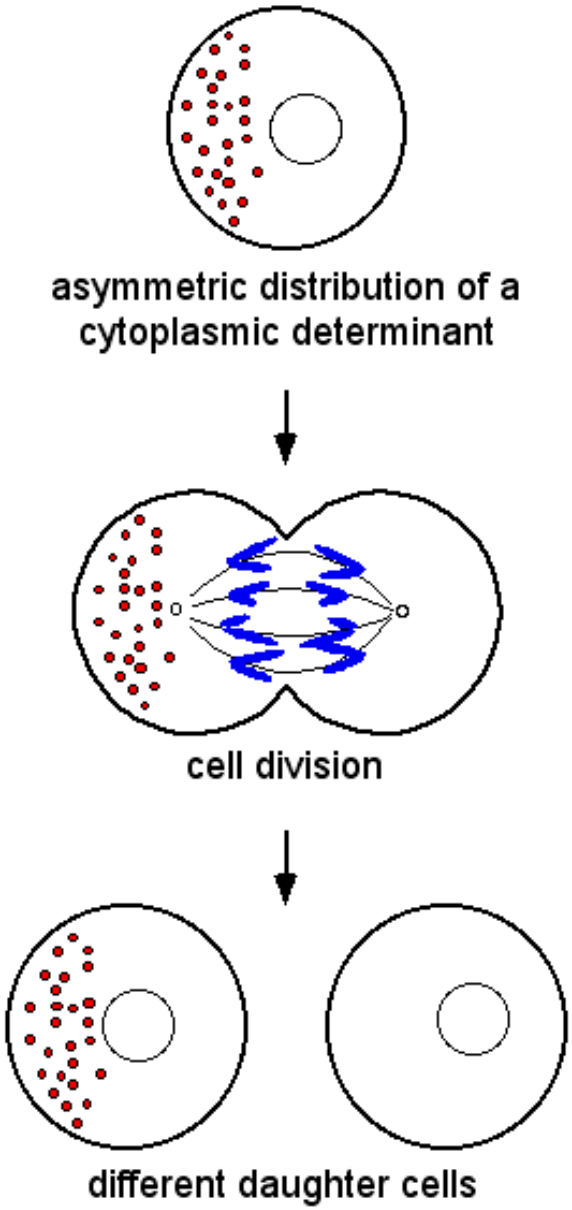
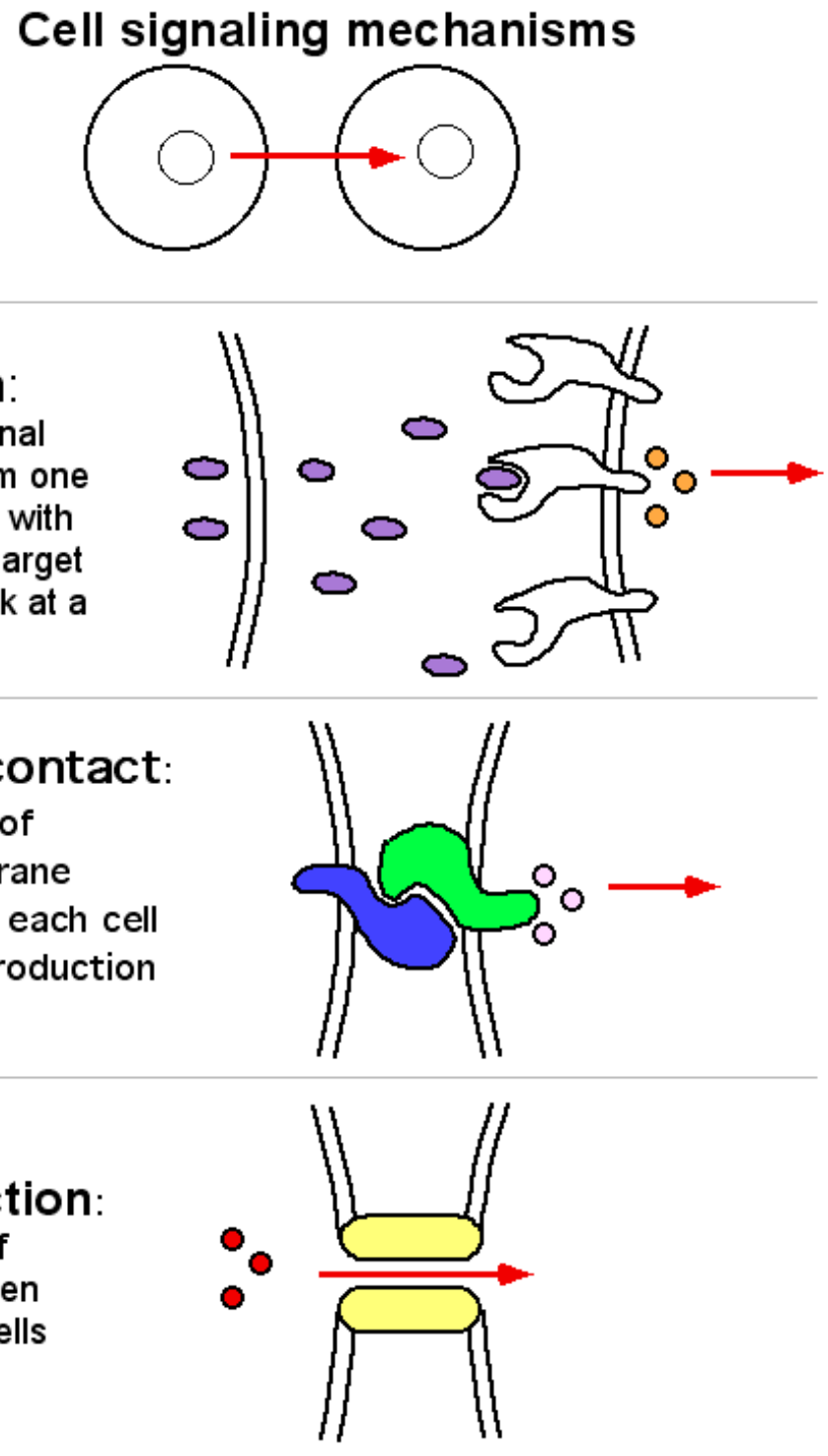
# Cell autonomous



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



# Mosaic Analysis

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

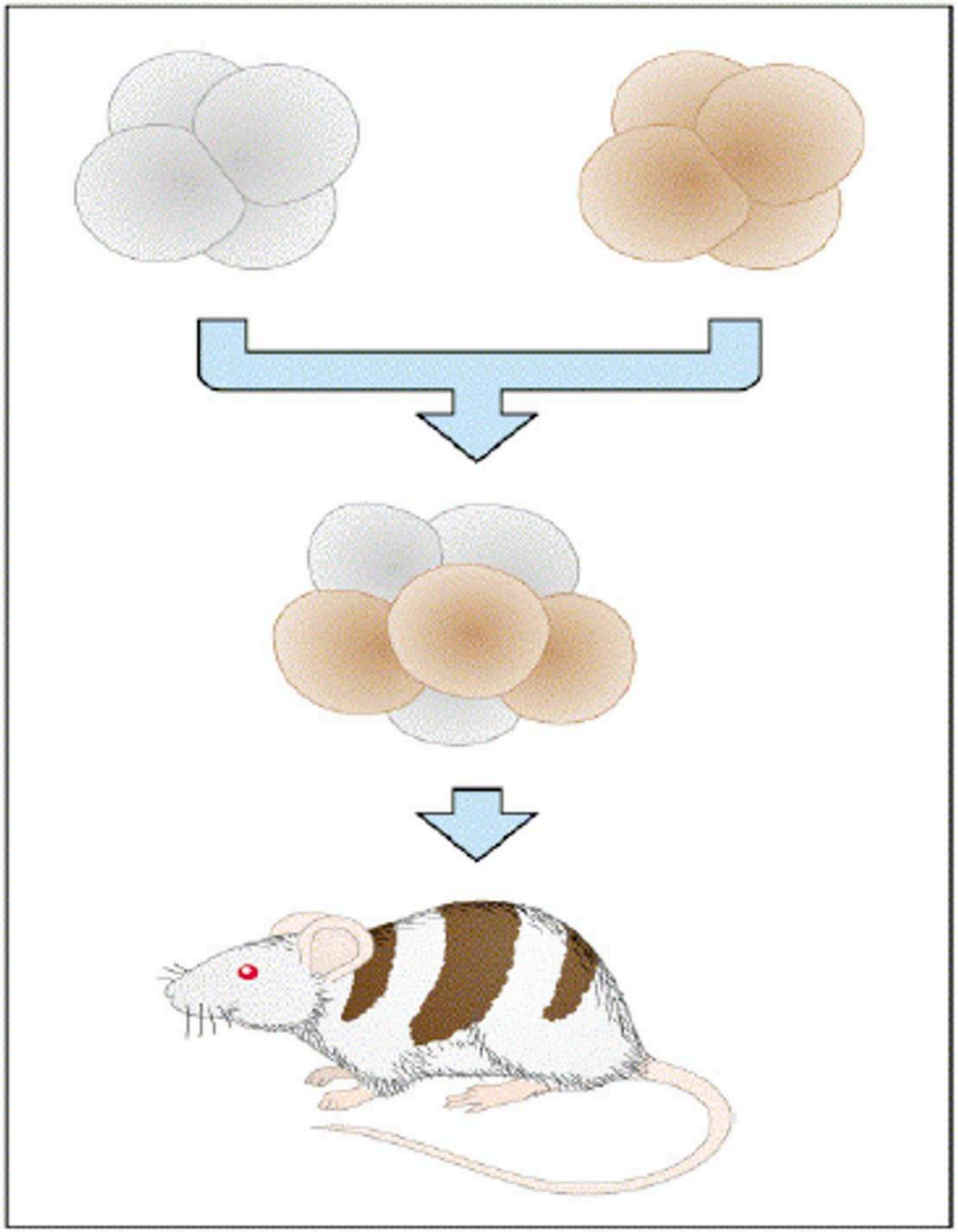
**Determine the function of a gene in specific tissues, even if homozygotes would be dead by this time in development.**

**Cell autonomous versus non-cell autonomous**

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

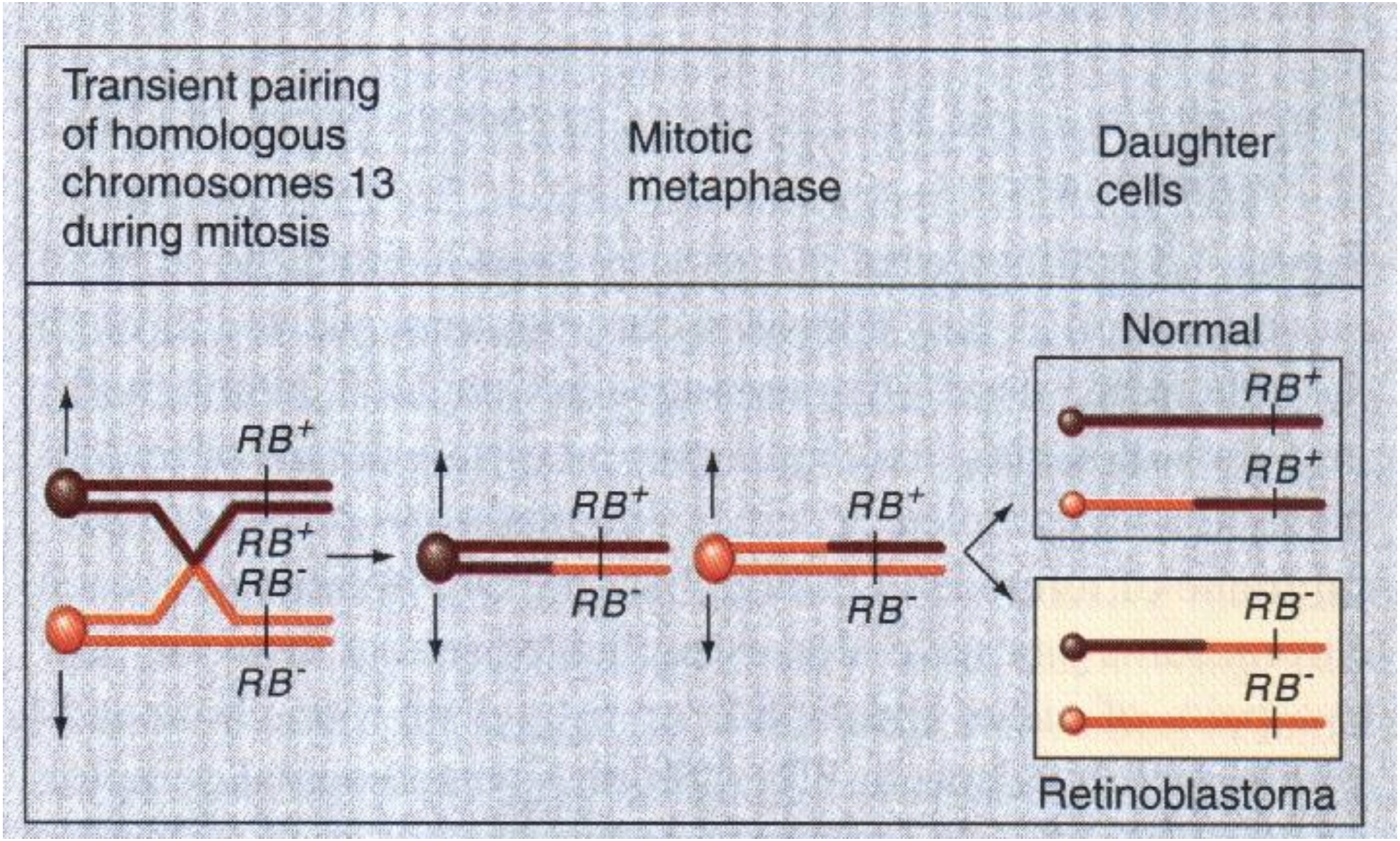
# Fusion of mouse embryos gives rise to a chimera/mosaic

