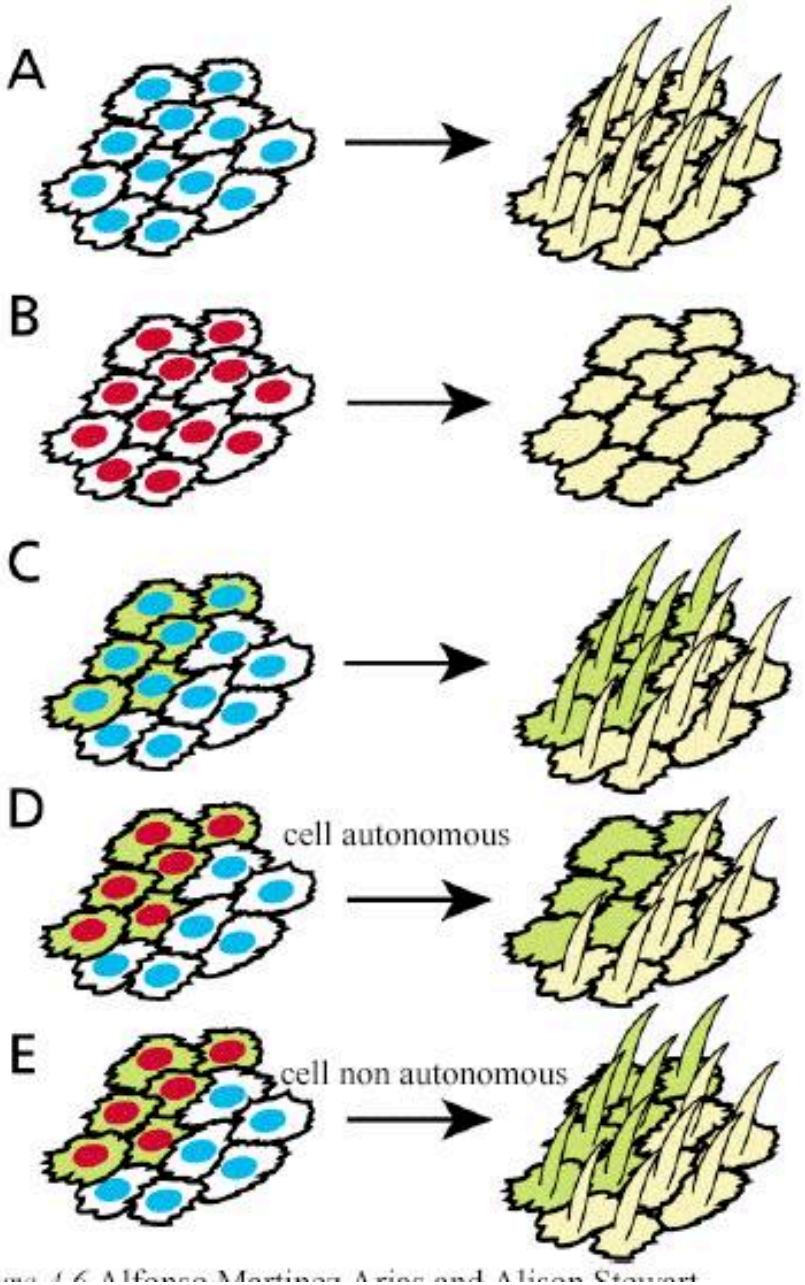
Induce mitotic recombination by X-rays

Formation of a homozygous mutant clone.

This will give rise to a population of "mutant" cells surrounded by wt cells.

A key to this experiment is having a cell marker to distinguish between mutant clones and wt clones.

# Cell autonomous vs. non cell autonomous




wt (blue nuclei) cells give rise to bristles

null mutation in gene "x" (red nuclei) leads to no bristles, therefore gene x is required for the production of bristles.

Create mosaic animals that have both wt and mutant cells (use a marker such as GFP (green cytoplasm) to distinguish between mutant and wt cells.

It is important that the marker does not interfere with normal development (panel c). If all mutant cells show phenotype then the gene acts cell autonomous. If the mutant cells show a "wt" phenotype then the gene can act non cell autonomous.

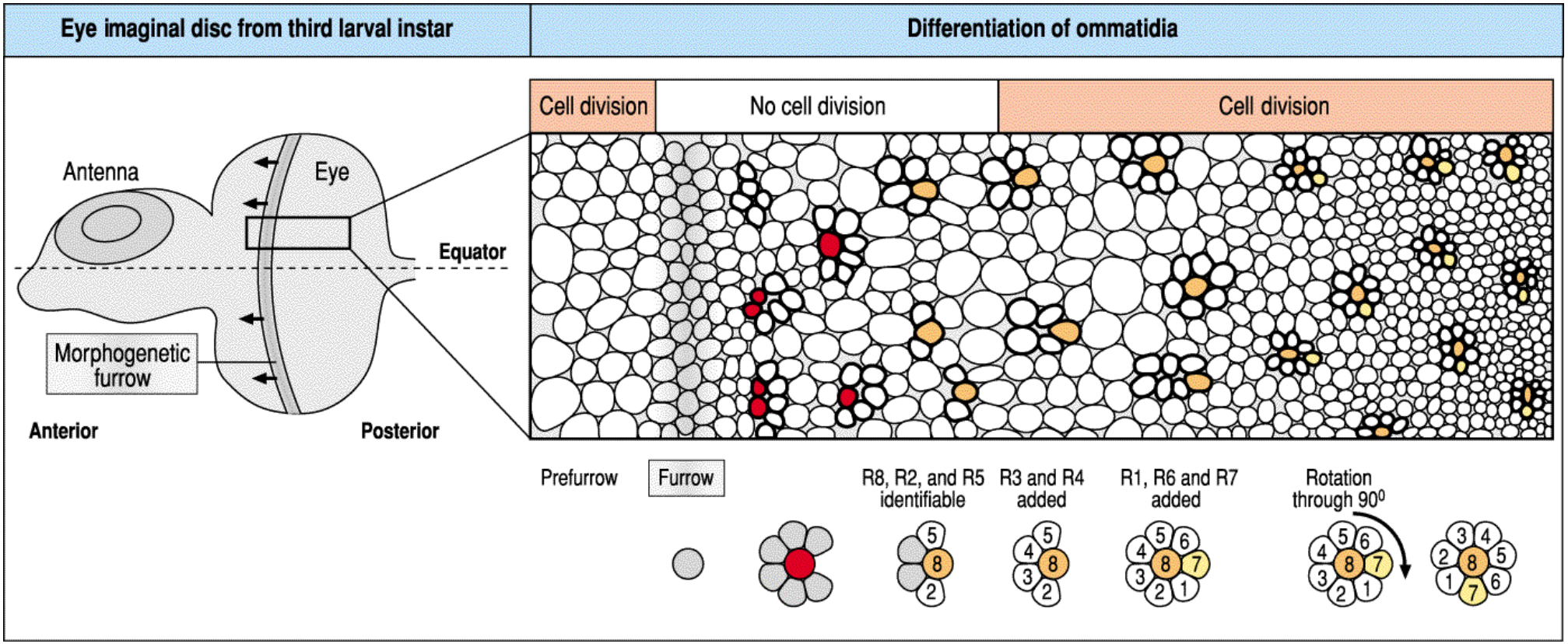
What can autonomy vs. cell autonomy tell you about your gene product?

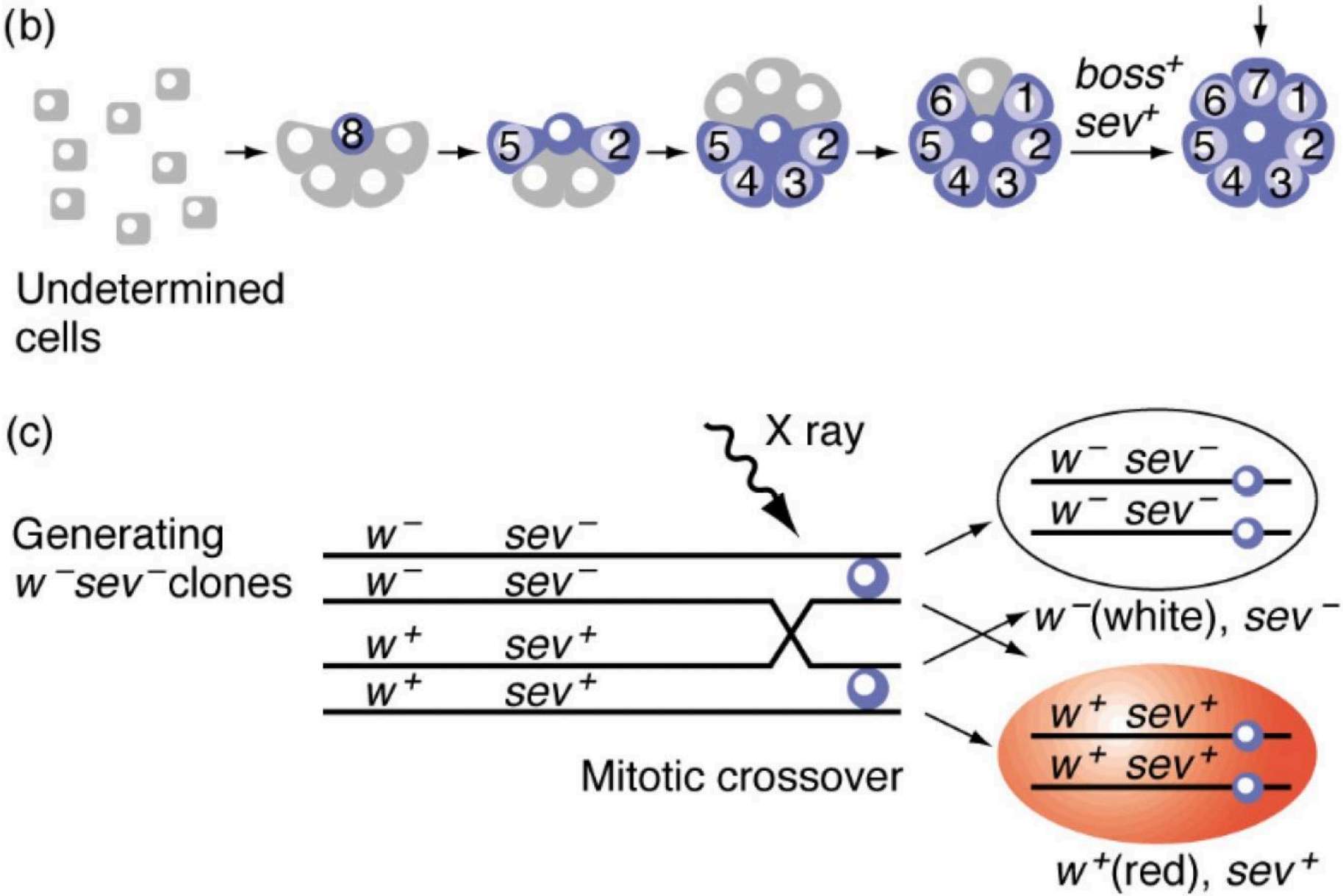
If the **genotype reflects phenotype** in a genetic mosaic experiment then we say the gene function is **cell autonomous**. Think of the gene as encoding a receptor on the cell surface. For example, it needs to be present on the cell to receive a signal to cause cell differentiation.

If **phenotype does not necessarily correspond to its genotype** this indicates that the gene function is **cell non-autonomous**. Think of the gene encoding a signaling molecule that can act on other cells. Thus wild type cells can produce the signal and "rescue" its phenotype.