**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.**



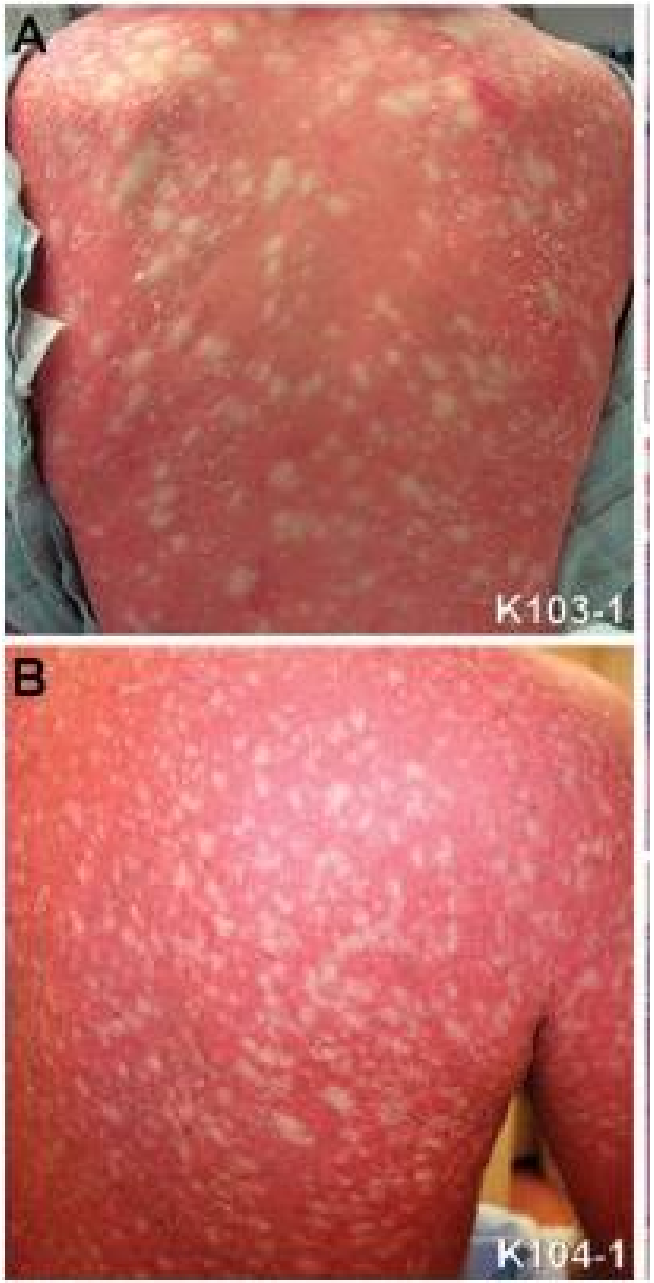
Don't confuse chimera with hybrid

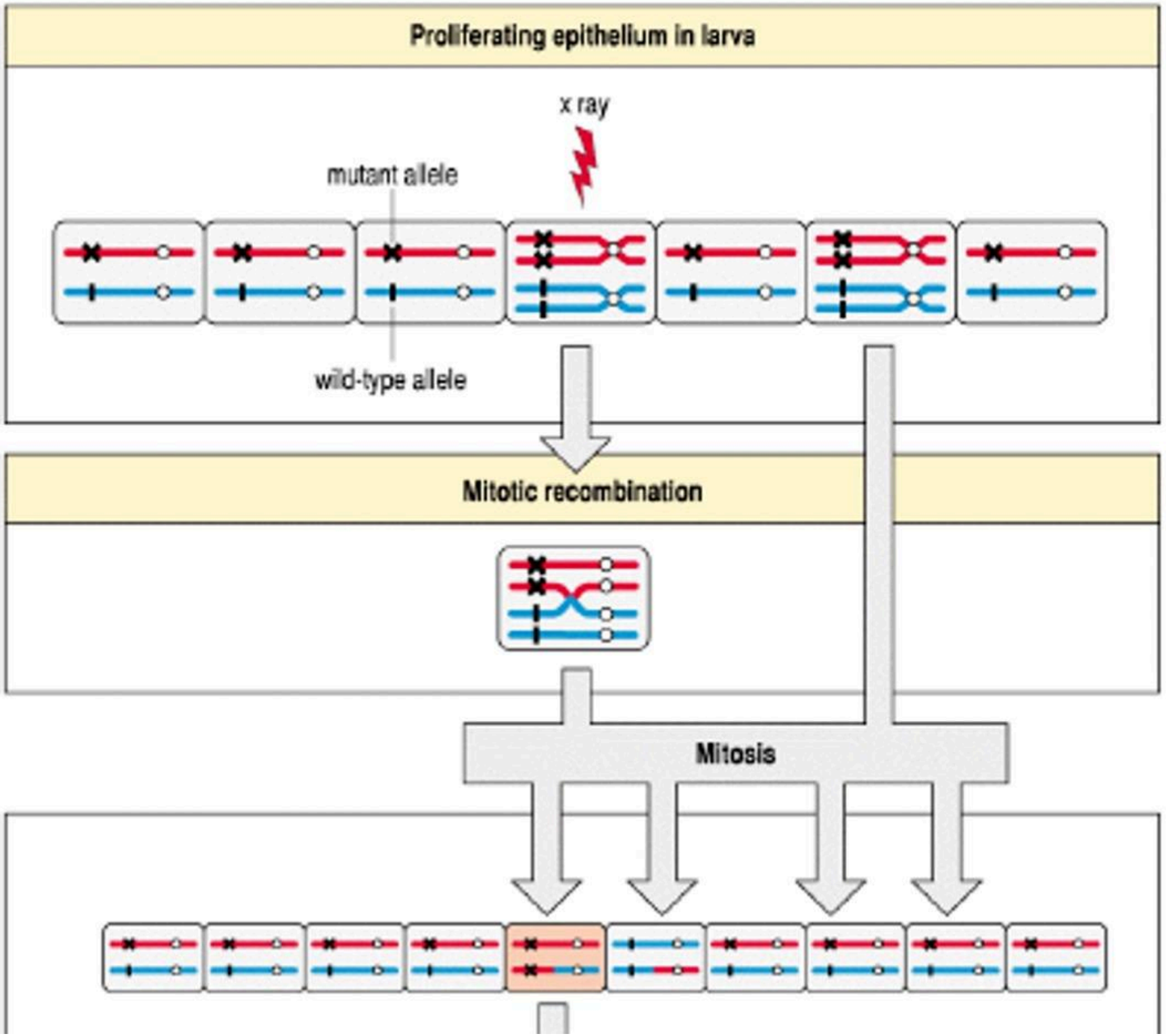
# Loss of heterozygosity for a tumor suppressor through mitotic recombination

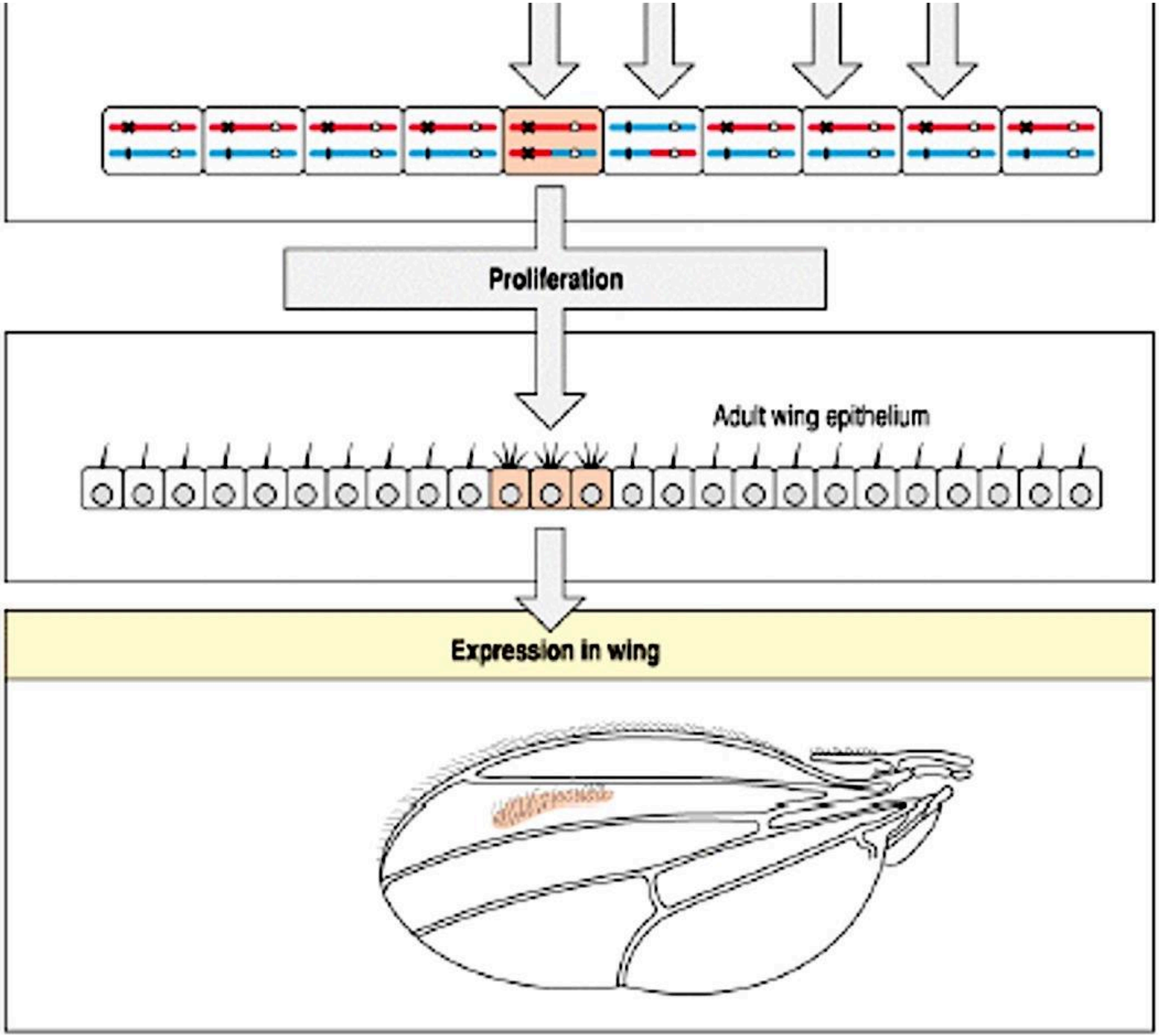


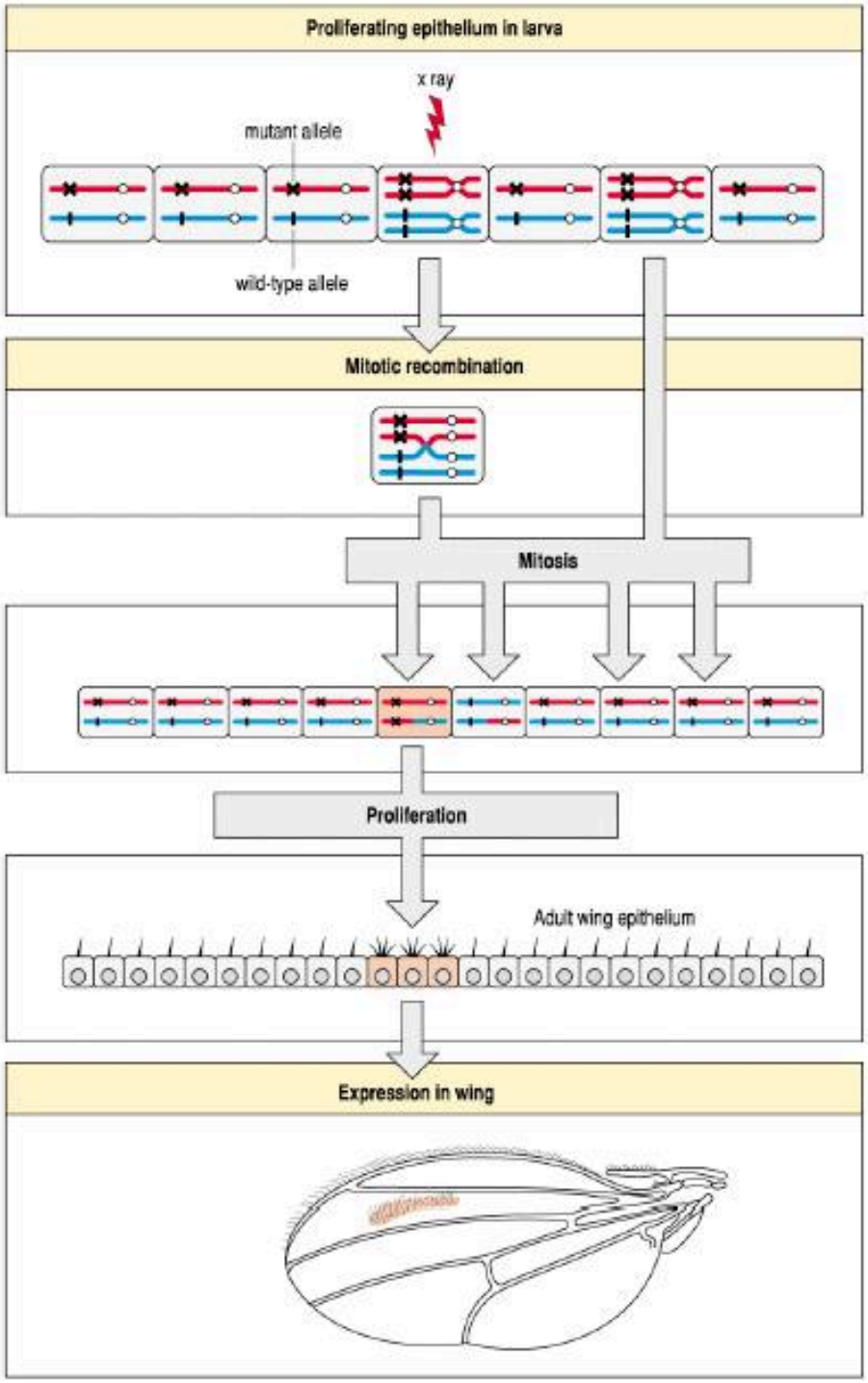
# A dominant condition reverting back to wildtype through mitotic recombination and loss of heterozygosity