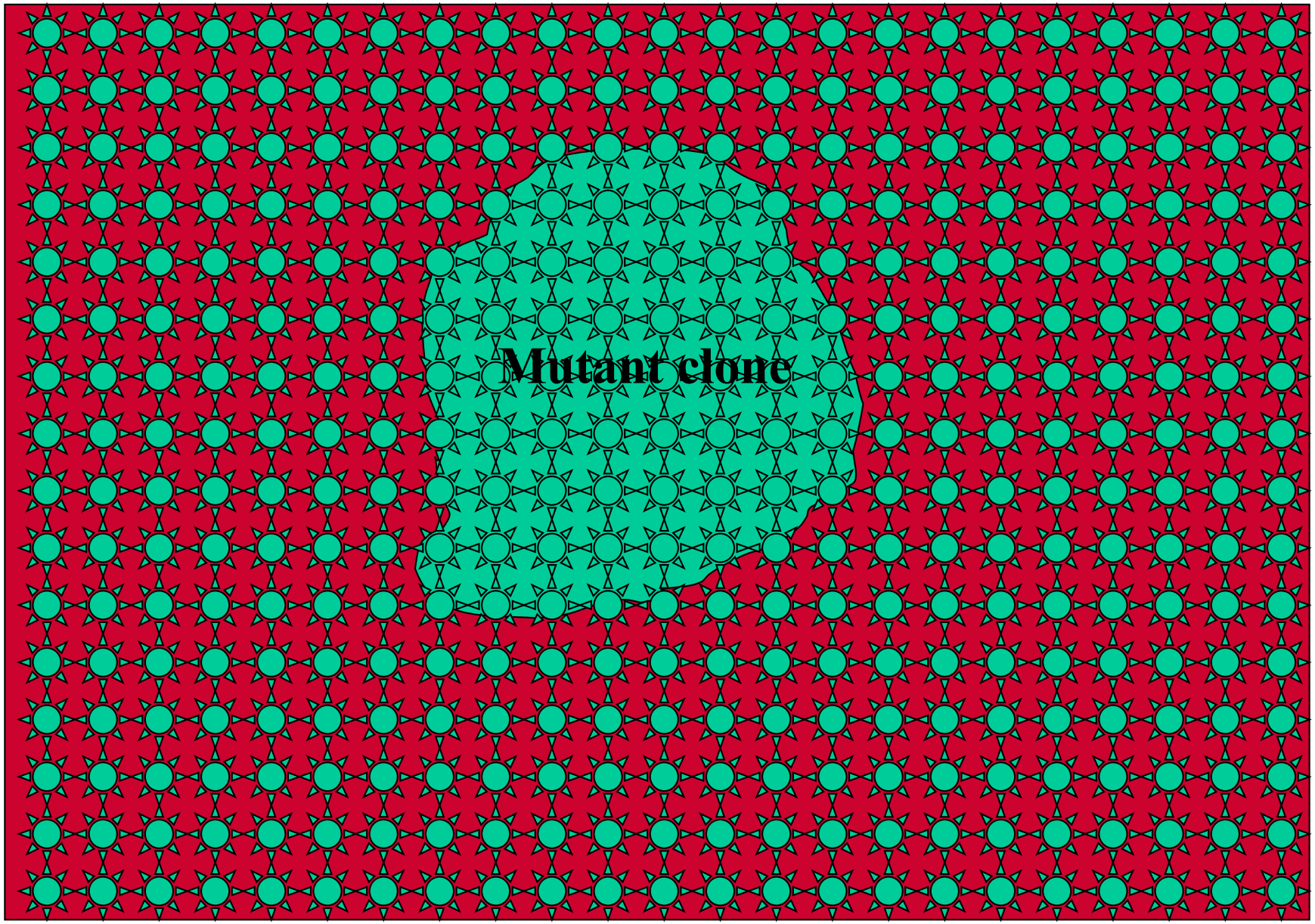
**Insulin as an example. It is produced by cells in the pancreas but acts non-autonomously throughout your body to regulate glucose uptake. Thus, loss of the insulin gene can be rescued by providing the protein through other means - like a needle.**

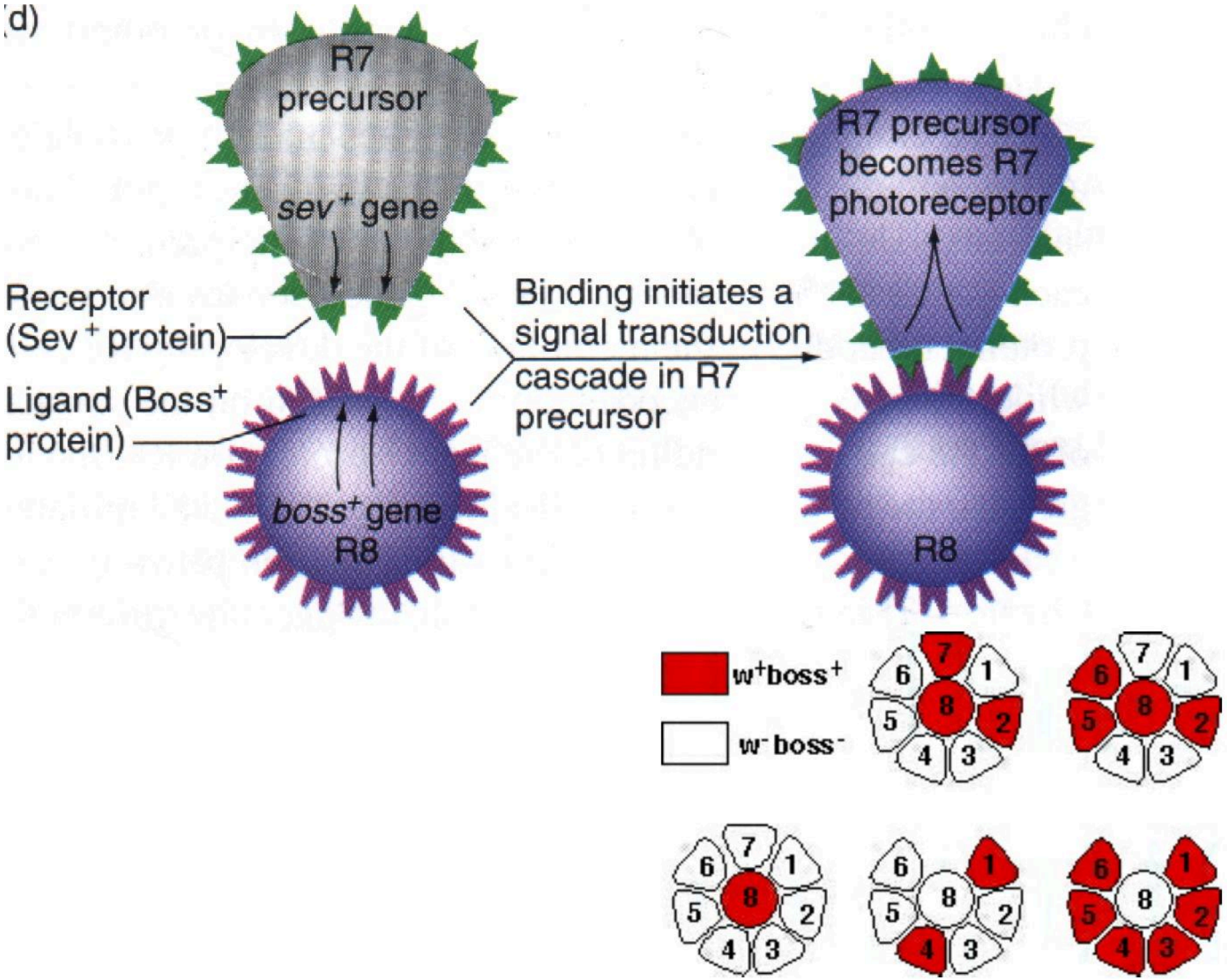
# Drosophila eye development



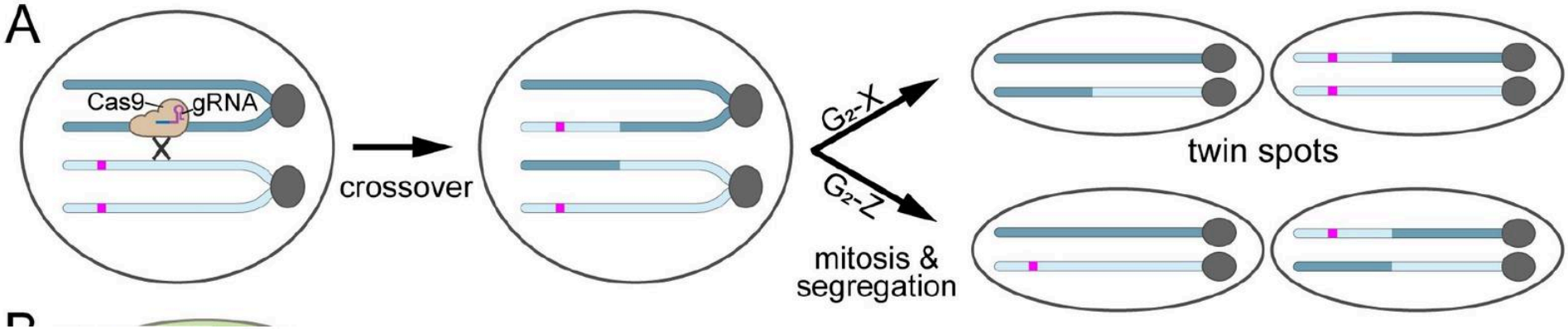

(b)
8
5
2
5
4
3
2
6
1
5
4
3
2
boss+
sev+
6
7
1
5
4
3
2
Undetermined cells
(c)
X ray
Generating w⁻sev⁻clones
w⁻
sev⁻
w⁻
sev⁻
w+
sev+
w+
sev+
Mitotic crossover
w⁻ sev⁻
w⁻ sev⁻
w⁻(white), sev⁻
w+ sev+
w+ sev+
w+(red), sev+
Phenotypes of mosaic facets
w+ sev+
w⁻ sev⁻