**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

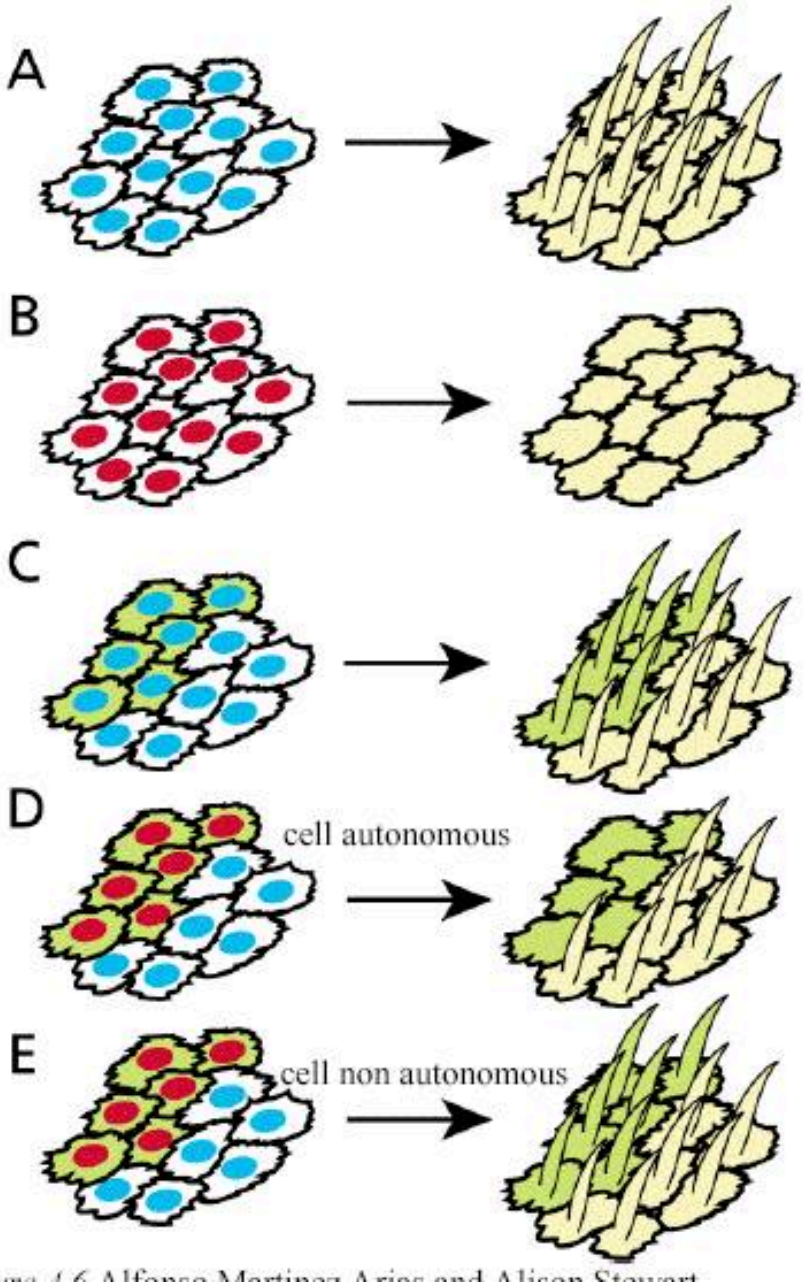
Induce mitotic recombination by X-rays

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



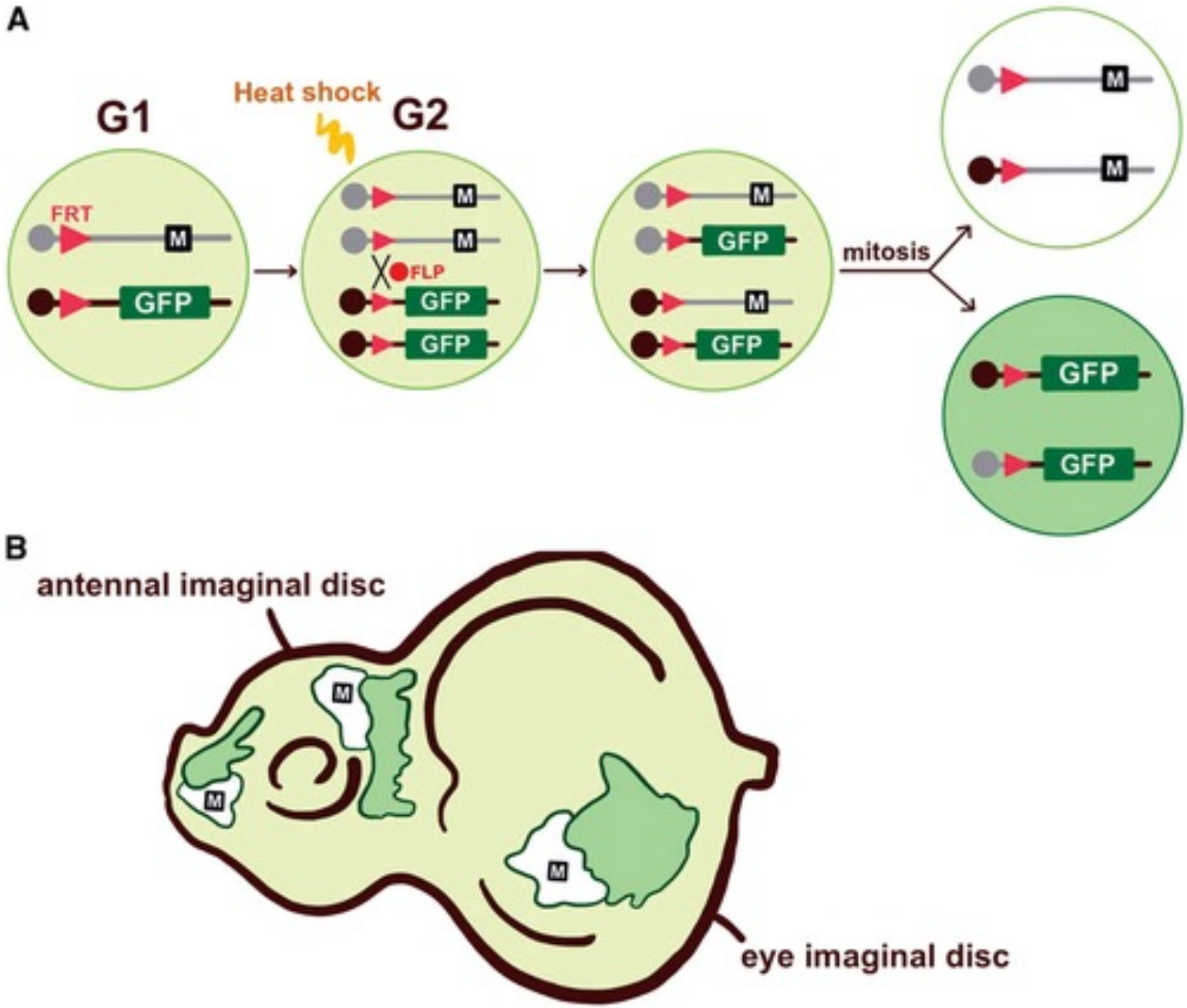

wt (blue nuclei) cells give rise to bristles

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.

Mutant clone marked by loss of GFP. Twin spot has two copies



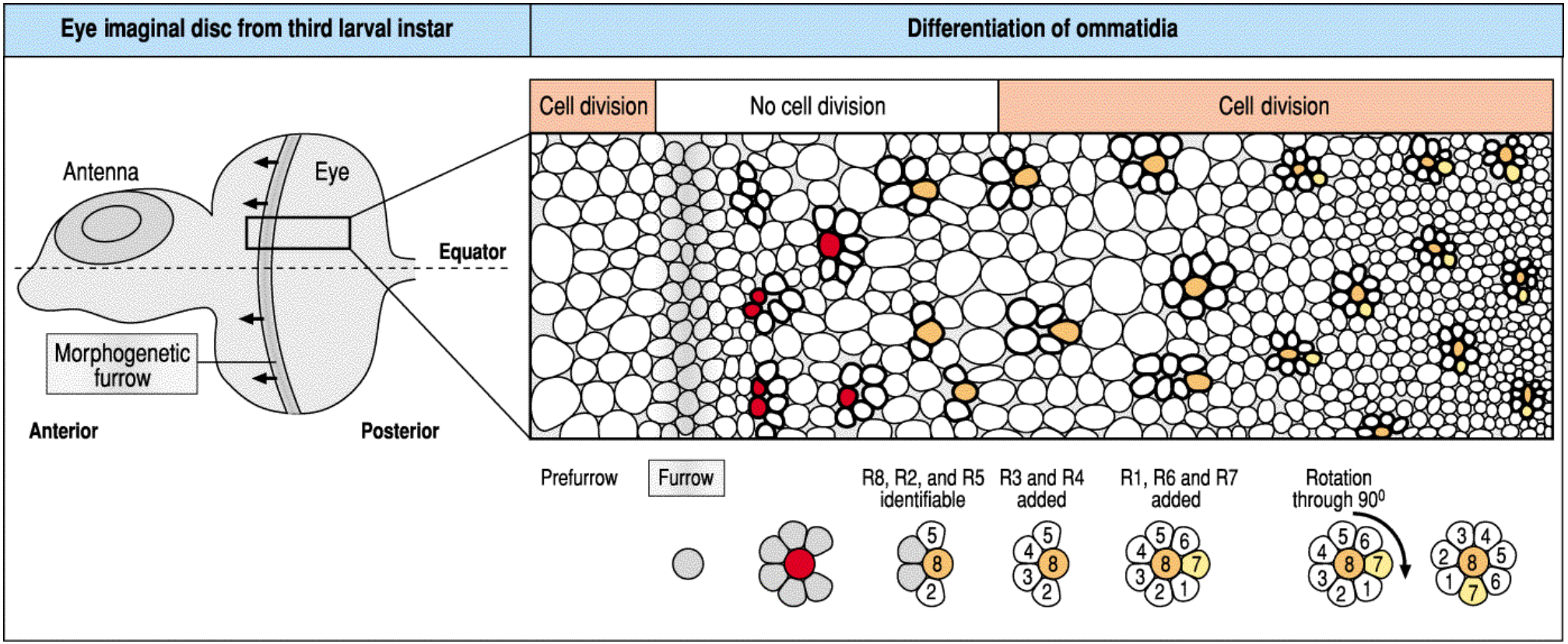
What can autonomy vs. cell autonomy tell you about your gene product?

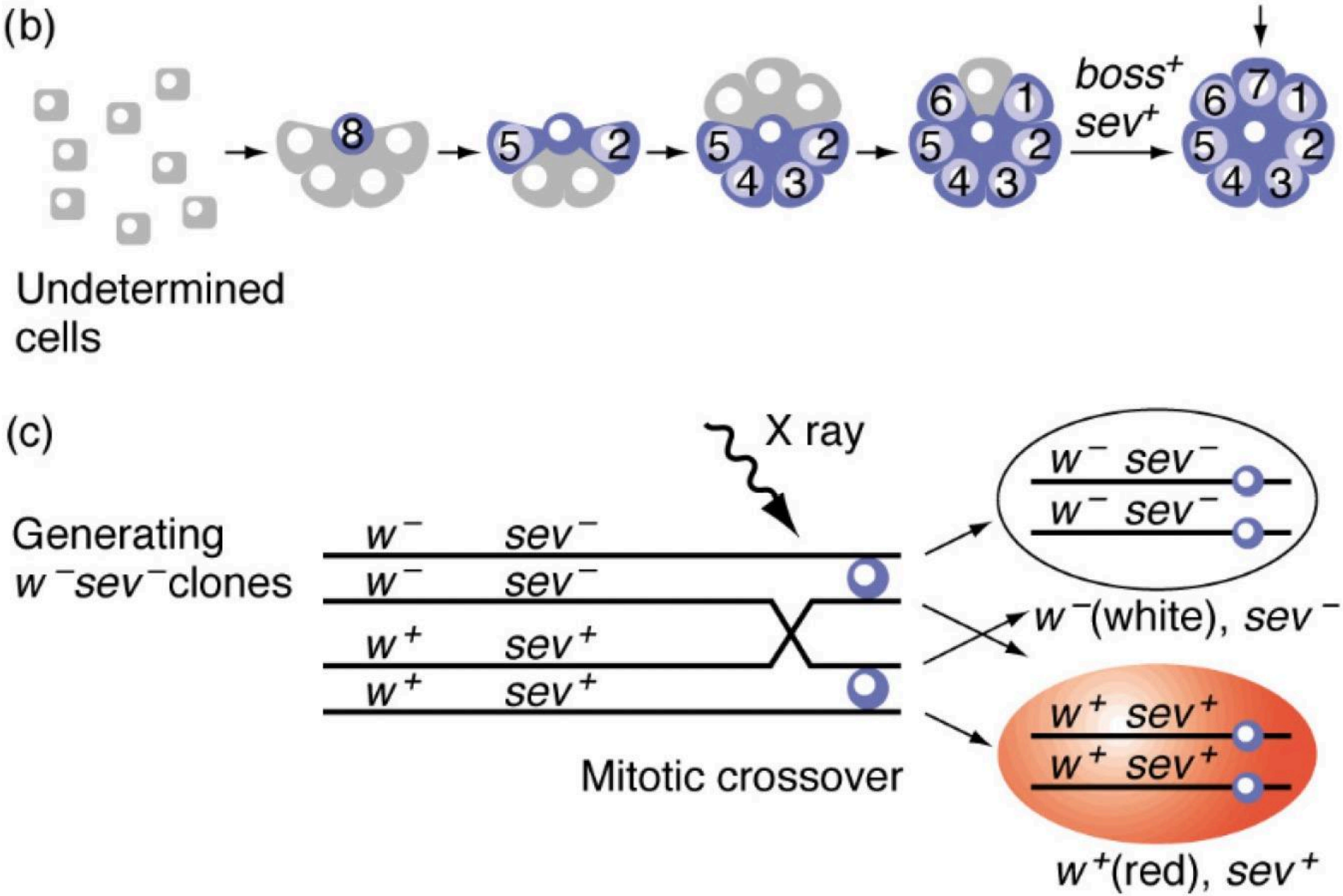
If the **genotype reflects phenotype** in a genetic mosaic experiment then we say the gene function is **cell autonomous**. Think of the gene as encoding a receptor on the cell surface. For example, it needs to be present on the cell to receive a signal to cause cell differentiation.

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.

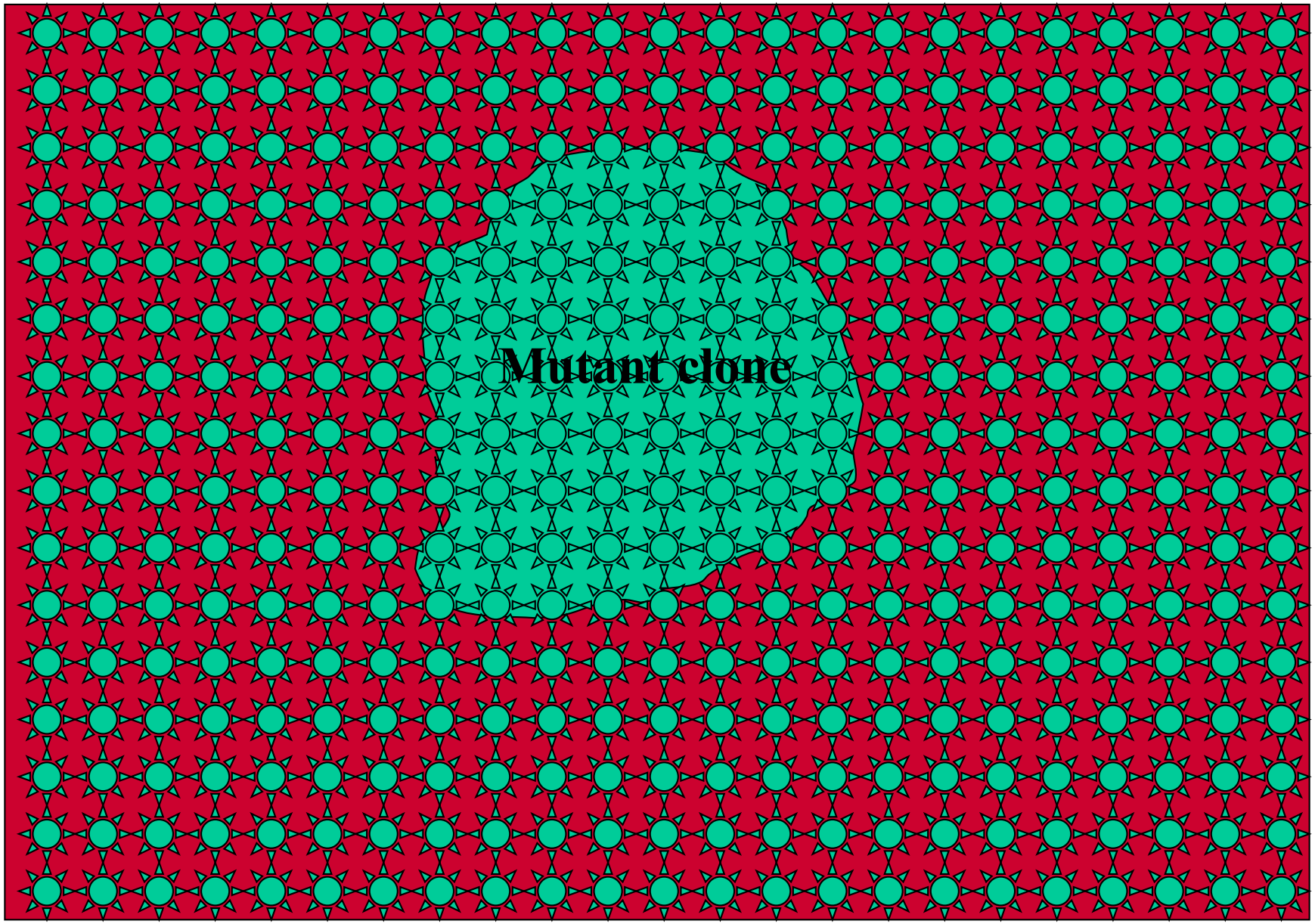
**Insulin as an example. It is produced by cells in the pancreas but acts non-autonomously throughout your body to regulate glucose uptake. Thus, loss of the insulin gene can be rescued by providing the protein through other means - like a needle.**

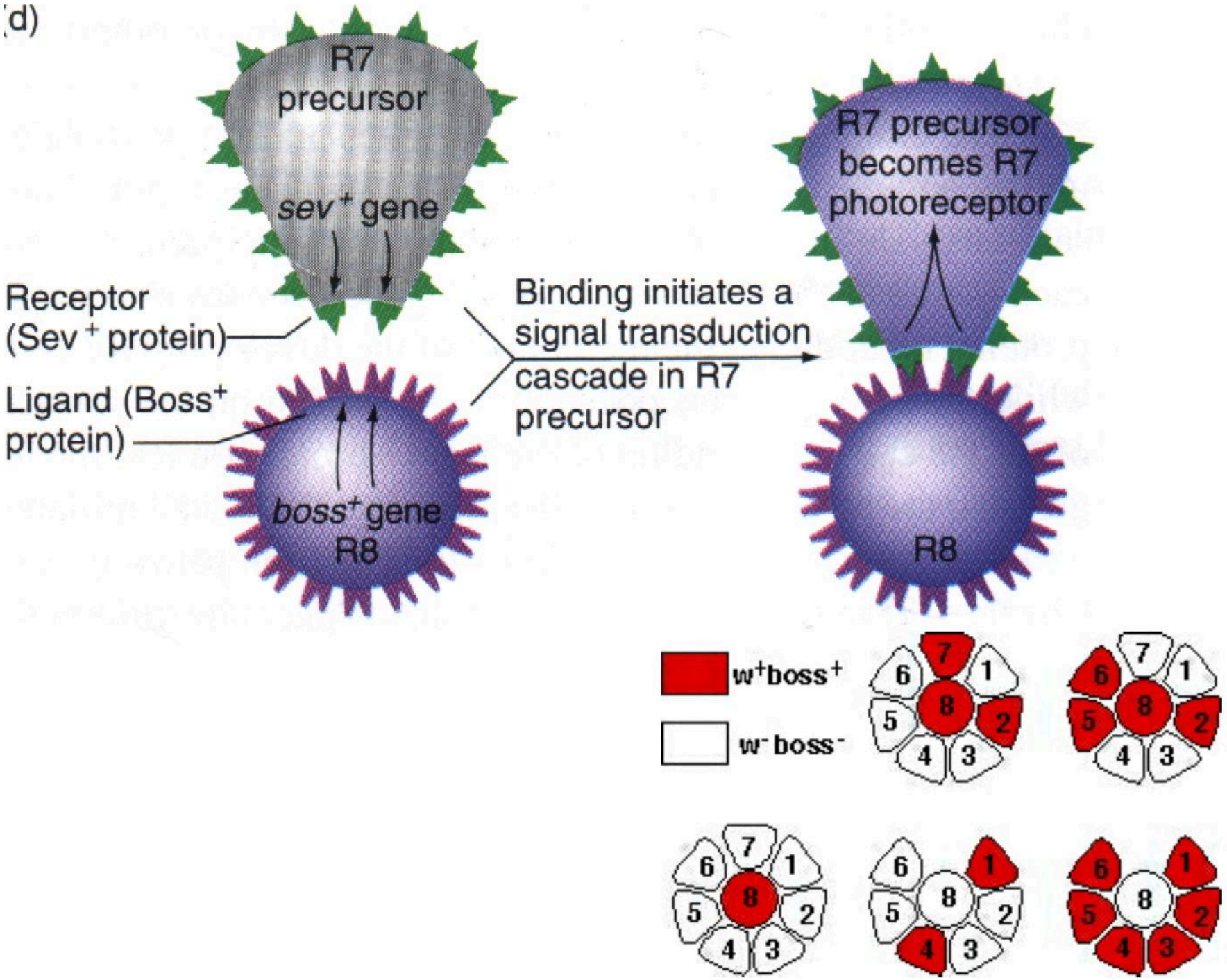
# Drosophila eye development



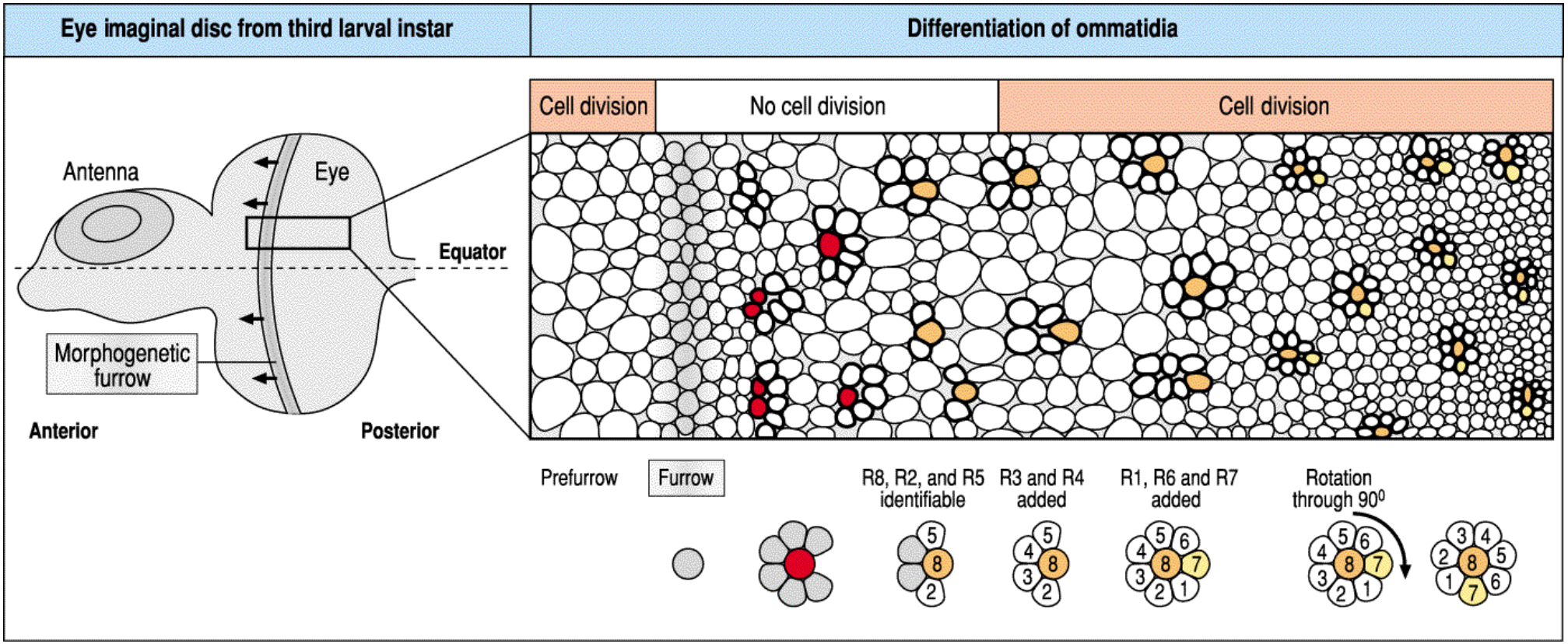

(b)
8
5
2
5
4
3
2
6
1
5
4
3
2
boss+
sev+
6
7
1
5
4
3
2
Undetermined cells
(c)
X ray
Generating w⁻sev⁻clones
w⁻
sev⁻
w⁻
sev⁻
w+
sev+
w+
sev+
Mitotic crossover
w⁻ sev⁻
w⁻ sev⁻
w⁻(white), sev⁻
w+ sev+
w+ sev+
w+(red), sev+
Phenotypes of mosaic facets
w+ sev+
w⁻ sev⁻

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



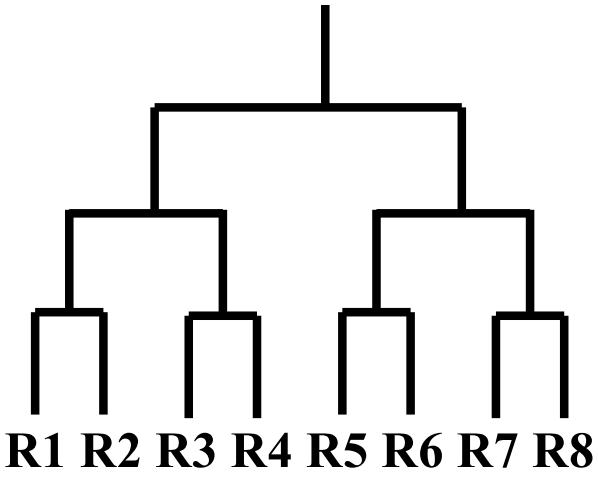

(d)
R7 precursor
sev+ gene
Receptor (Sev+ protein)
Ligand (Boss+ protein)
boss+ gene
R8
Binding initiates a signal transduction cascade in R7 precursor
R7 precursor becomes R7 photoreceptor
R8
w+boss+
w- boss-
1
2
3
4
5
6
7
8

# Drosophila eye development

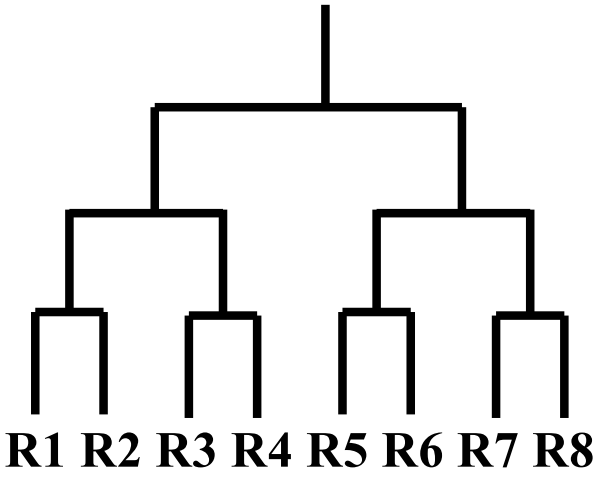


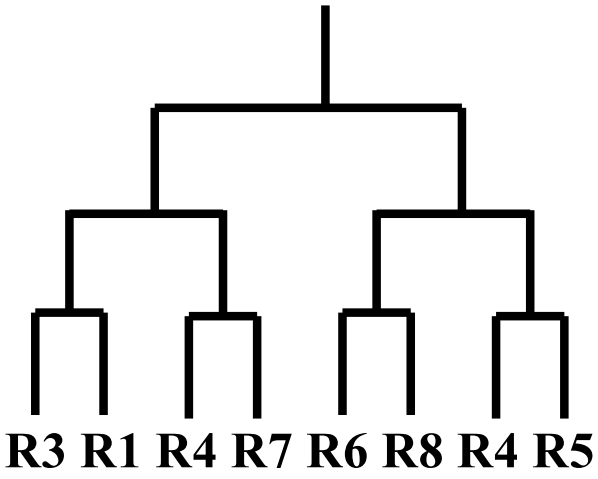
# How do cells in the fly eye develop: an invariant lineage, or no lineage restrictions?

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




**No lineage restrictions?**

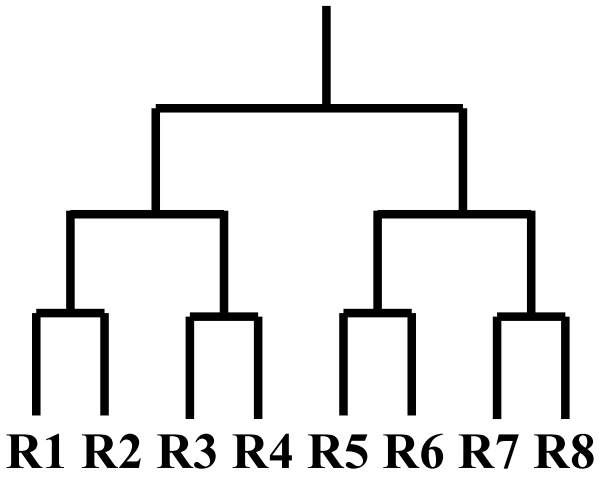


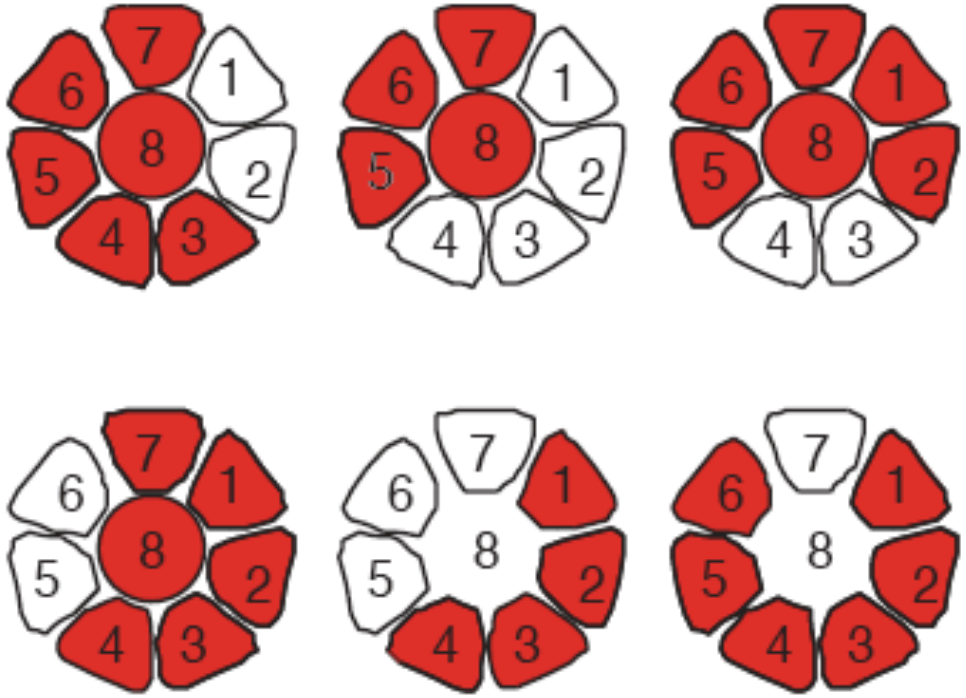




**And all other combinations!**

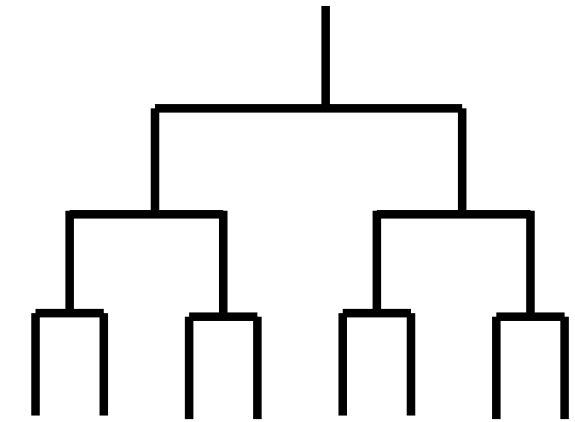
# Irradiate *white*/+ animals and study mosaic eyes.

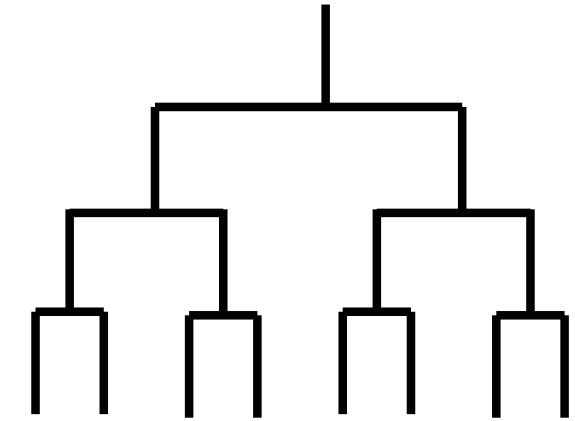
## If invariant lineage, then lineage relationships will be maintained in *white* clones