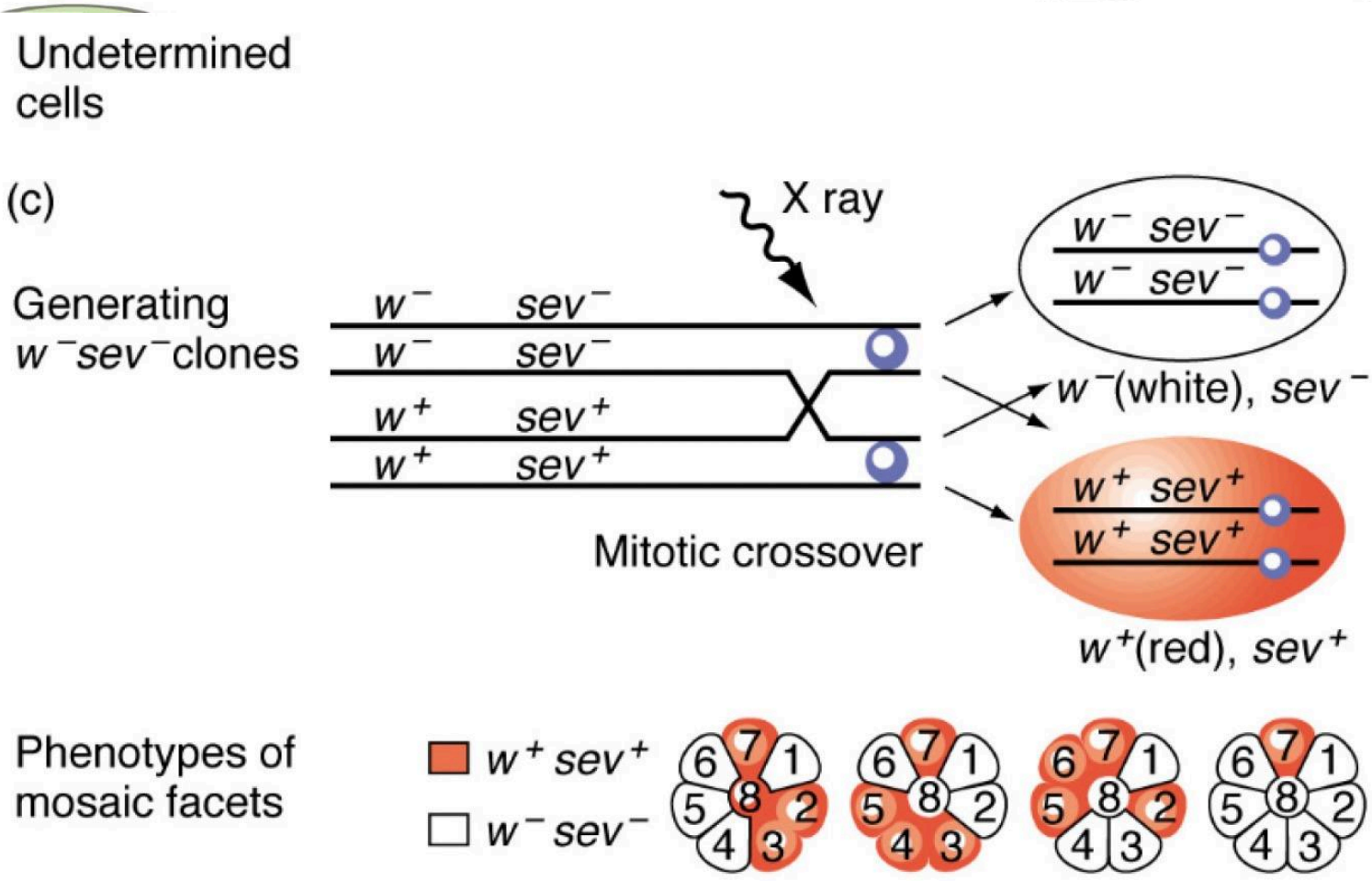
**Analyze ommatidia at the periphery of mutant clone. These will be mosaic, containing both wild-type (red) and mutant (white) cells.**



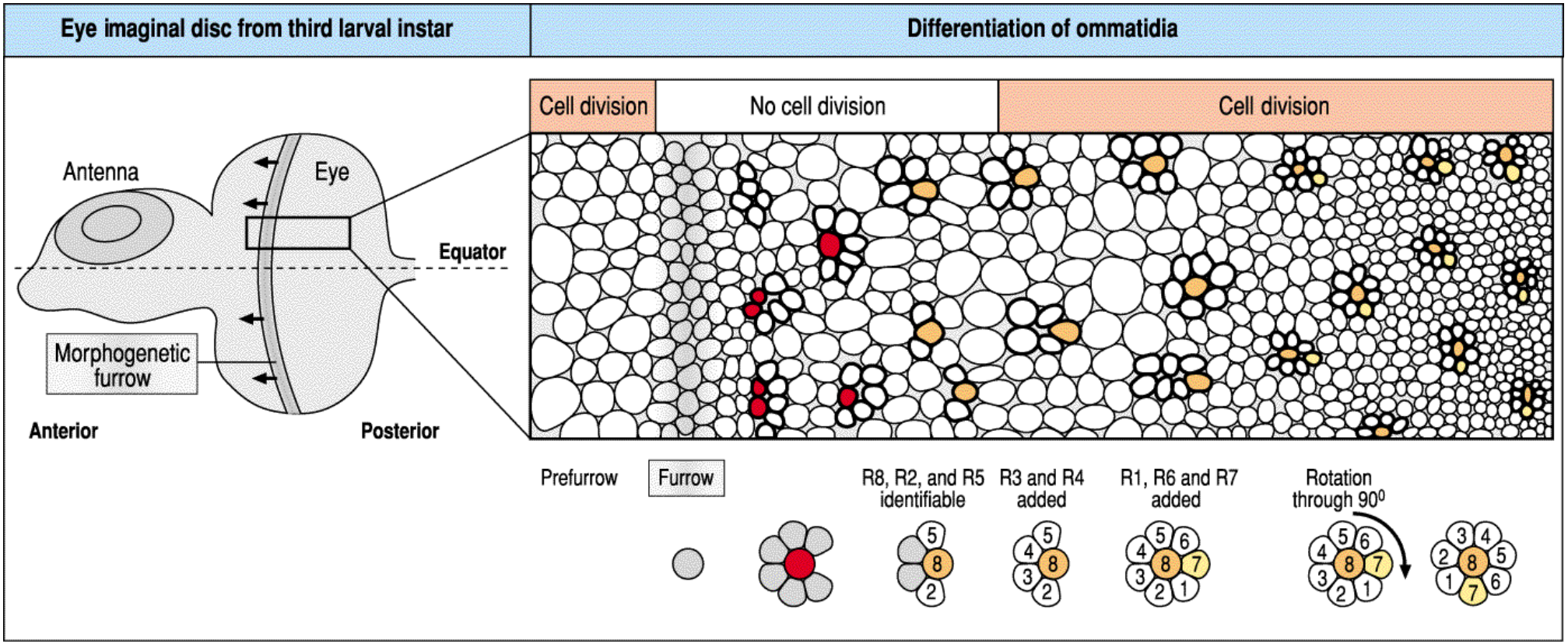

(d)
R7 precursor
sev+ gene
Receptor (Sev+ protein)
Ligand (Boss+ protein)
boss+ gene
R8
Binding initiates a signal transduction cascade in R7 precursor
R7 precursor becomes R7 photoreceptor
R8
w+boss+
w- boss-
1
2
3
4
5
6
7
8

# Targeting the location of DNA breaks for recombination



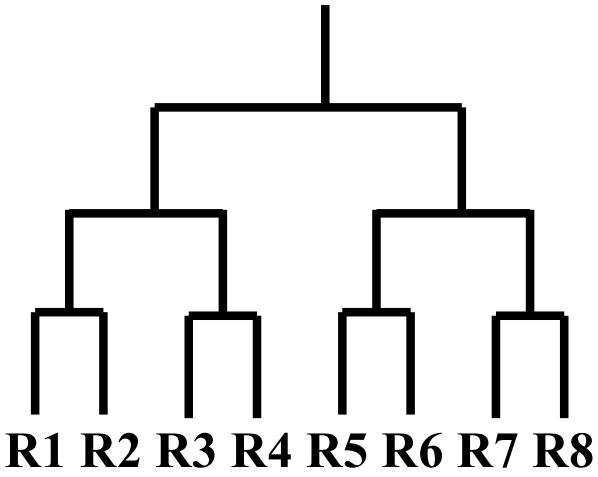



# Drosophila eye development

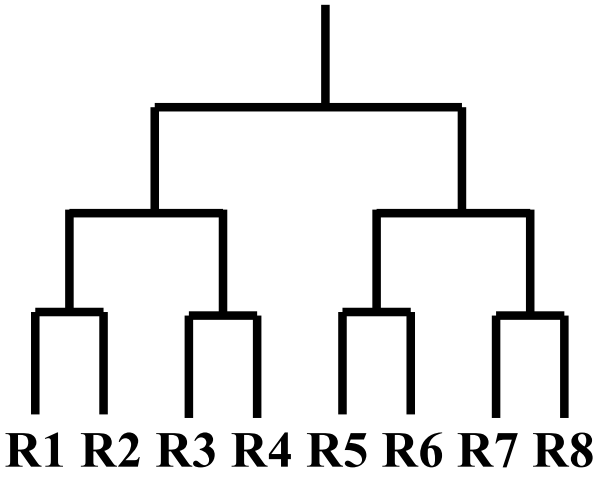


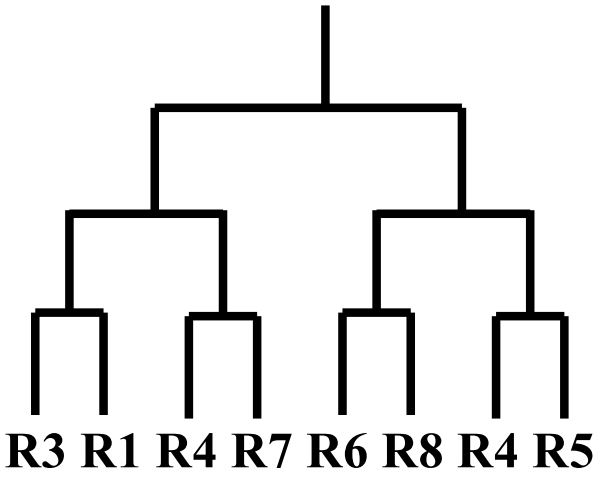
# How do cells in the fly eye develop: an invariant lineage, or no lineage restrictions?

**Invariant lineage?**
**Like *C. elegans***




**No lineage restrictions?**

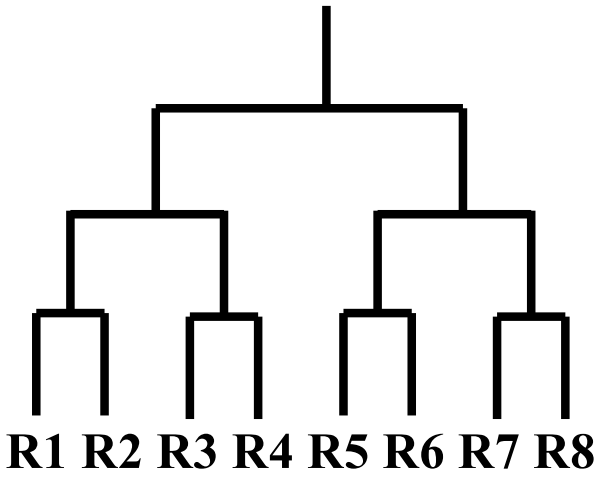


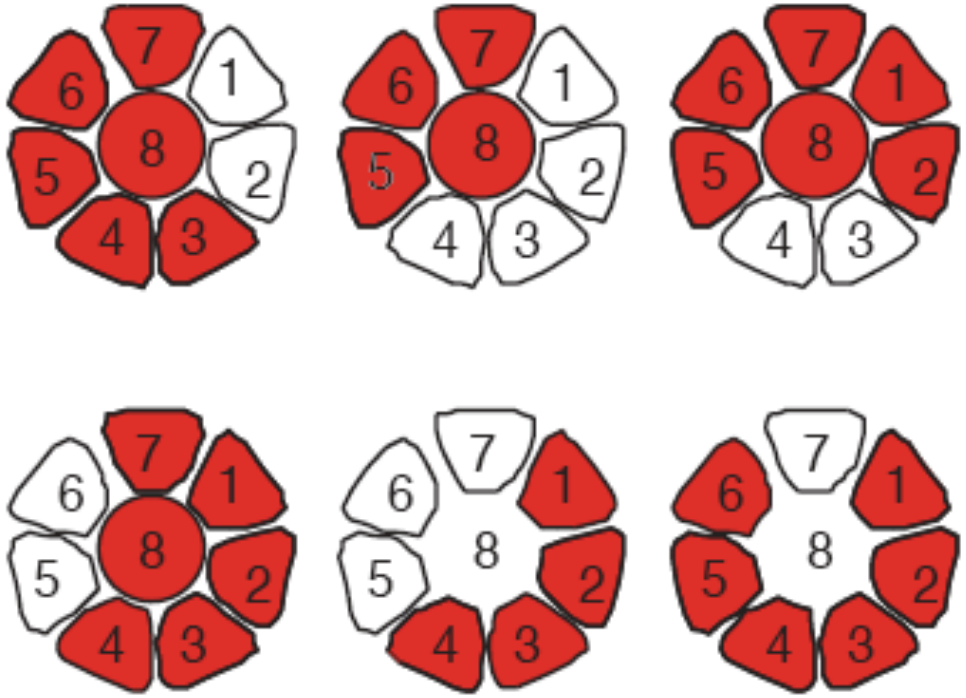




**And all other combinations!**

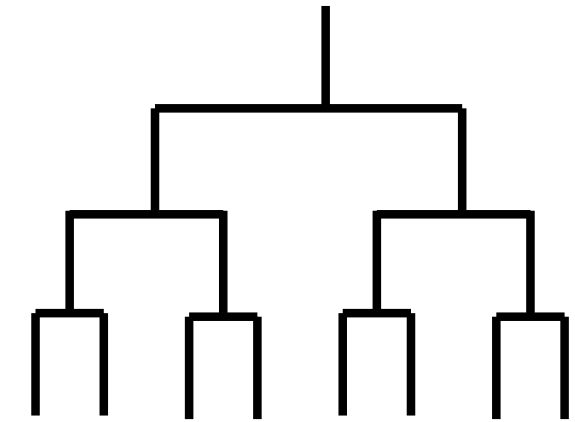
# Irradiate *white*/+ animals and study mosaic eyes.