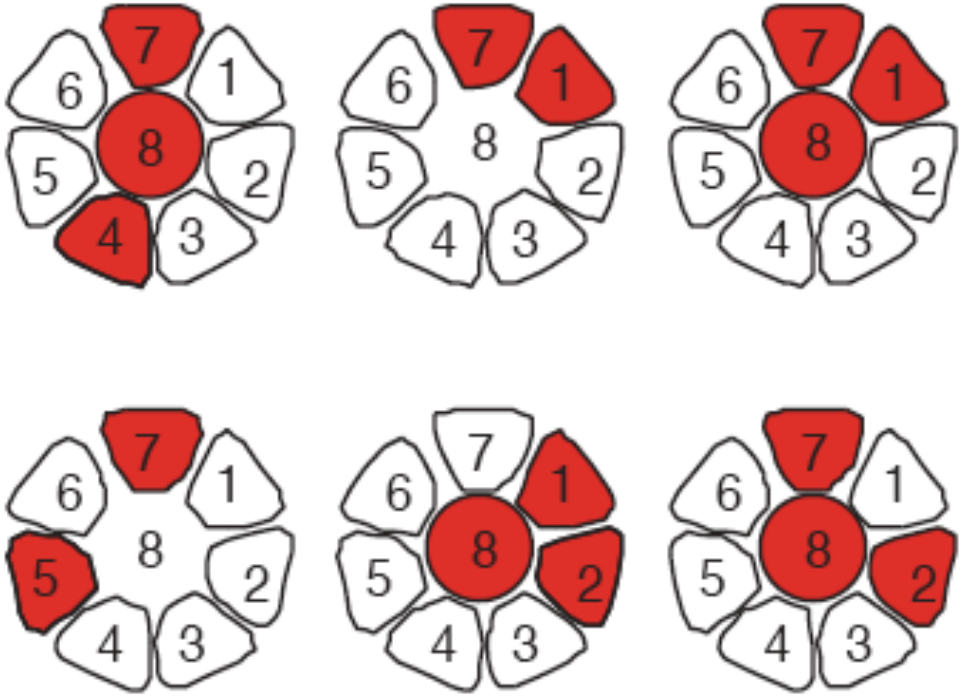



**If variant, then there will be no lineage relationships among cells in clone.**



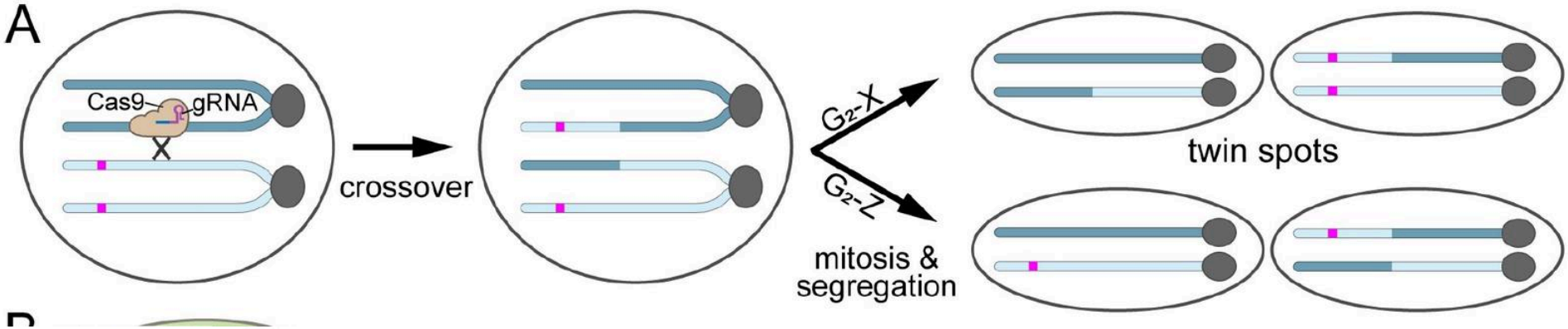



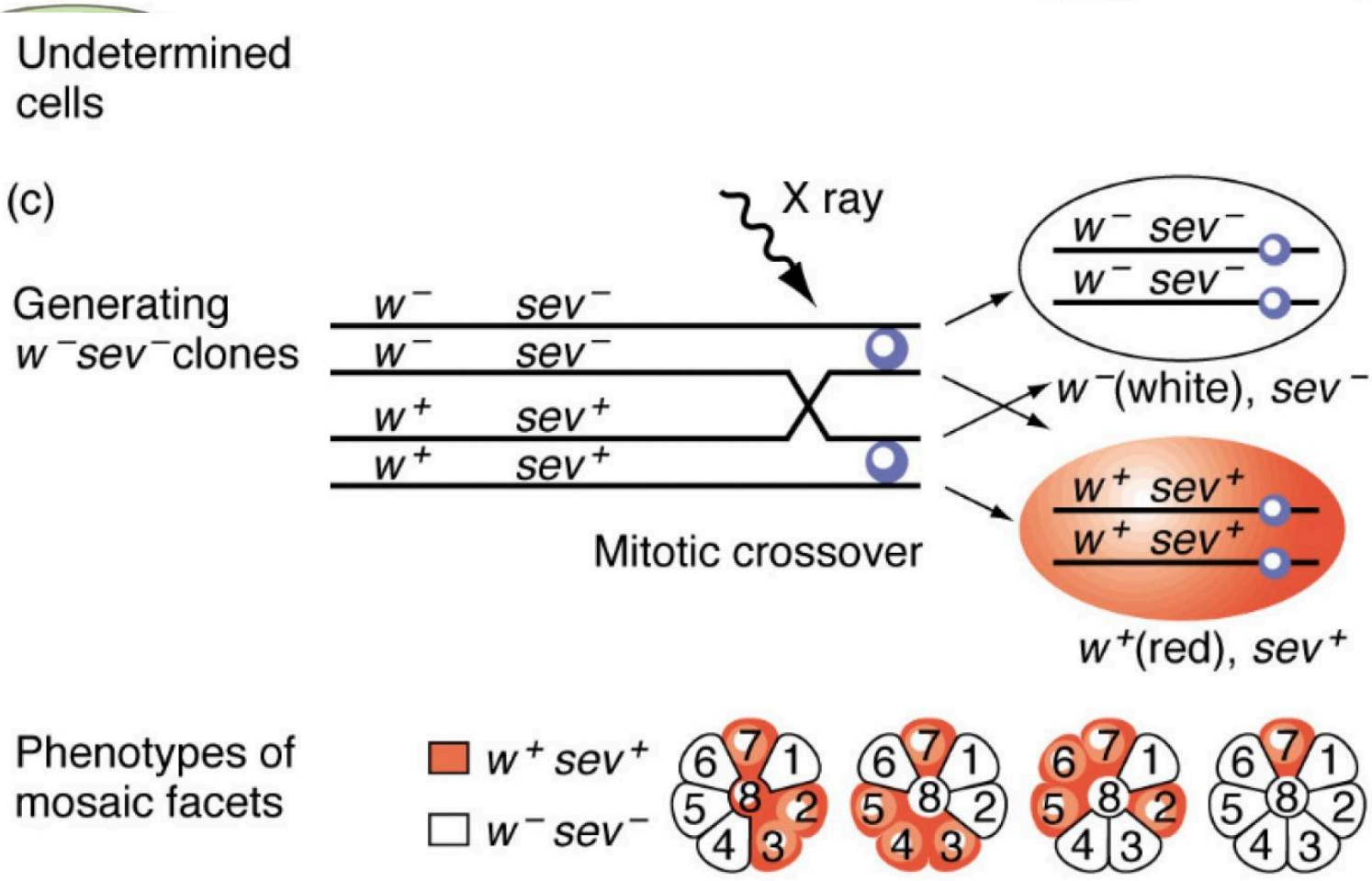

Etc…



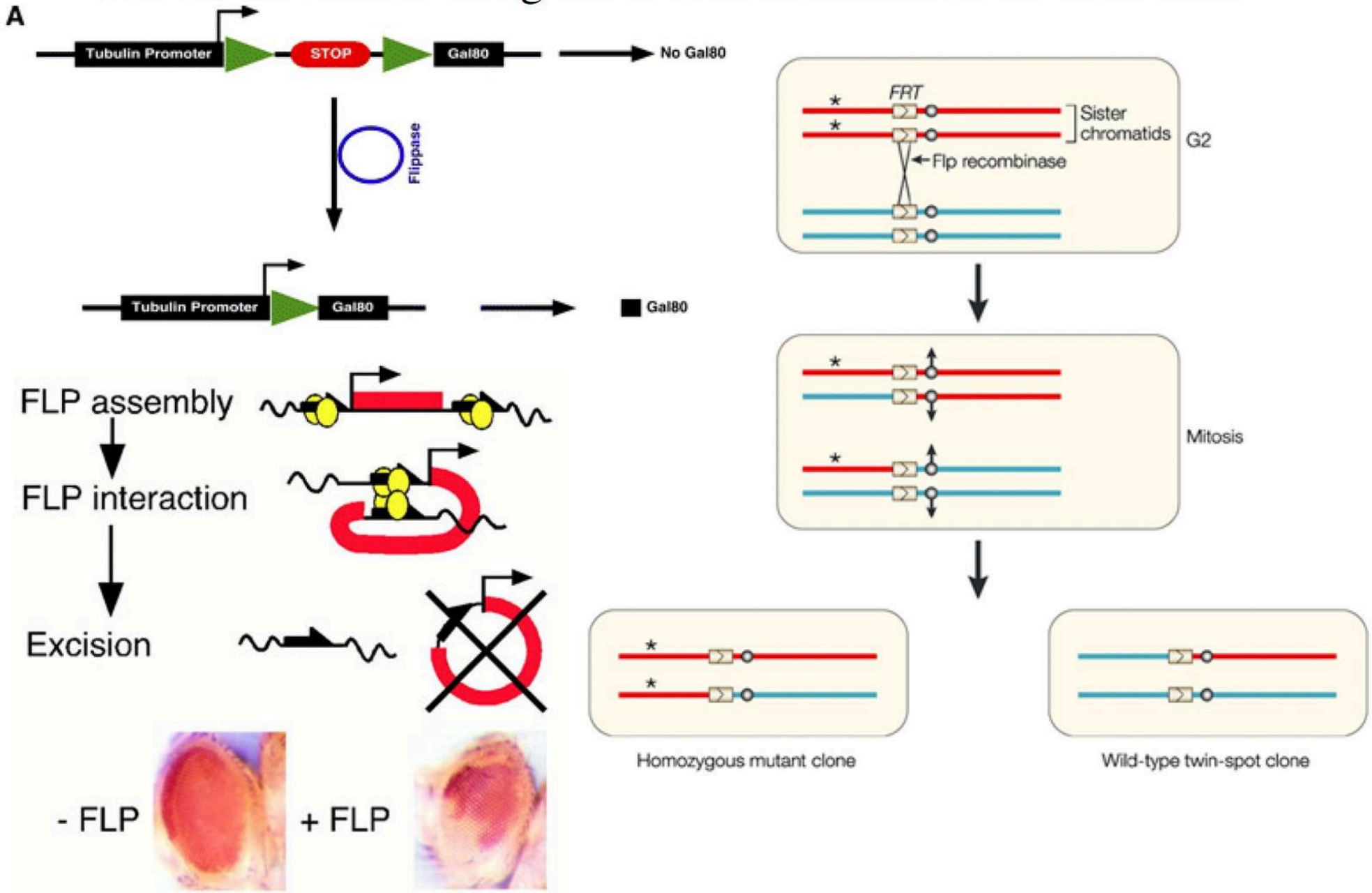

**This is what happens!**
**Cells are specified entirely**
**By cell interactions.**

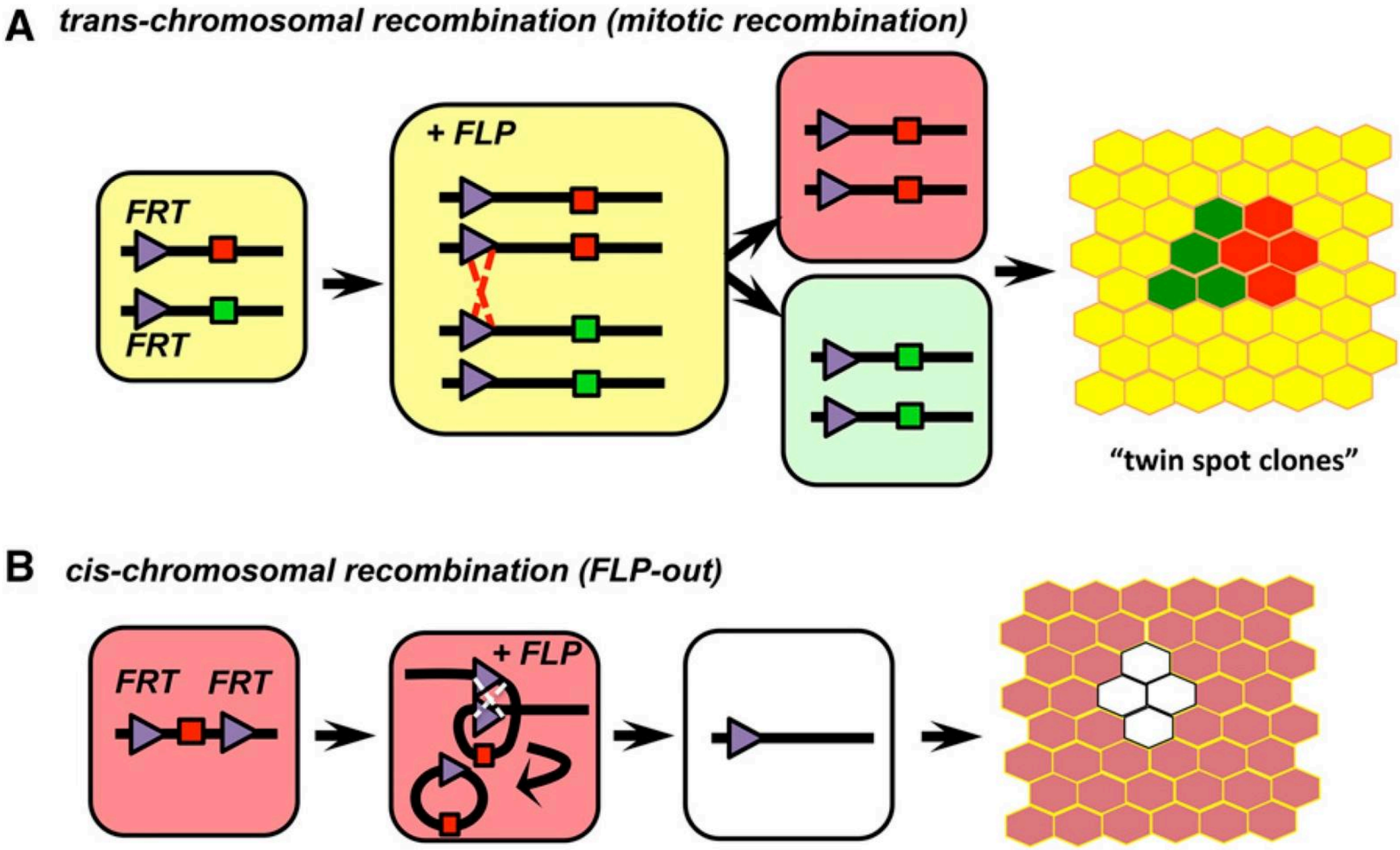
# Targeting the location of DNA breaks for recombination



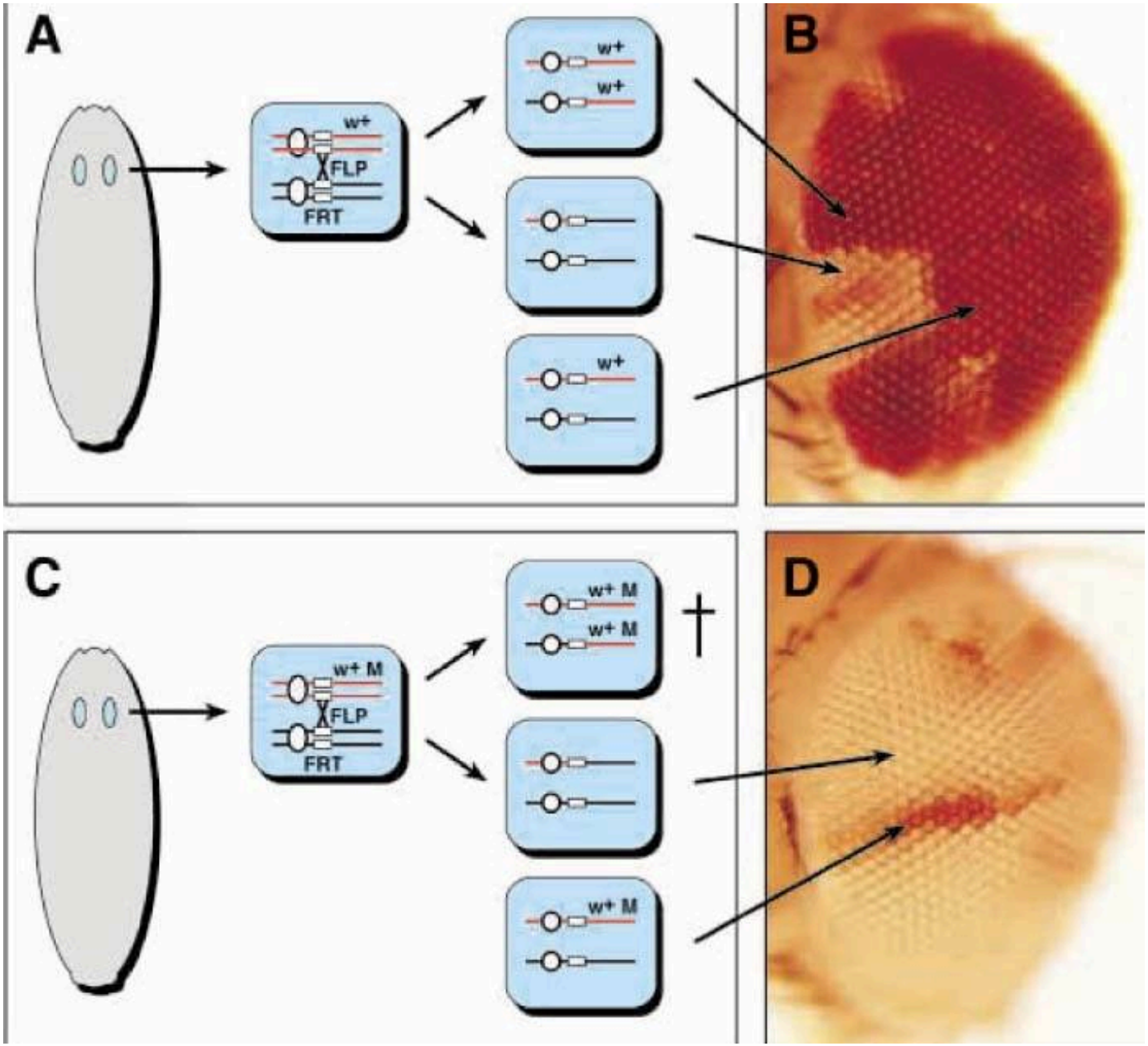



FLP is a site-specific recombinase that acts on target sites known as FRTs to bring about recombination in cis or in trans

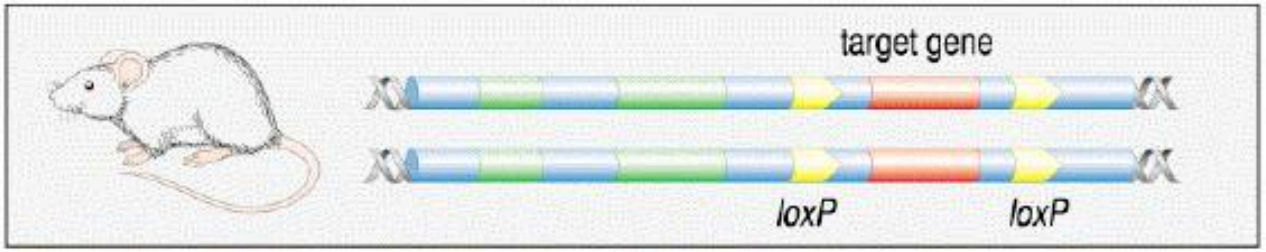



A
trans-chromosomal recombination (mitotic recombination)
FRT
FRT
+ FLP
"twin spot clones"
B
cis-chromosomal recombination (FLP-out)
FRT FRT
+ FLP

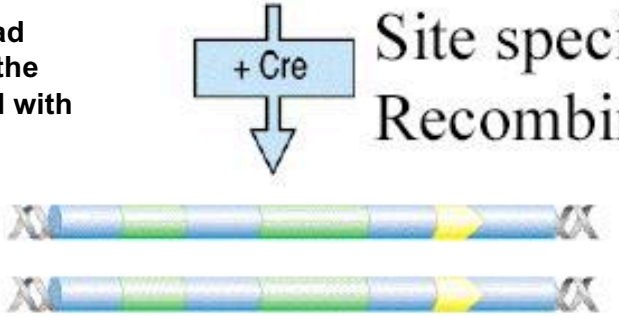
# FLP recombinase expressed from the *eyeless* promoter results in recombination just in the developing eye.



# 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.**

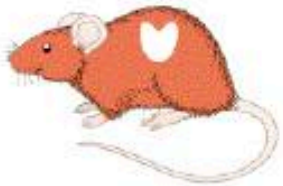



Site specific Recombinase

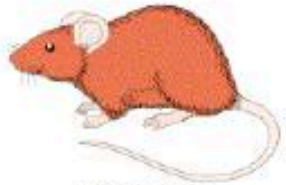
For example
to knock out gene
in a specific tissue

**Heart-specific Cre**

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



**Ubiquitous inducible Cre**



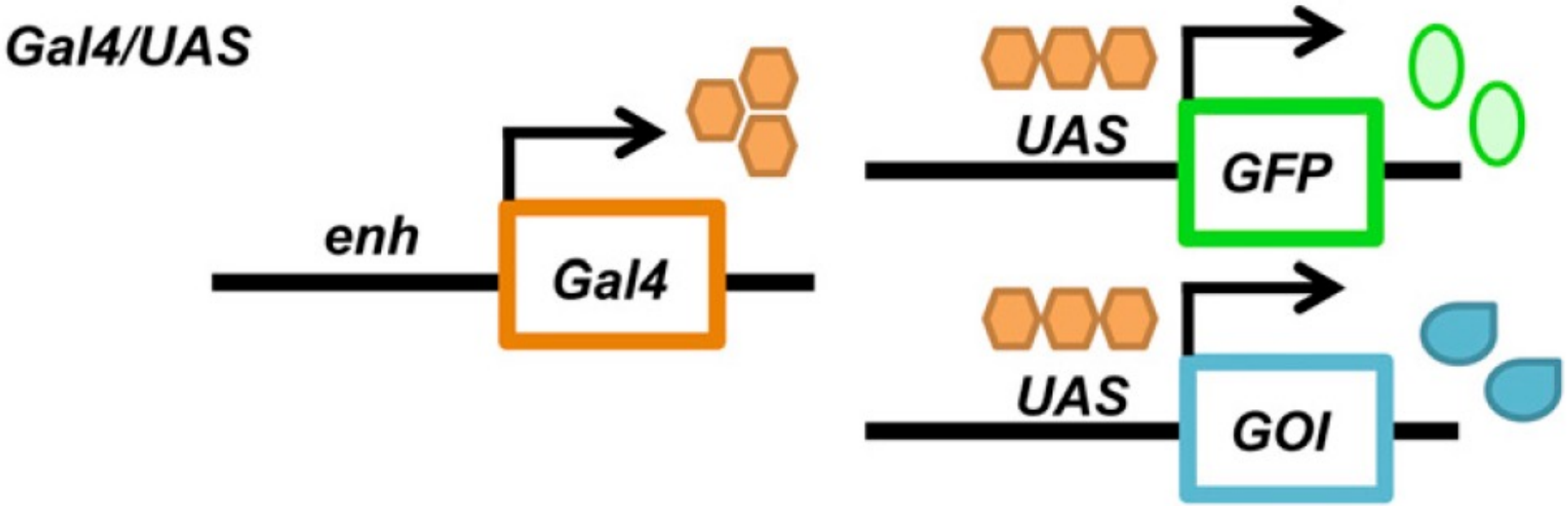
+ inducer

target gene expressed  target gene deleted

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

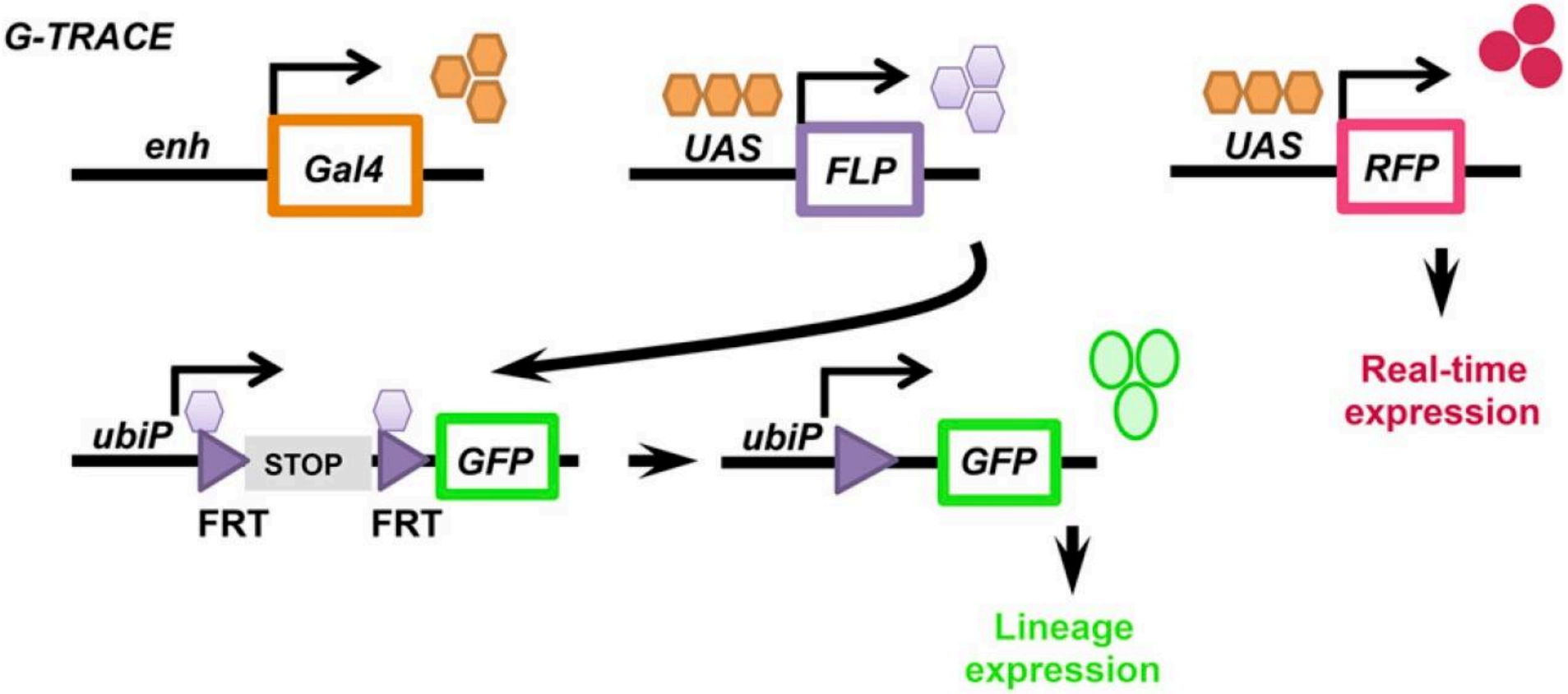
**Cre is a site-specific recombinase that acts on loxP sites. Recombination between direct repeats results in loss of the target gene.**

**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.**



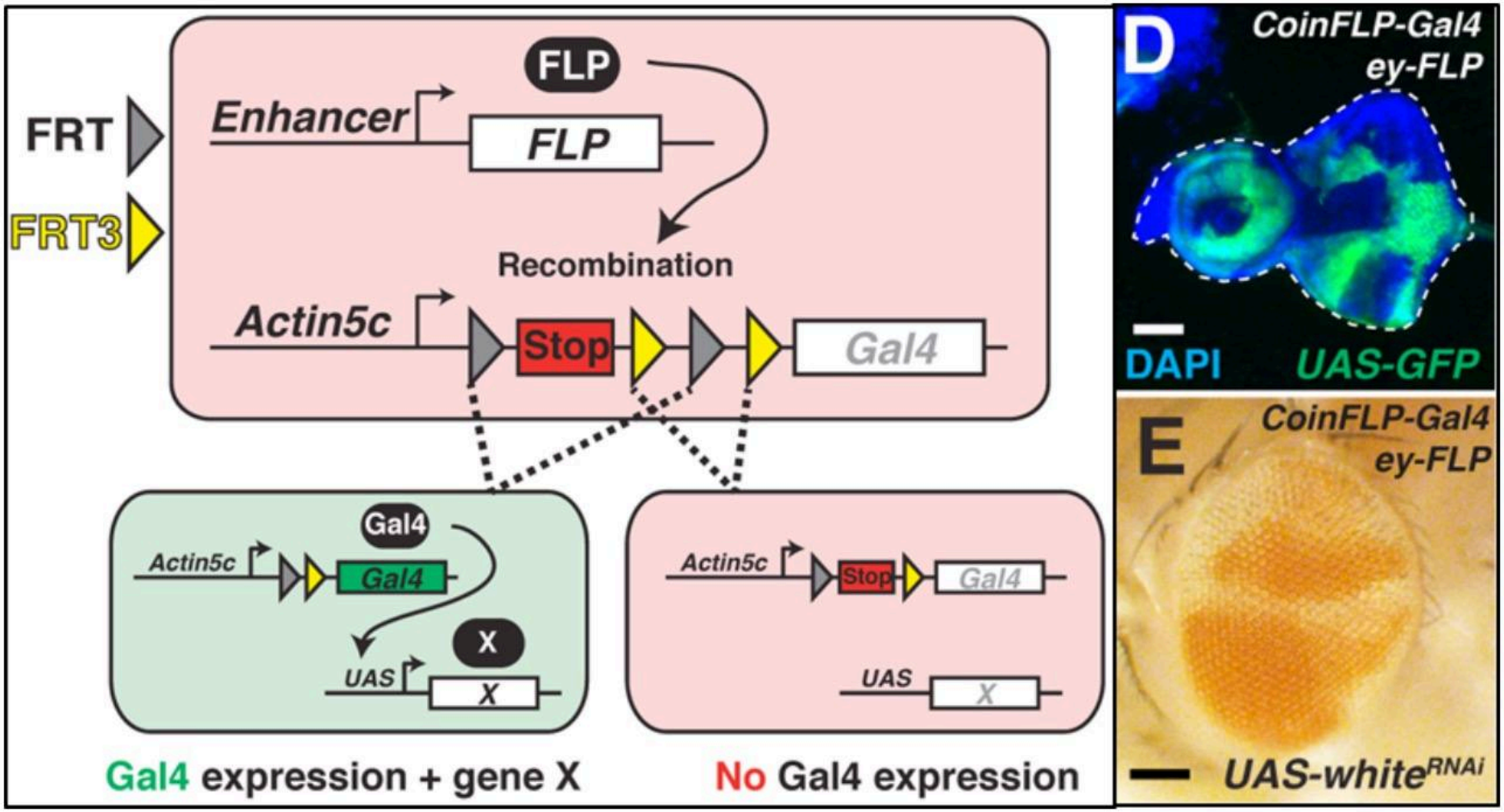
# Marking a cell lineage and visualizing a pattern of gene expression

tells you where gal4
is currently expressed

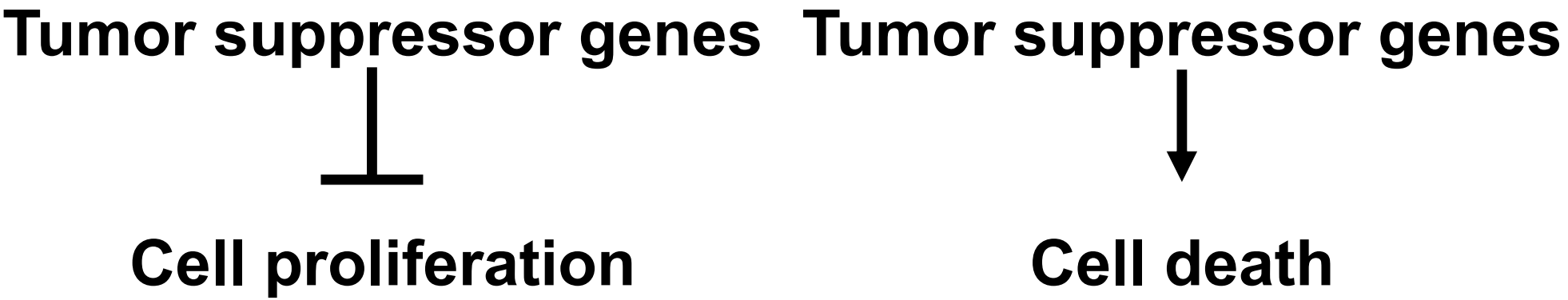



tells you where gal4 was expressed in the past
(what is the fate of this lineage of cells?)

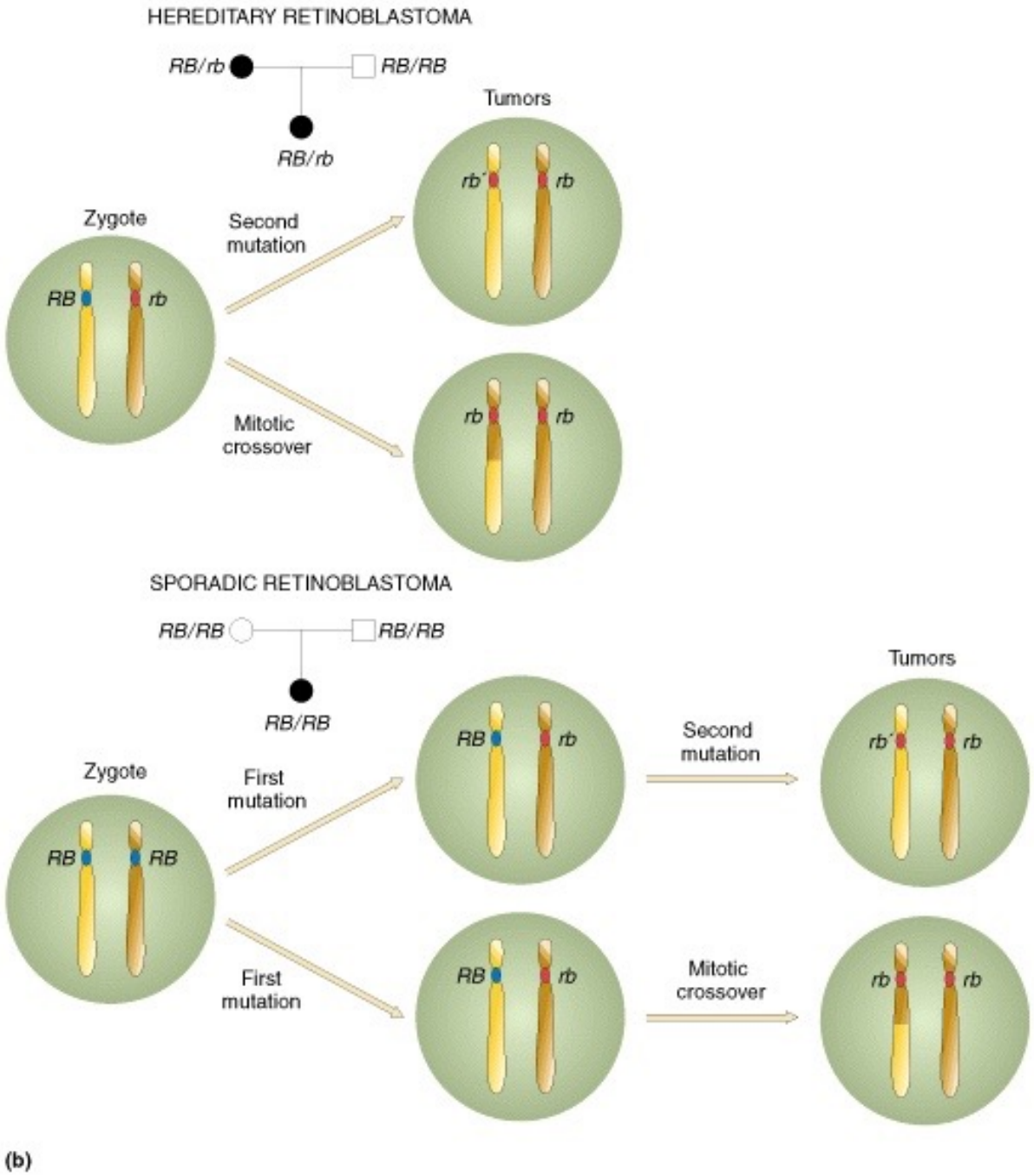
# Coinflip: turning on spots of expression of a gene of interest