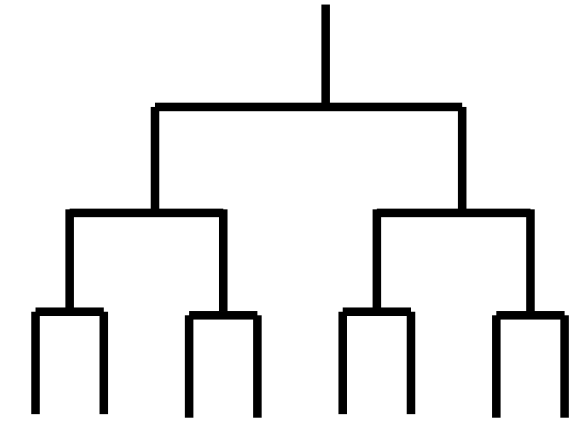
## If invariant lineage, then lineage relationships will be maintained in *white* clones



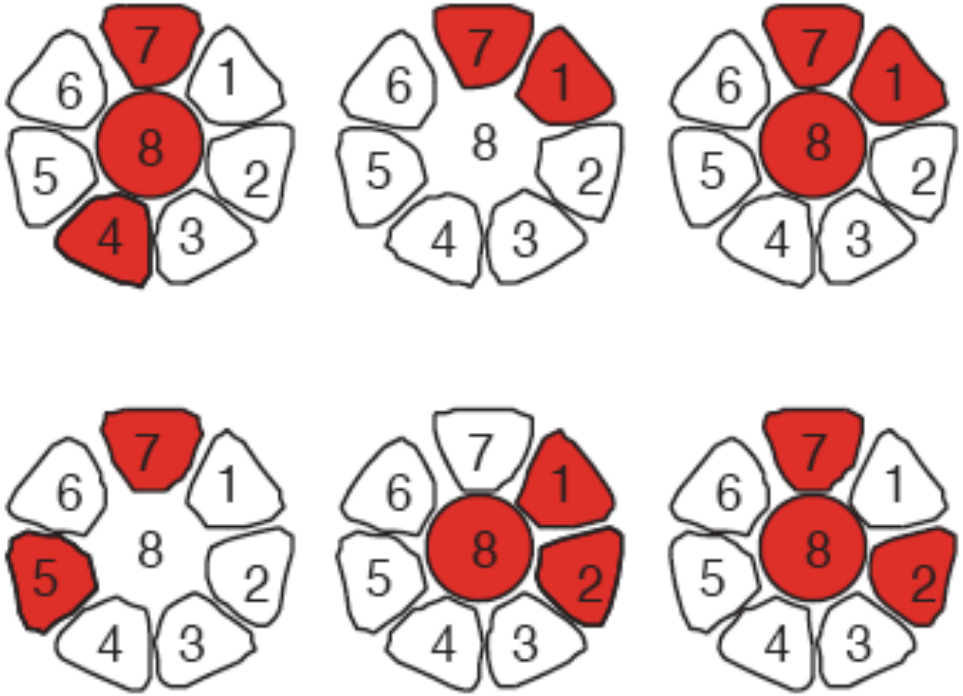



**If variant, then there will be no lineage relationships among cells in clone.**



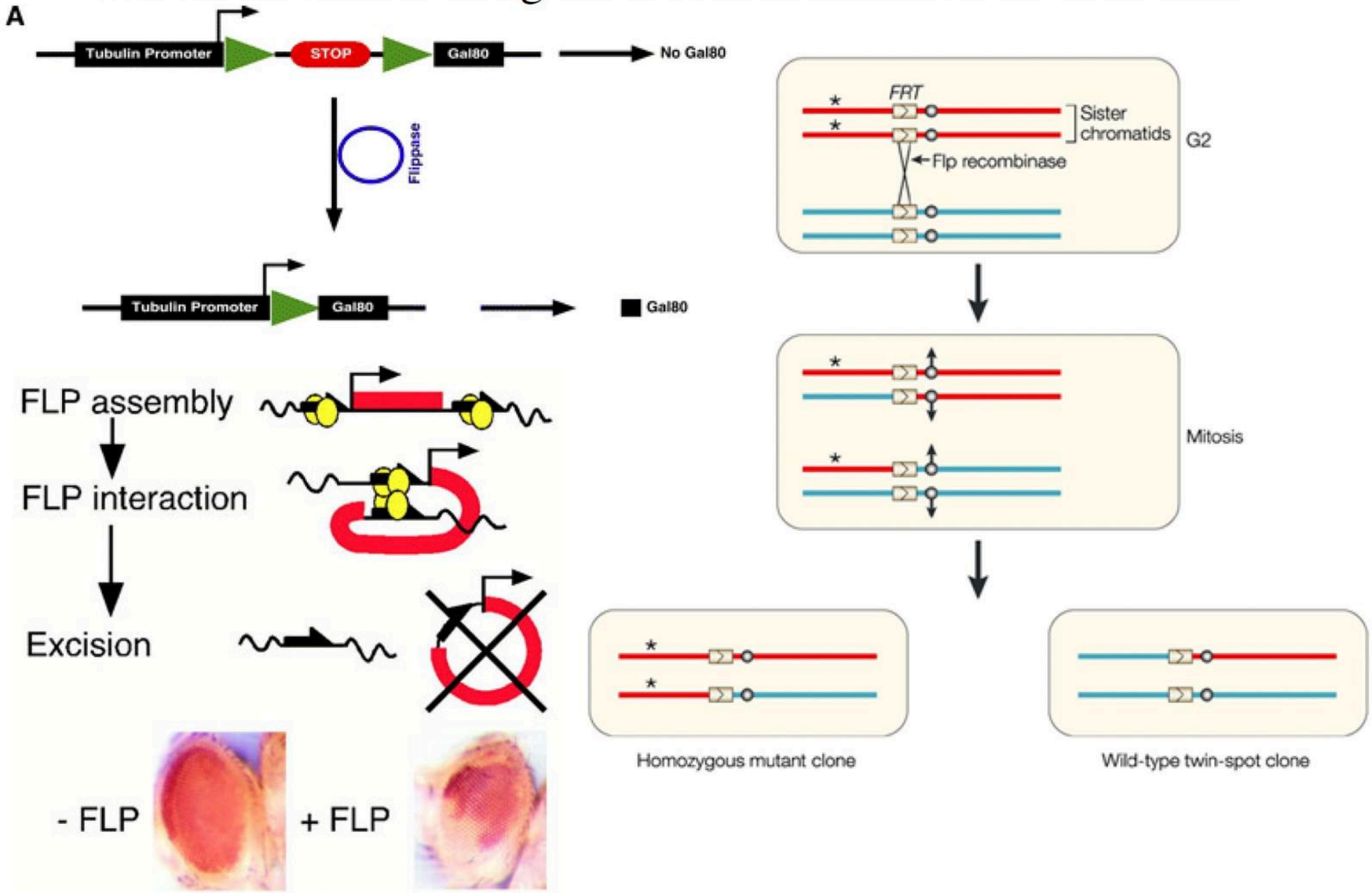



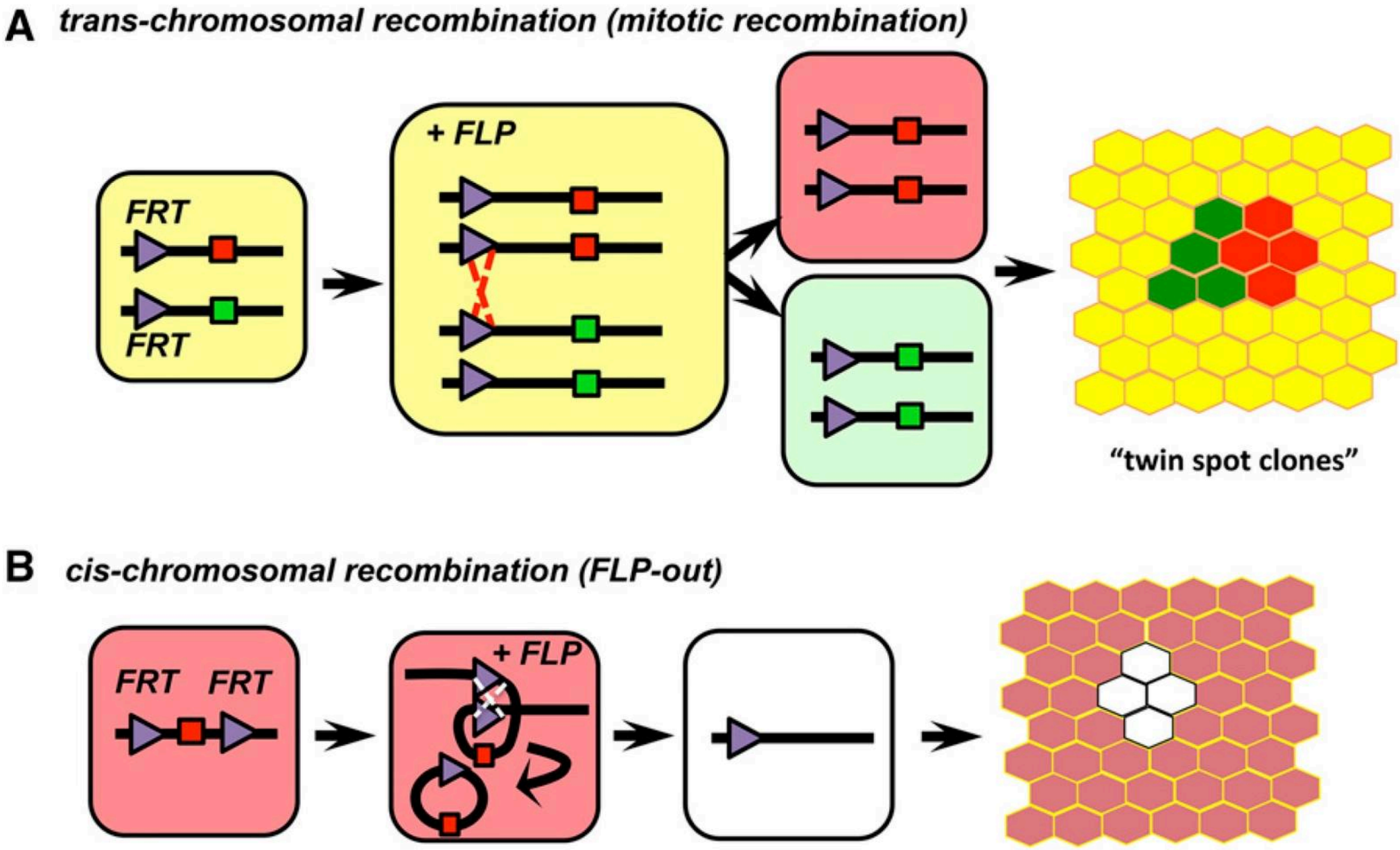

Etc…



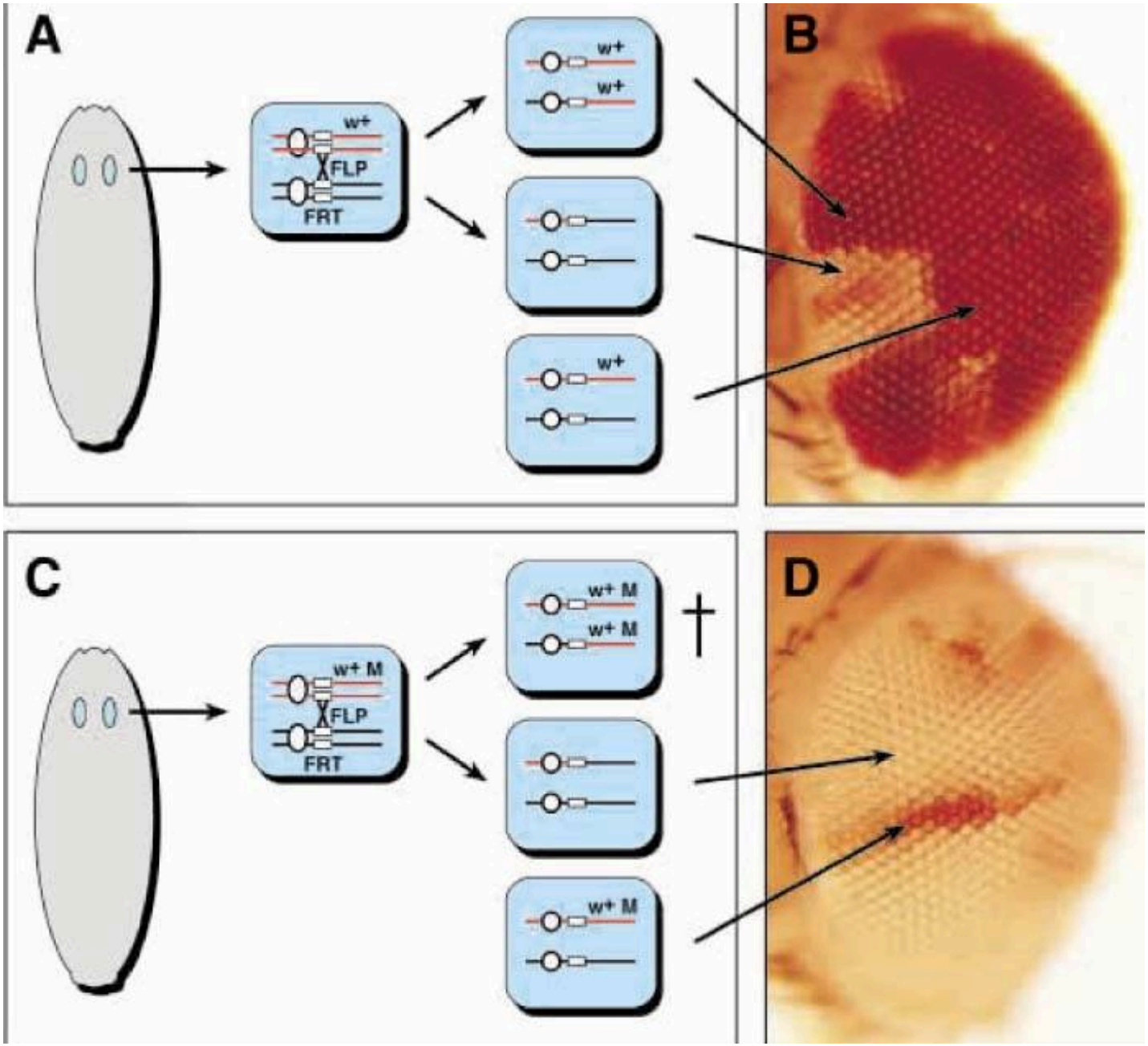

**This is what happens!**
**Cells are specified entirely**
**By cell interactions.**

FLP is a site-specific recombinase that acts on target sites known as FRTs to bring about recombination in cis or in trans

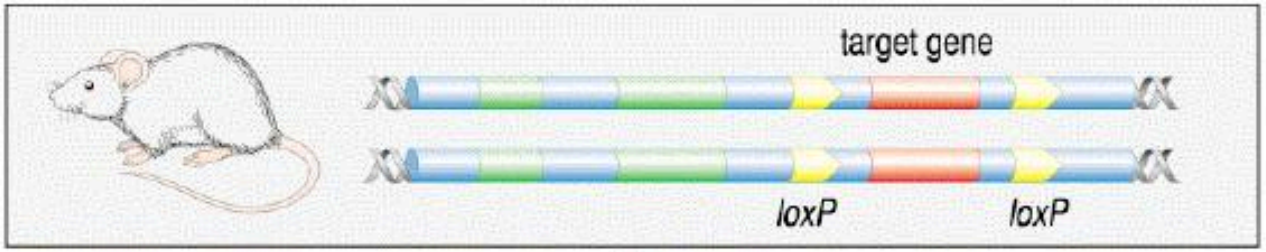



A
trans-chromosomal recombination (mitotic recombination)
FRT
FRT
+ FLP
"twin spot clones"
B
cis-chromosomal recombination (FLP-out)
FRT FRT
+ FLP

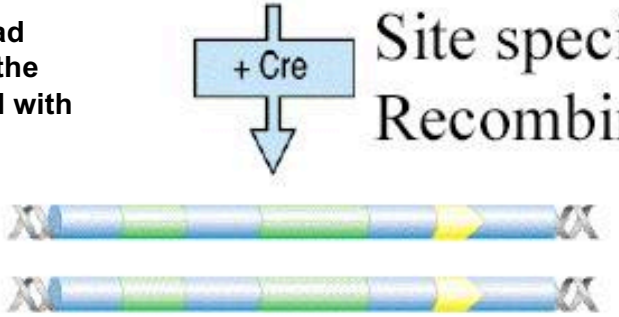
# FLP recombinase expressed from the *eyeless* promoter results in recombination just in the developing eye.



# Mosaics in mice




**The above mouse has had its endogenous copy of the gene of interest replaced with one that is flanked by loxP sites.**

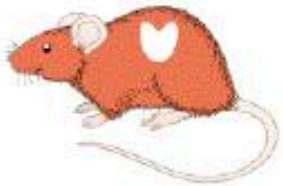



Site specific Recombinase

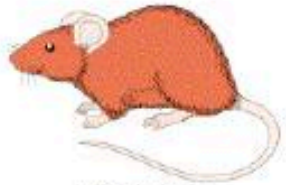
For example
to knock out gene
in a specific tissue

**Heart-specific Cre**

eg. cross to another transgenic mouse that expresses Cre only in heart tissue.



**Ubiquitous inducible Cre**



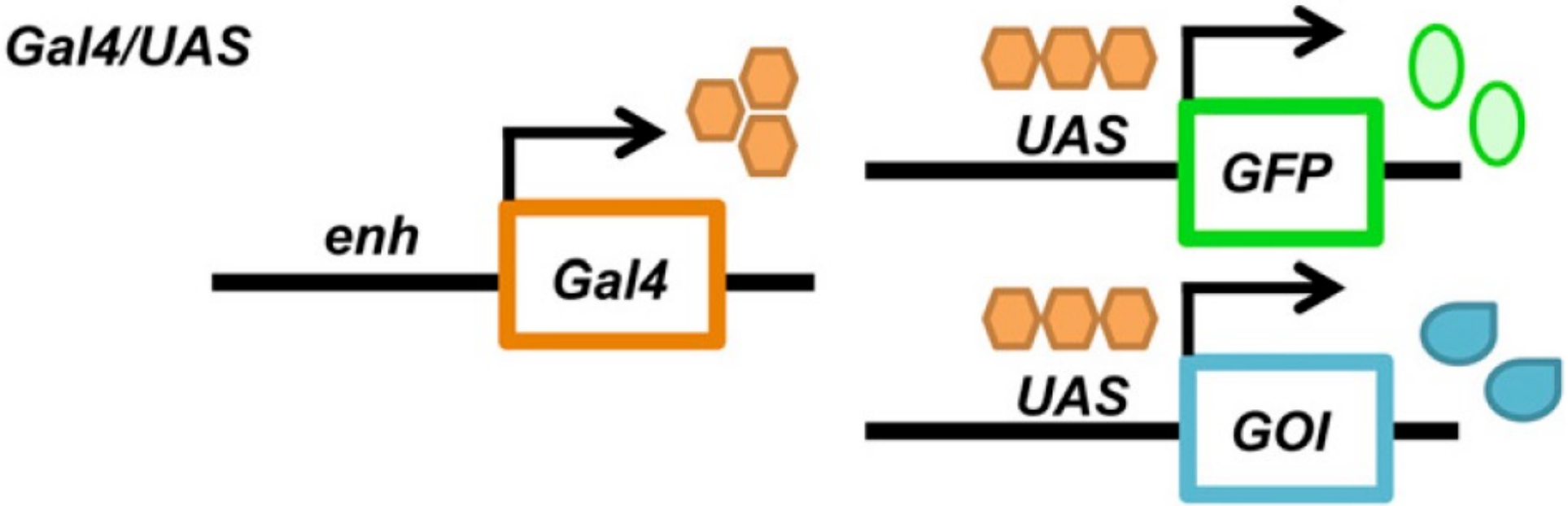
+ inducer

target gene expressed  target gene deleted

**Time of gene action can be determined by removing gene at specific time points**

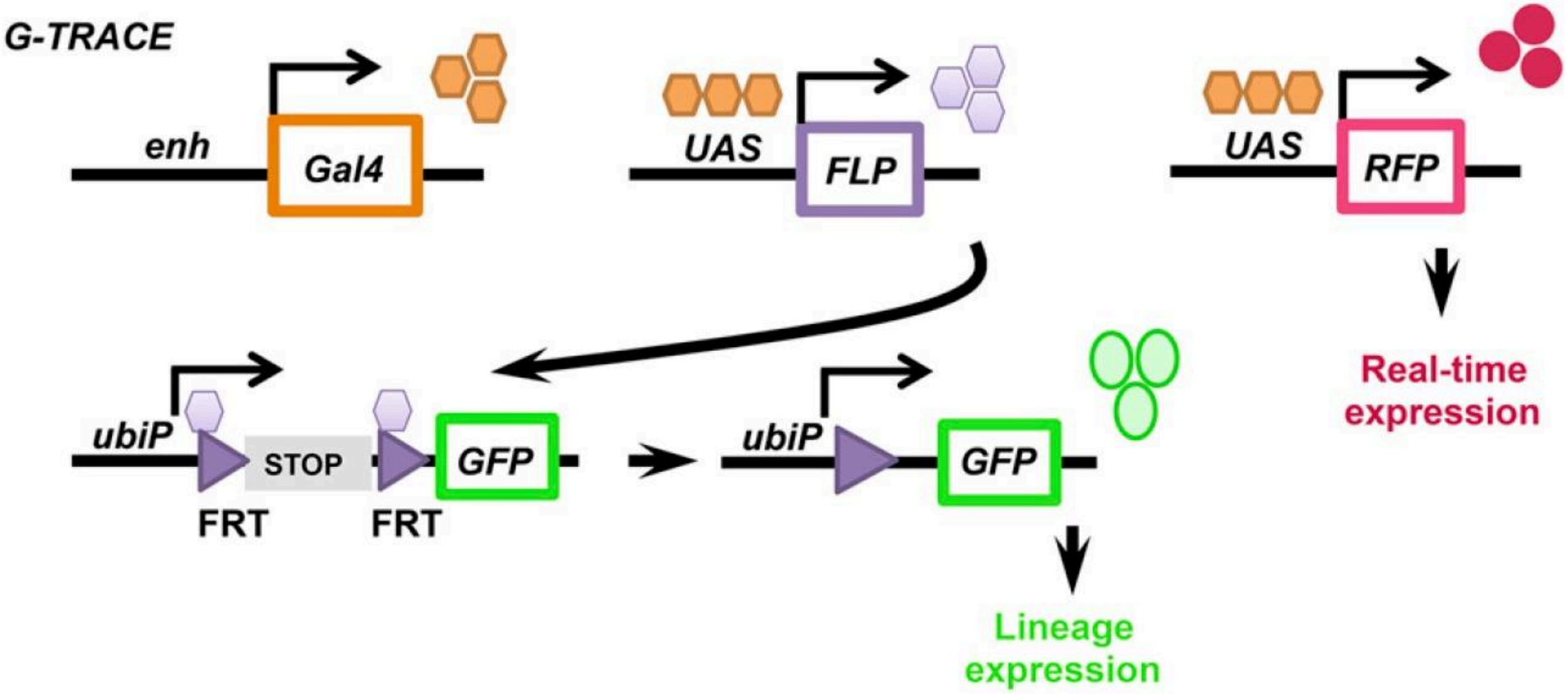
**Cre is a site-specific recombinase that acts on loxP sites. Recombination between direct repeats results in loss of the target gene.**