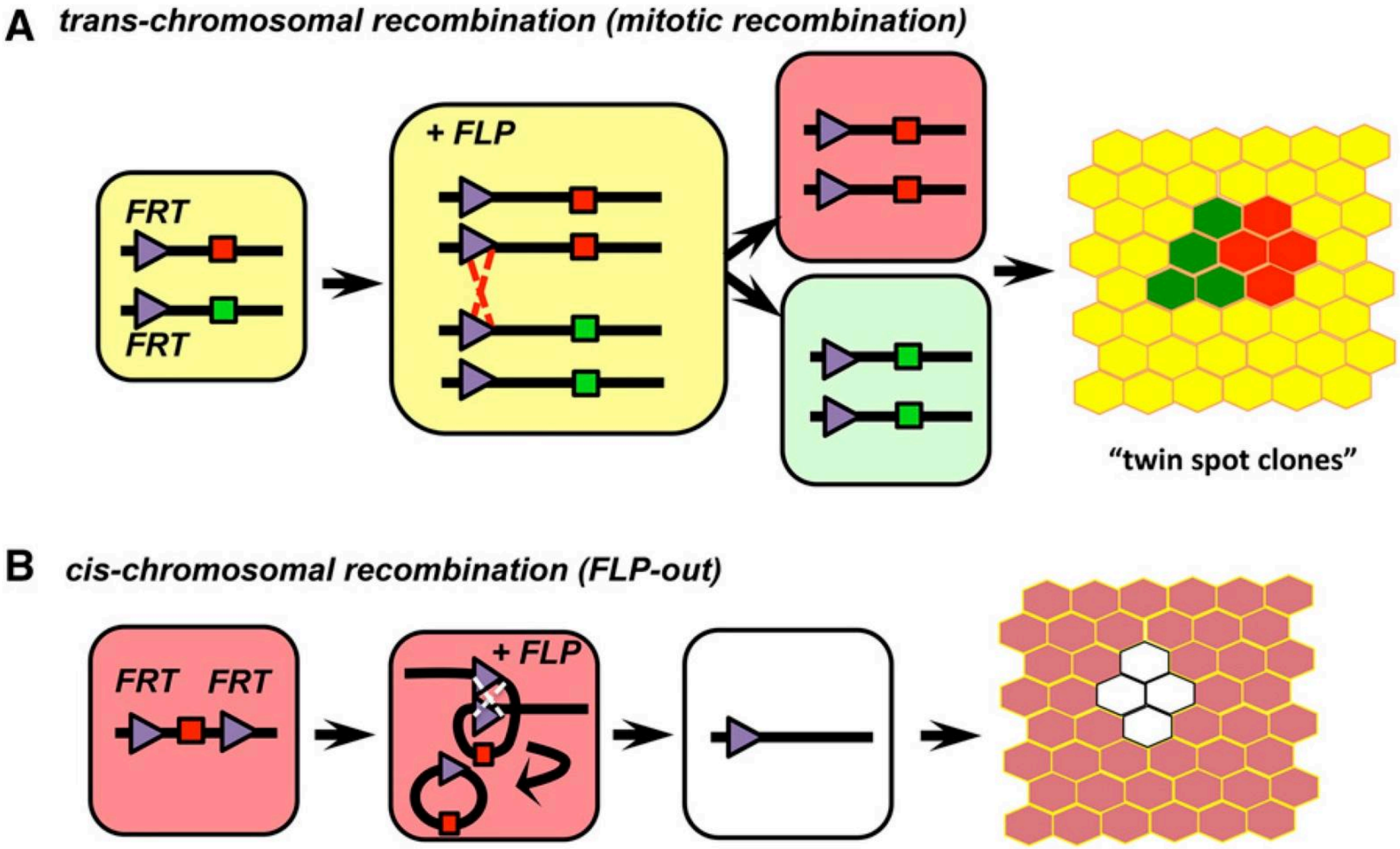
# Tumor suppressor genes can either inhibit cell proliferation or promote cell death.



# How can we identify mutations in tumor suppressors ?

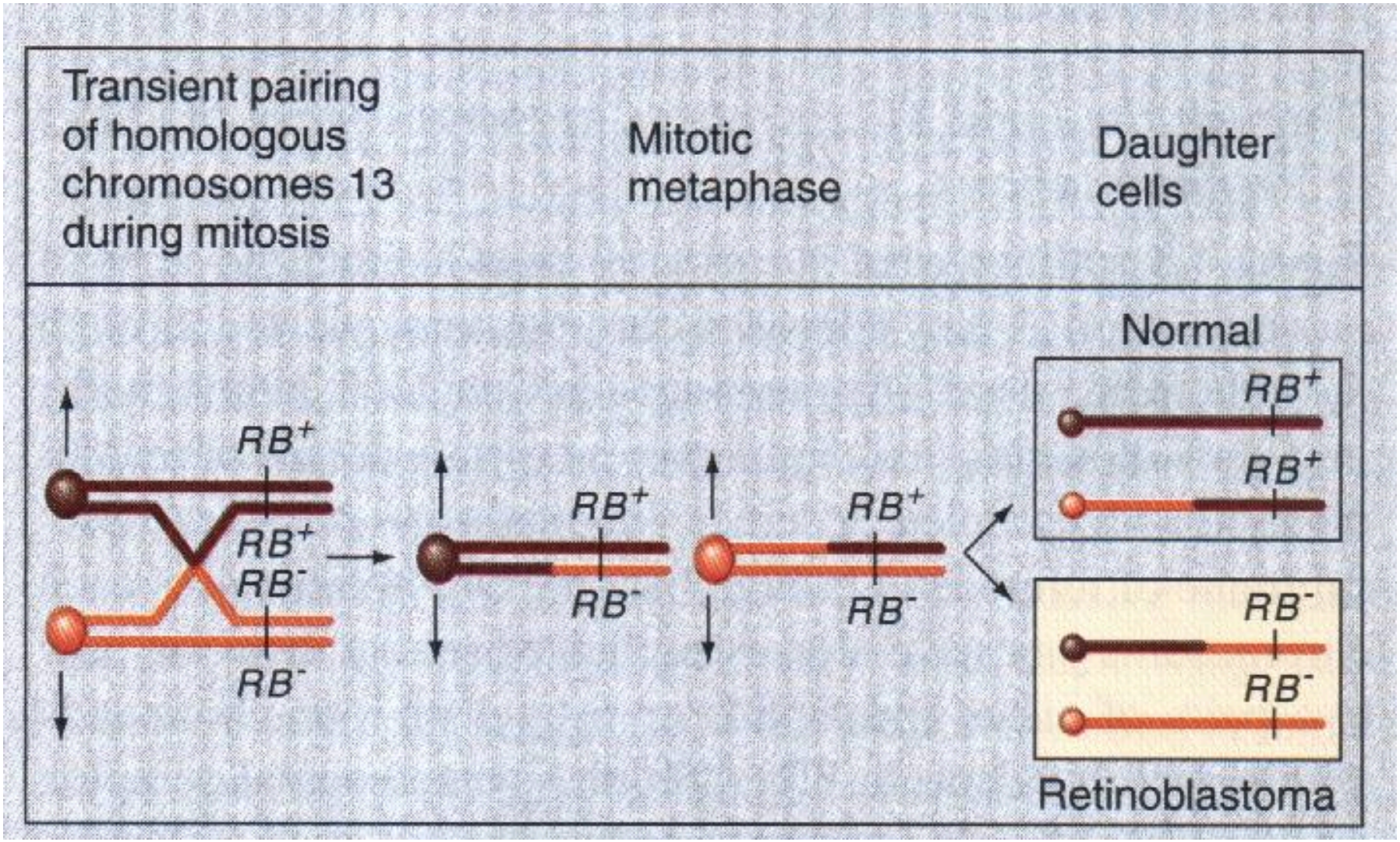


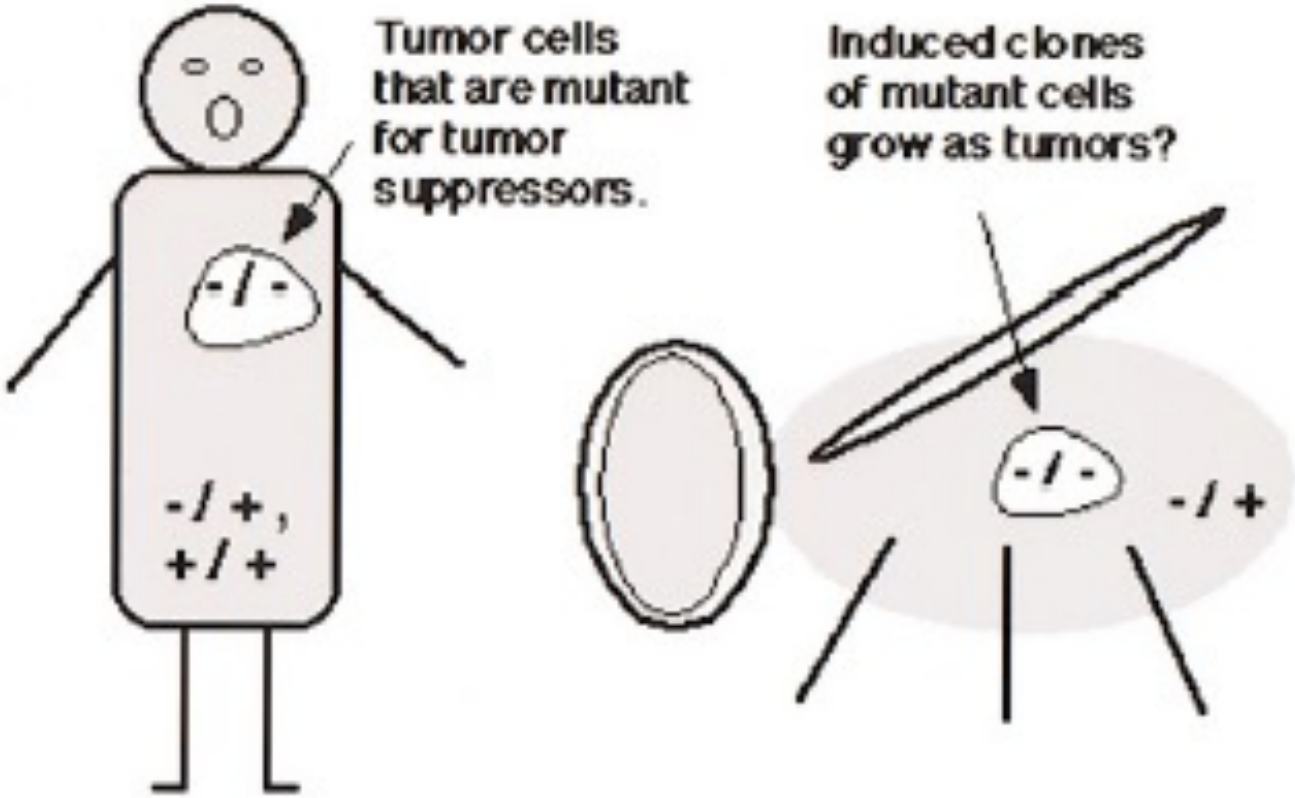
# Expression of FLP recombinase can stimulate mitotic recombination at FRT sites.



# 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.**

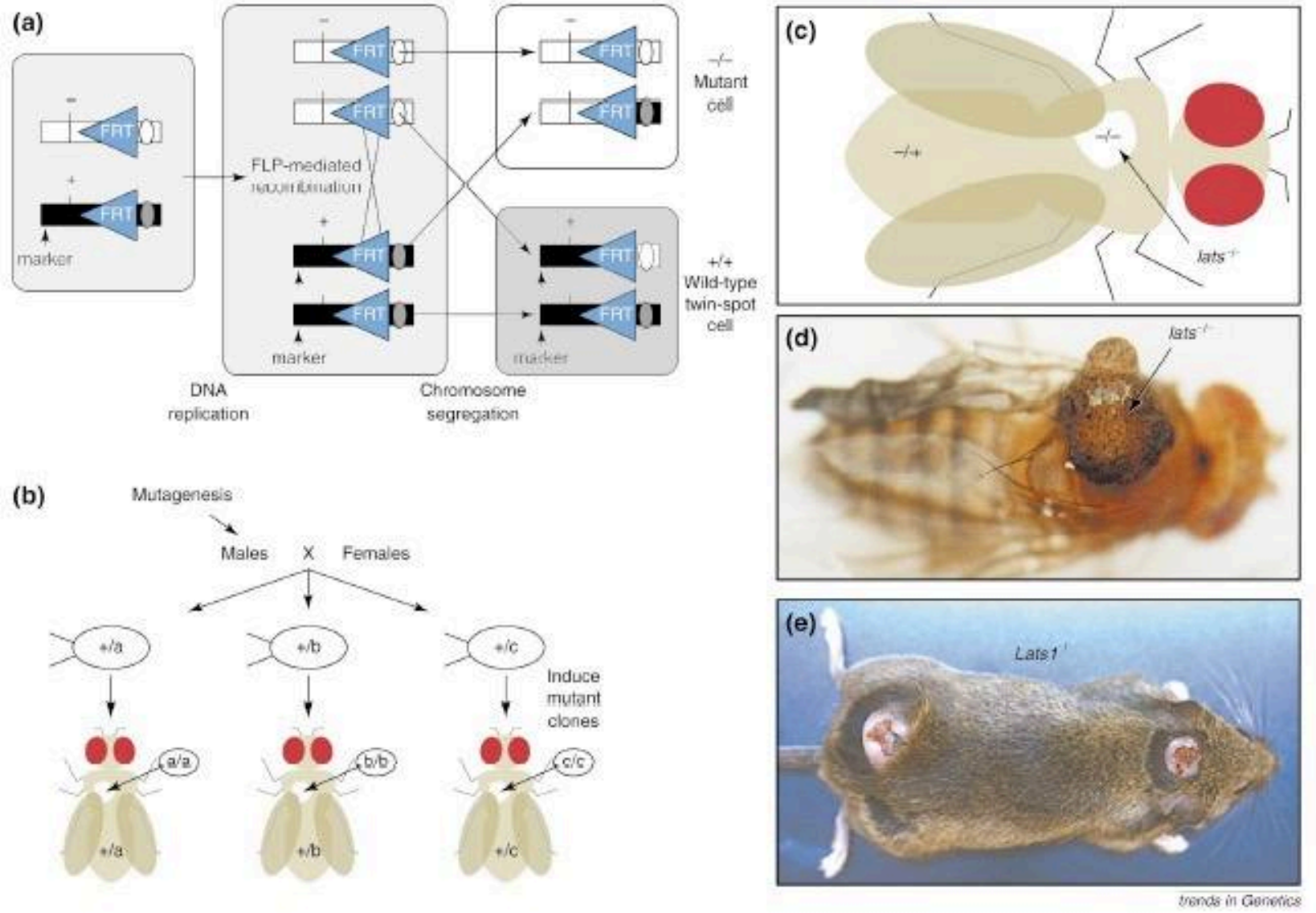





Tumor patients

Screen for tumor suppressors in mosaic flies

# A single generation screen for (recessive) tumor suppressors




(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).