**Tissue-specific expression of GAL4 can be used to turn up (overexpress) or knock down (RNAi) expression of specific genes. GFP tells you where expression occurred.**



# Marking a cell lineage and visualizing a pattern of gene expression

tells you where gal4
is currently expressed

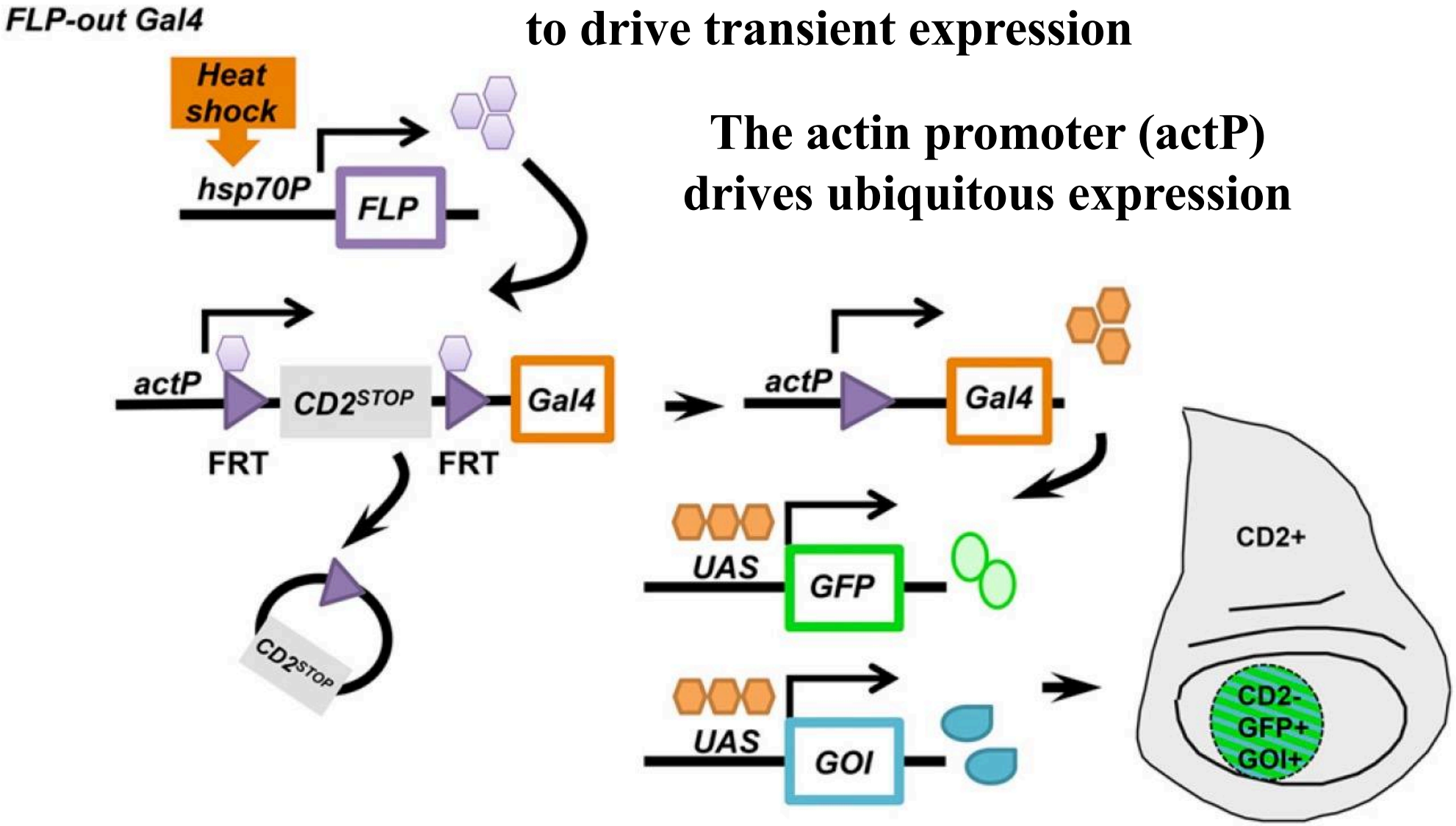



tells you where gal4 was expressed in the past
(what is the fate of this lineage of cells?)

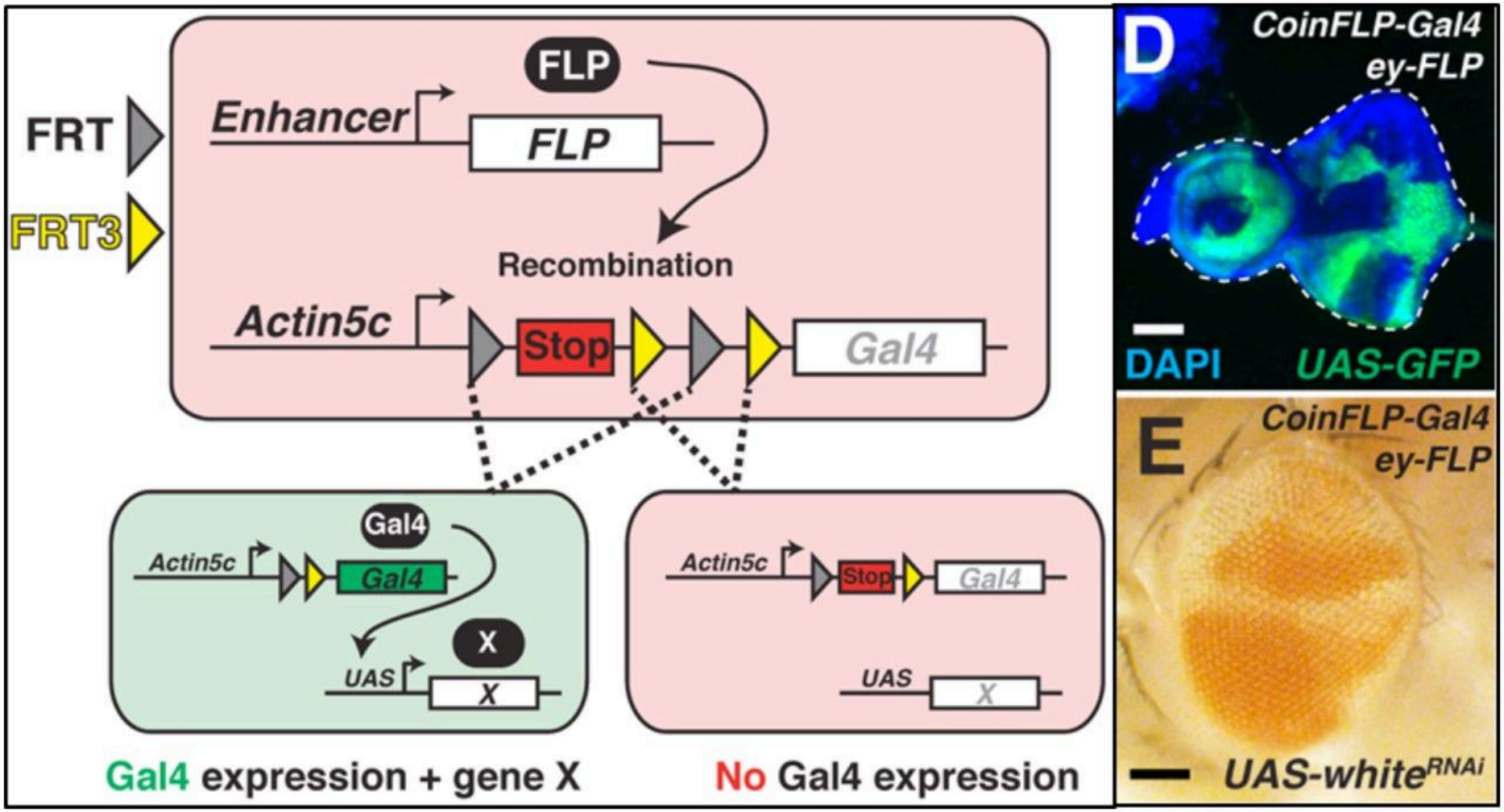
# Turning on gene expression in a specific tissue and visualizing that pattern of expression

**Heat shock promoter used (with heat shock) to drive transient expression**

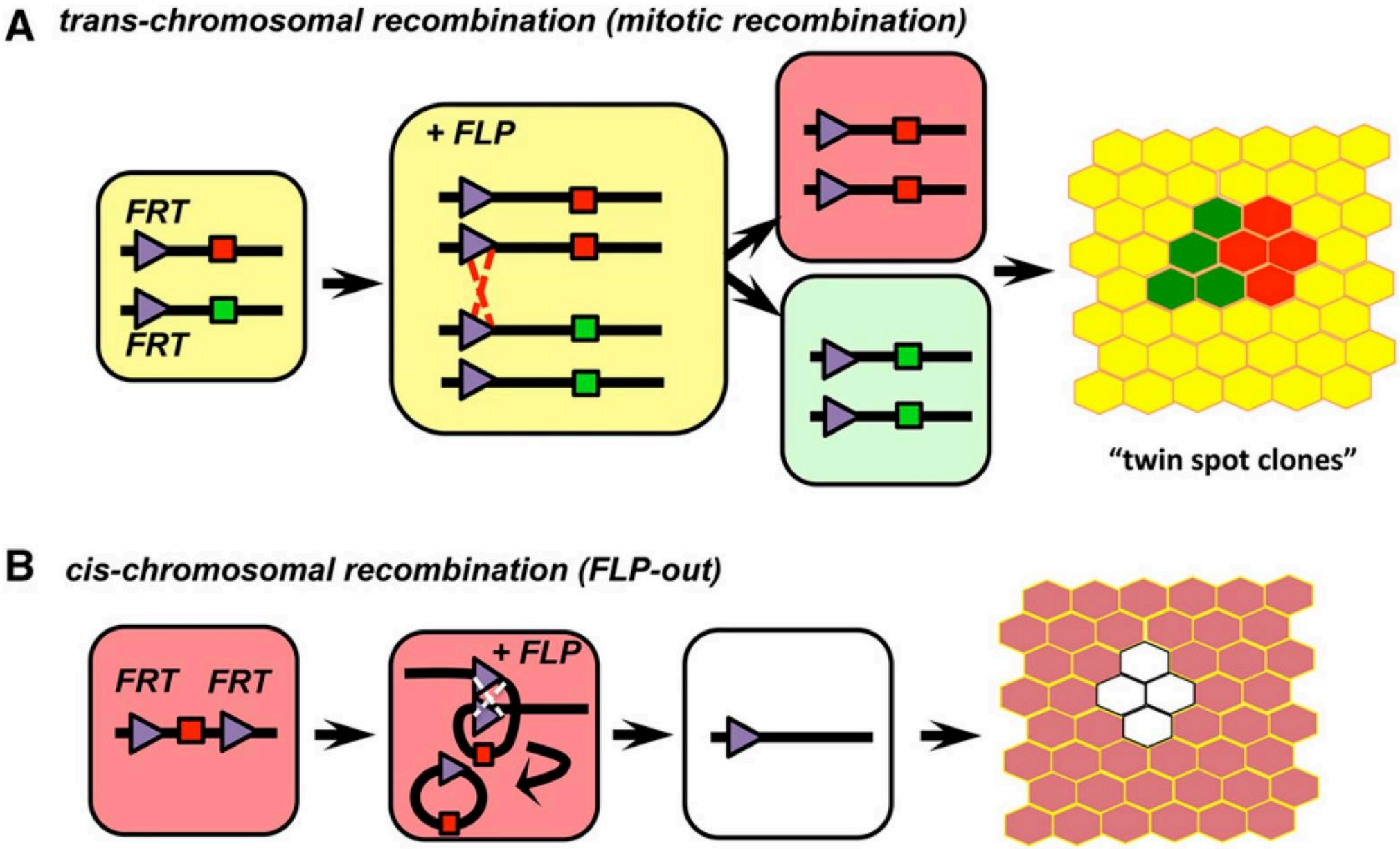
**The actin promoter (actP) drives ubiquitous expression**



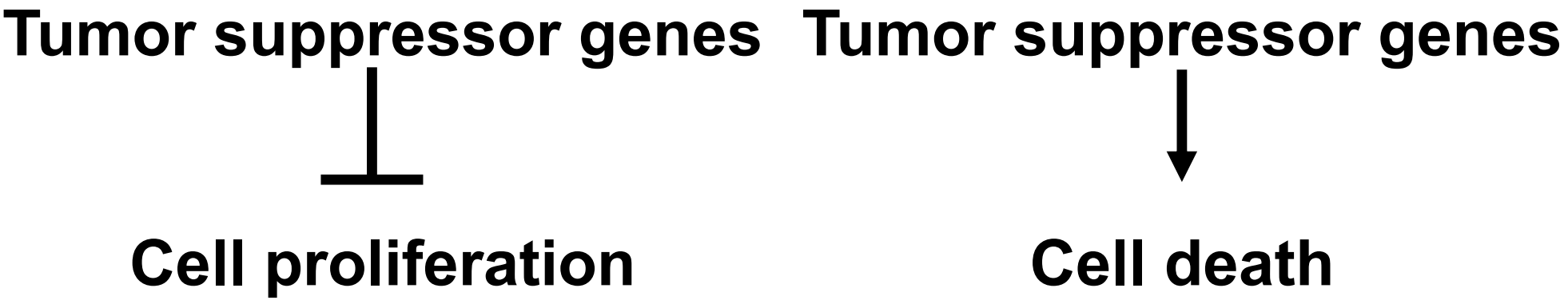
# Coinflip: turning on spots of expression of a gene of interest



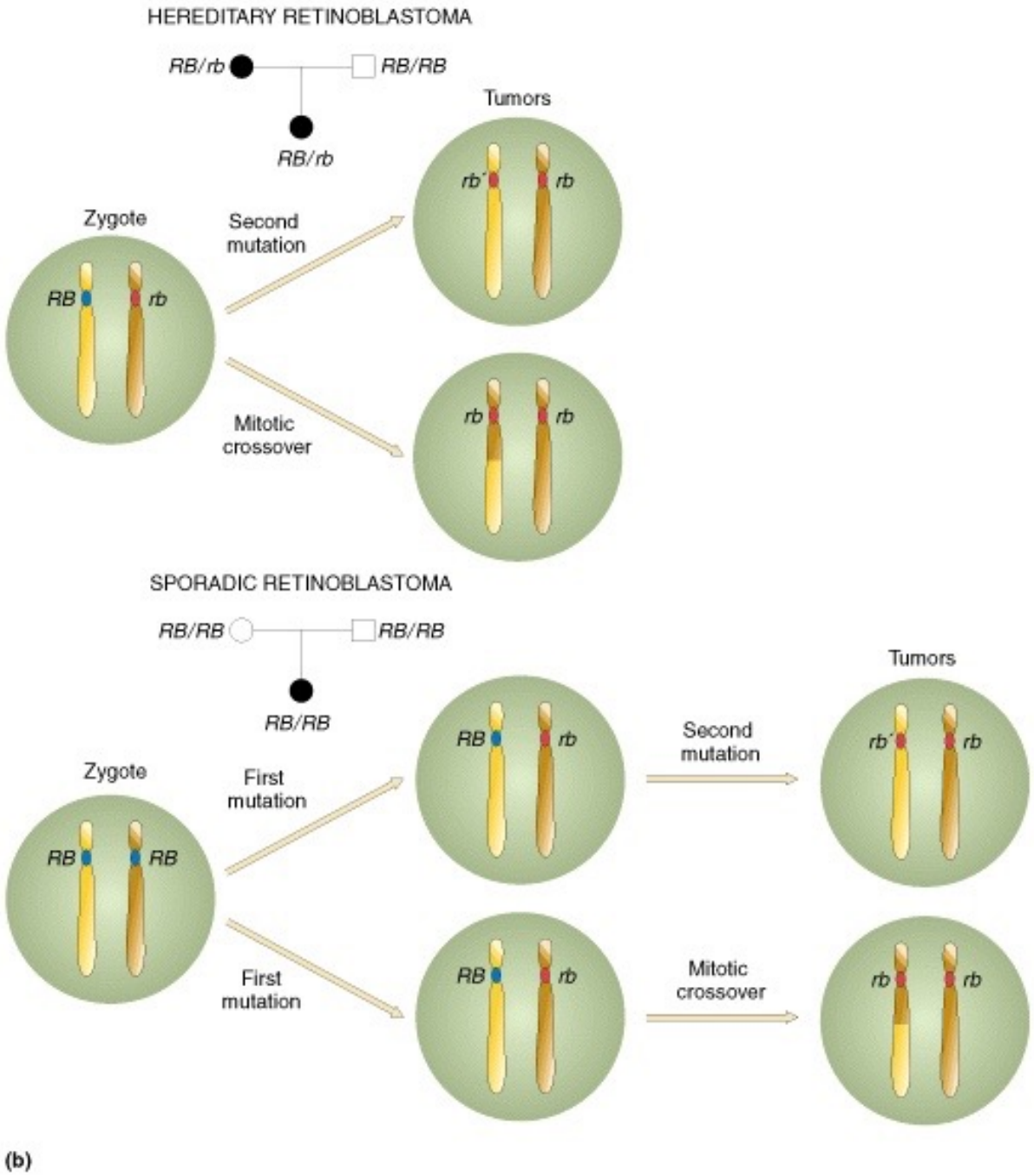
# Expression of FLP recombinase can stimulate mitotic recombination at FRT sites.



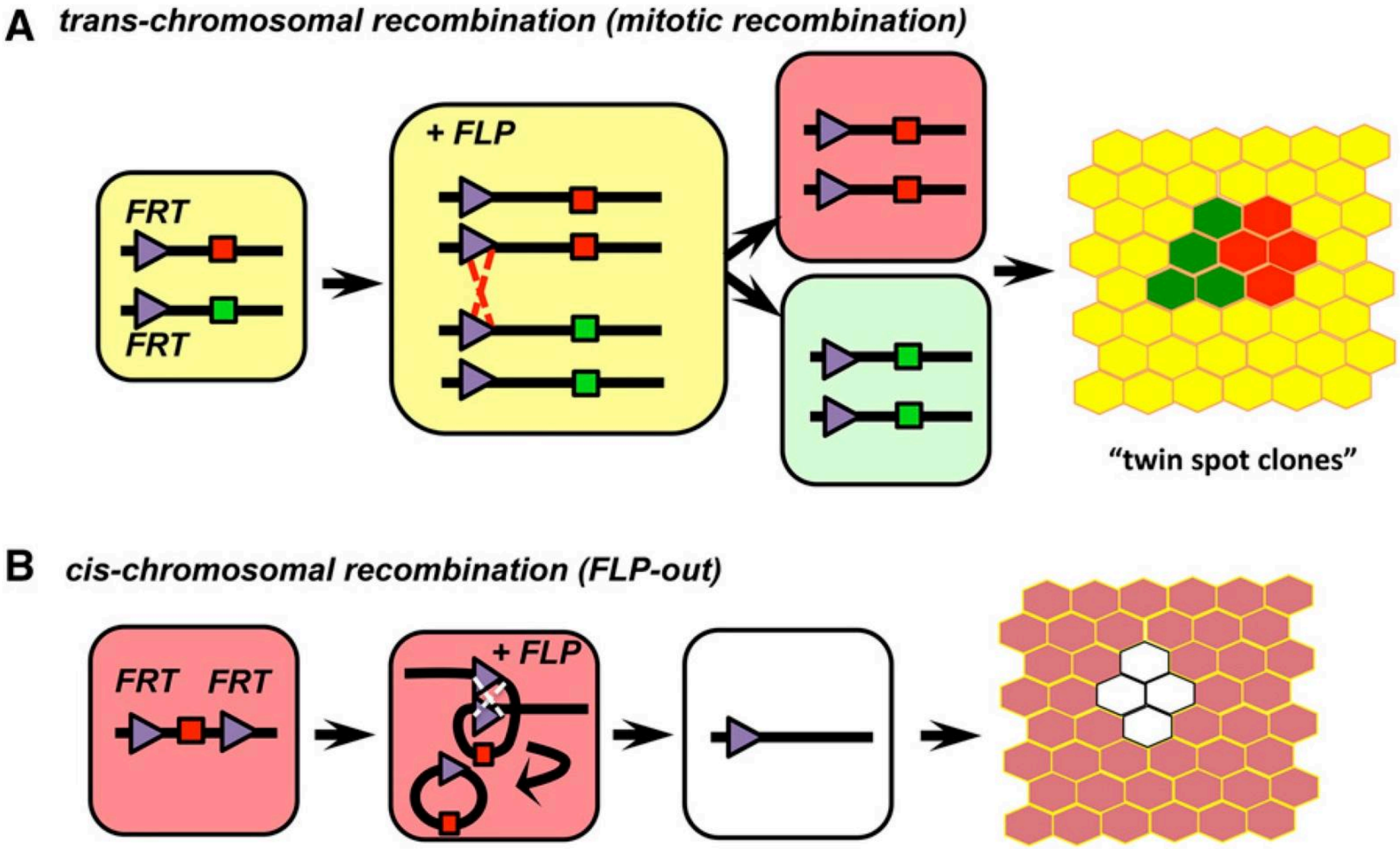
# Tumor suppressor genes can either inhibit cell proliferation or promote cell death.



# How can we identify mutations in tumor suppressors ?

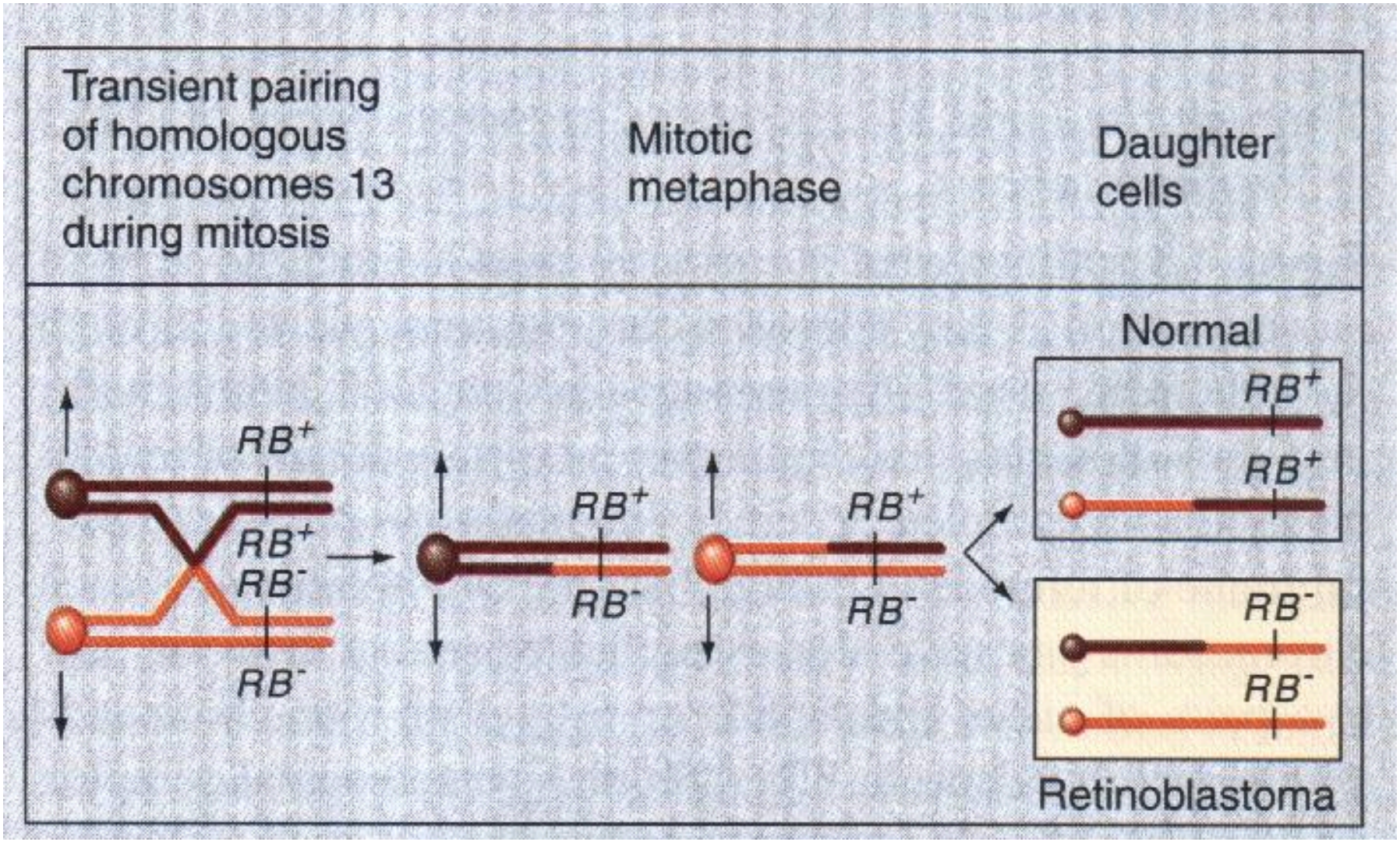


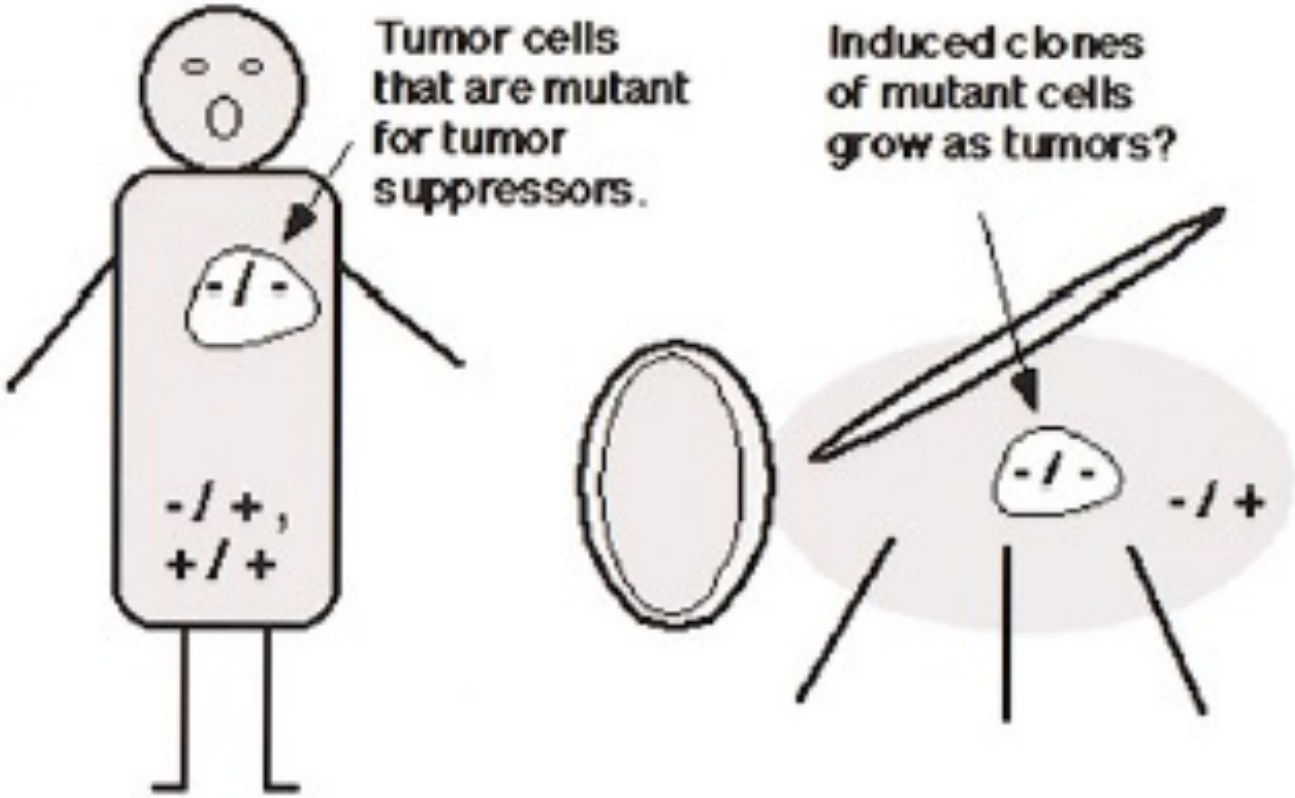
# Expression of FLP recombinase can stimulate mitotic recombination at FRT sites.



# Mitotic recombination-mediated loss of a tumor suppressor results in the appearance of tumors.

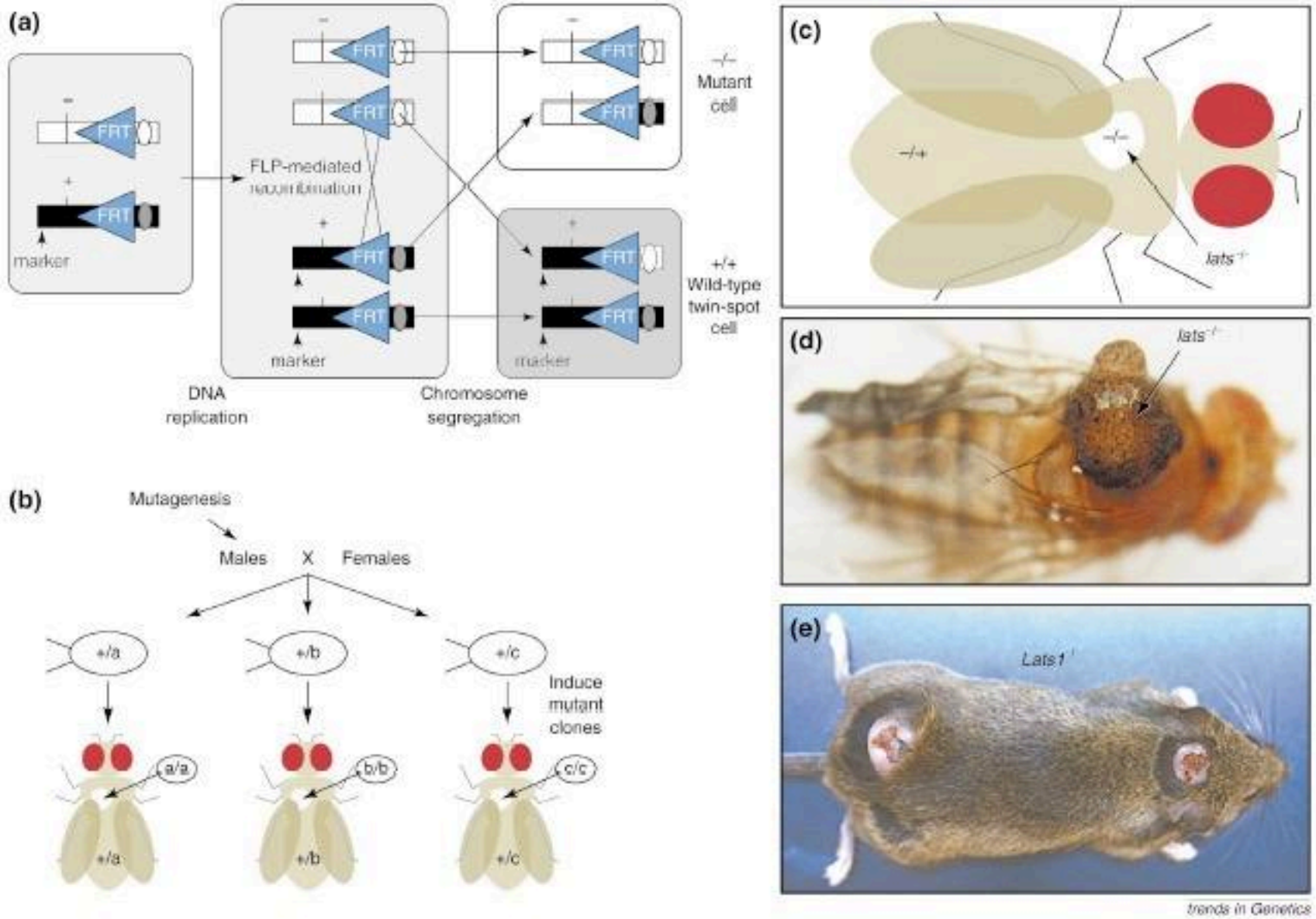
**Lets use this phenotype, and the fact that we can use the FLP-FRT system to carry out mitotic recombination at high frequency, as a way of screening for new tumor suppressor genes.**






Tumor patients

Screen for tumor suppressors in mosaic flies




(a) Induction of homozygous mutant clones in heterozygous animals by FLP/FRT-mediated mitotic recombination. Homologous chromosomes are illustrated as white and black bars. High frequency mitotic recombination between chromosome arms can be induced at the FLP recombination target (FRT) sites (arrowhead) by the induction of FLP enzyme. After chromosome segregation, a daughter cell homozygous for the mutant gene (-/-) can be produced. The mutant cell also lacks the marker gene, allowing it to be distinguished from the wild-type twin-spot cell (+/+) or the heterozygous cells. (b) The FLP/FRT-mediated mitotic recombination can be used to generate mosaic animals that carry clones of cells that are homozygous for independently induced mutations. Mutations in tumor suppressors or other genes of interest can be identified in mosaic animals of the first generation. Mosaic flies are a good model for patients with cancer predisposition syndromes such as those heterozygous for mutated tumor suppressor genes. Loss of the wild-type copy of the tumor suppressor in these patients, or in the fly model (c), can lead to the development of a tumor (white patch). (d) The tumor suppressor function of *large tumor suppressor* (*lats*) is conserved in mammals as (e) mice mutant for the *Lats1* homolog develop soft-tissue sarcomas (see text).