# How are pathways constructed?

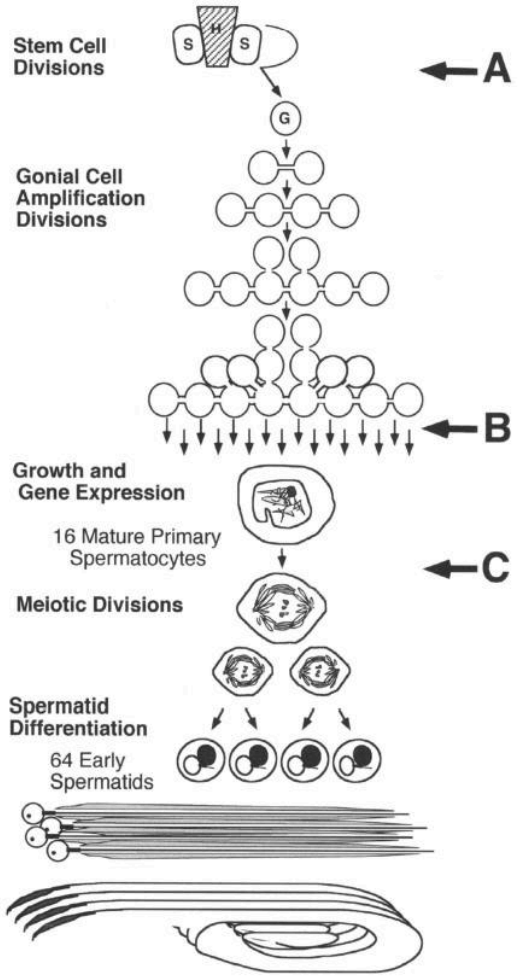
1. In a regulatory or switch pathway the upstream gene product alters the activity of the downstream target. Therefore, the downstream gene is epistatic.

2. In a biosynthetic pathway products of the early steps in the pathway are substrates for the later steps. Therefore, the upstream gene is  epistatic.

3. In a developmental pathway early events in development are required for later events to occur. Therefore, the upstream gene is epistatic.

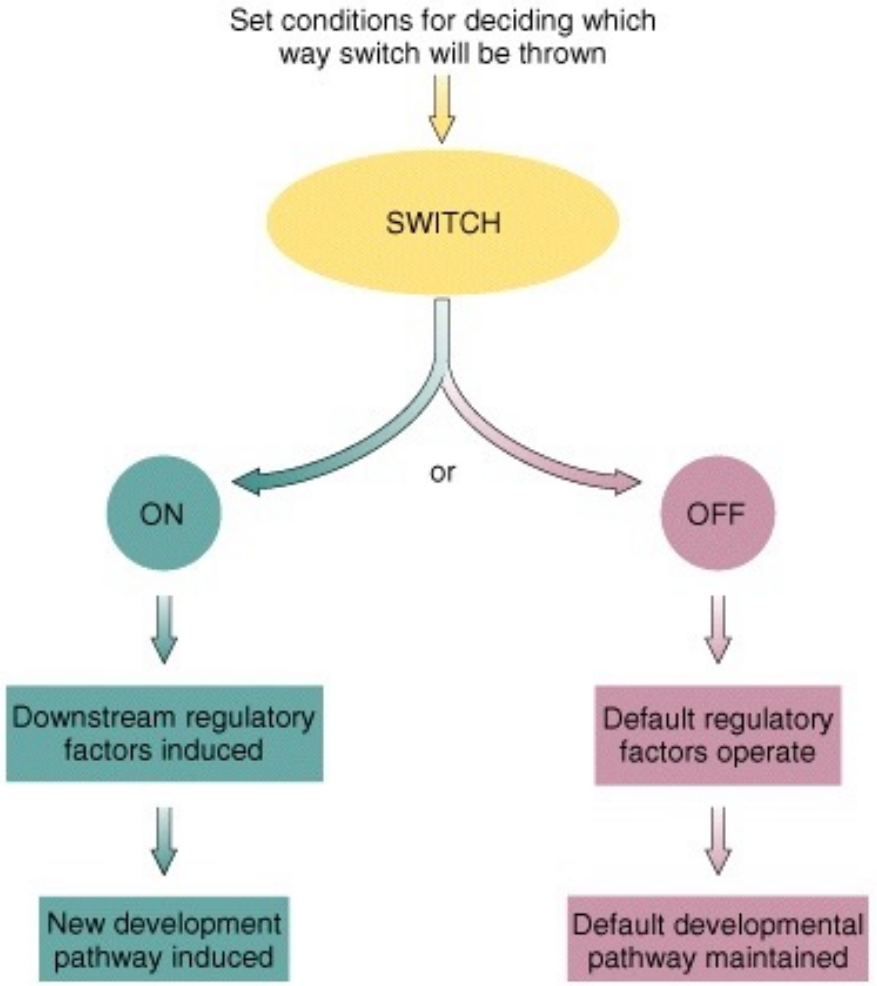
# Two kinds of pathways

In a biosynthetic/developmental pathway the goal is to make a final product



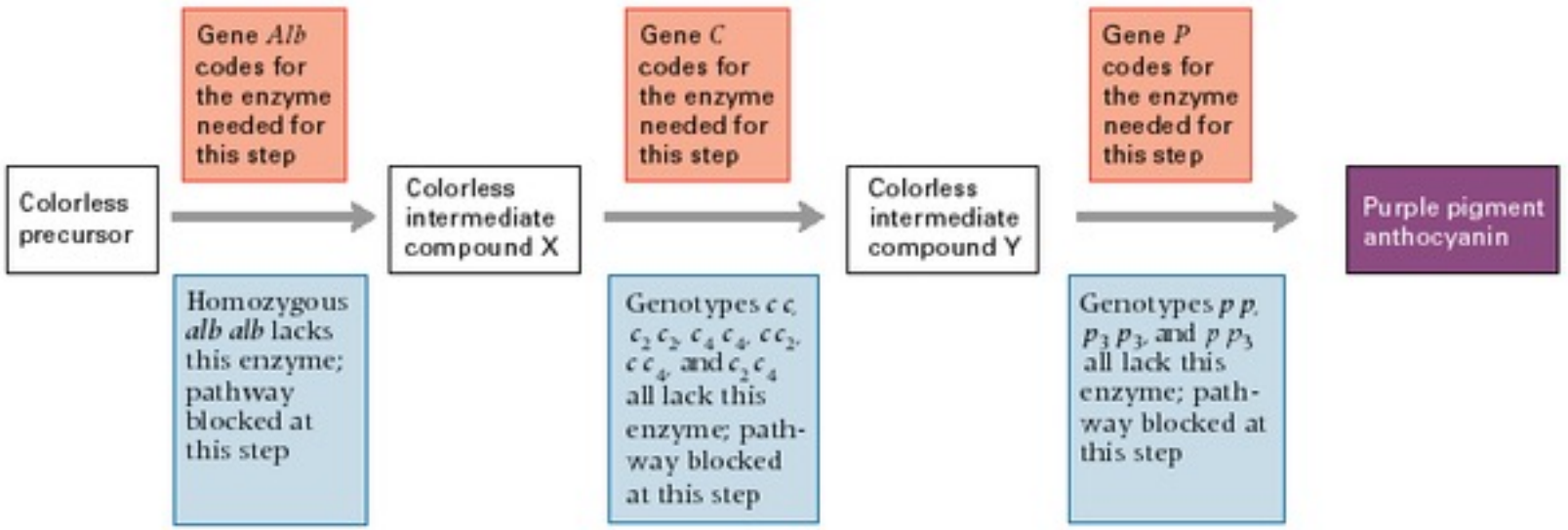

gure 1. Overview of wild type spermatogenesis in *Dro-bhila*, showing three major cell fate transitions. Male

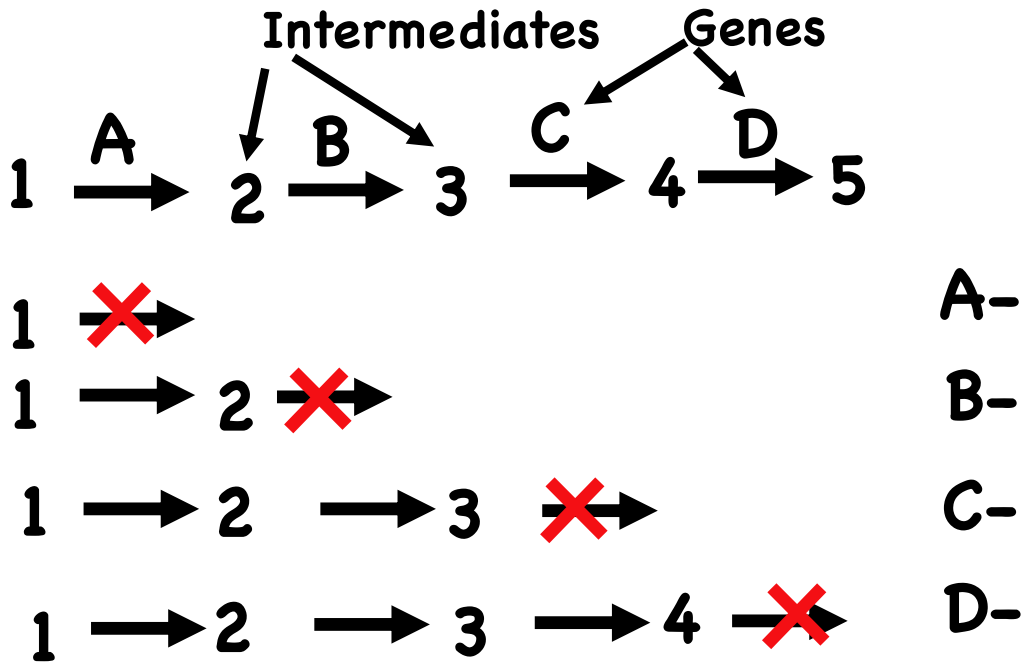
In a switch pathway the goal is to choose between alternative cell fates or states



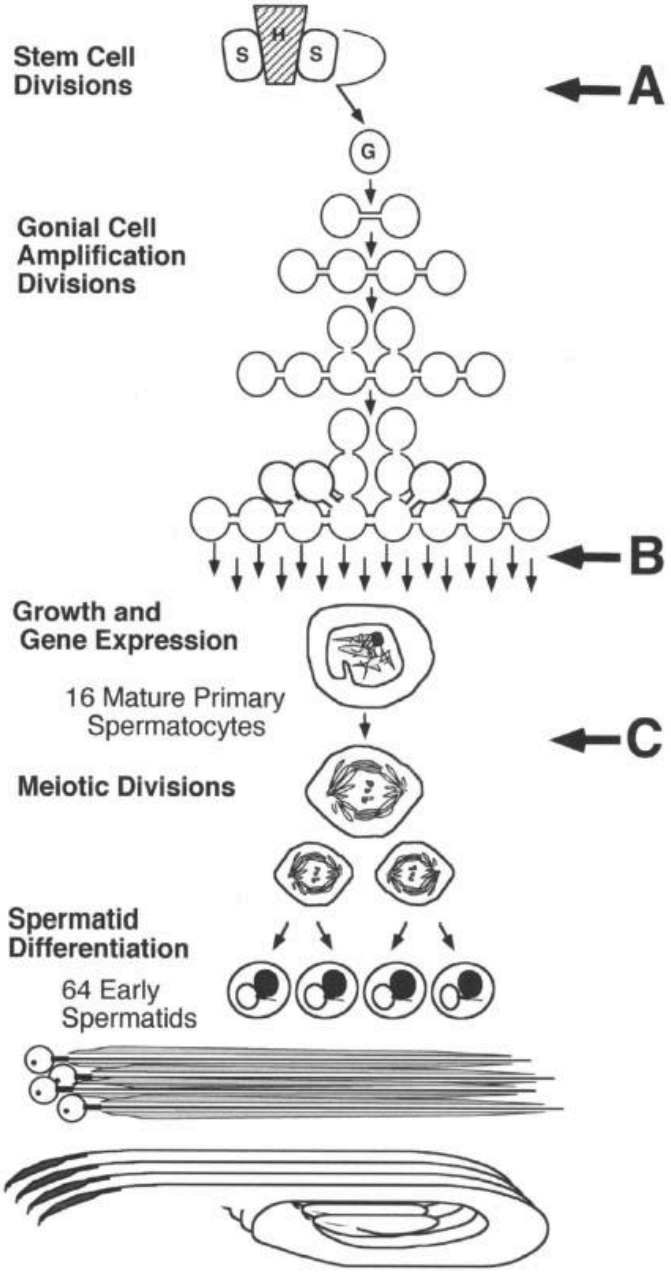
# In a biosynthetic pathway the goal is to make a final product

## Pathway mutations result in the buildup of intermediates

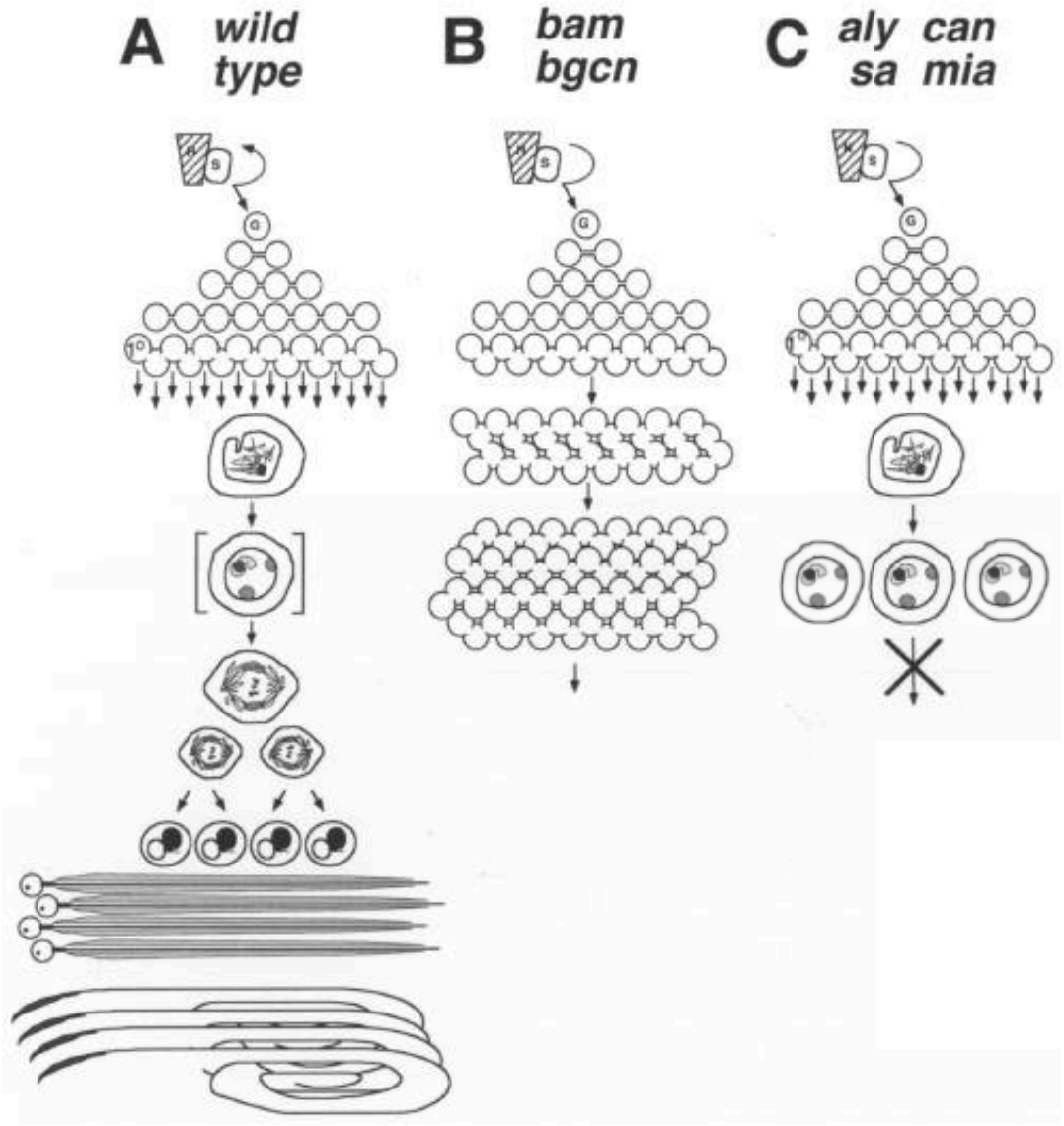


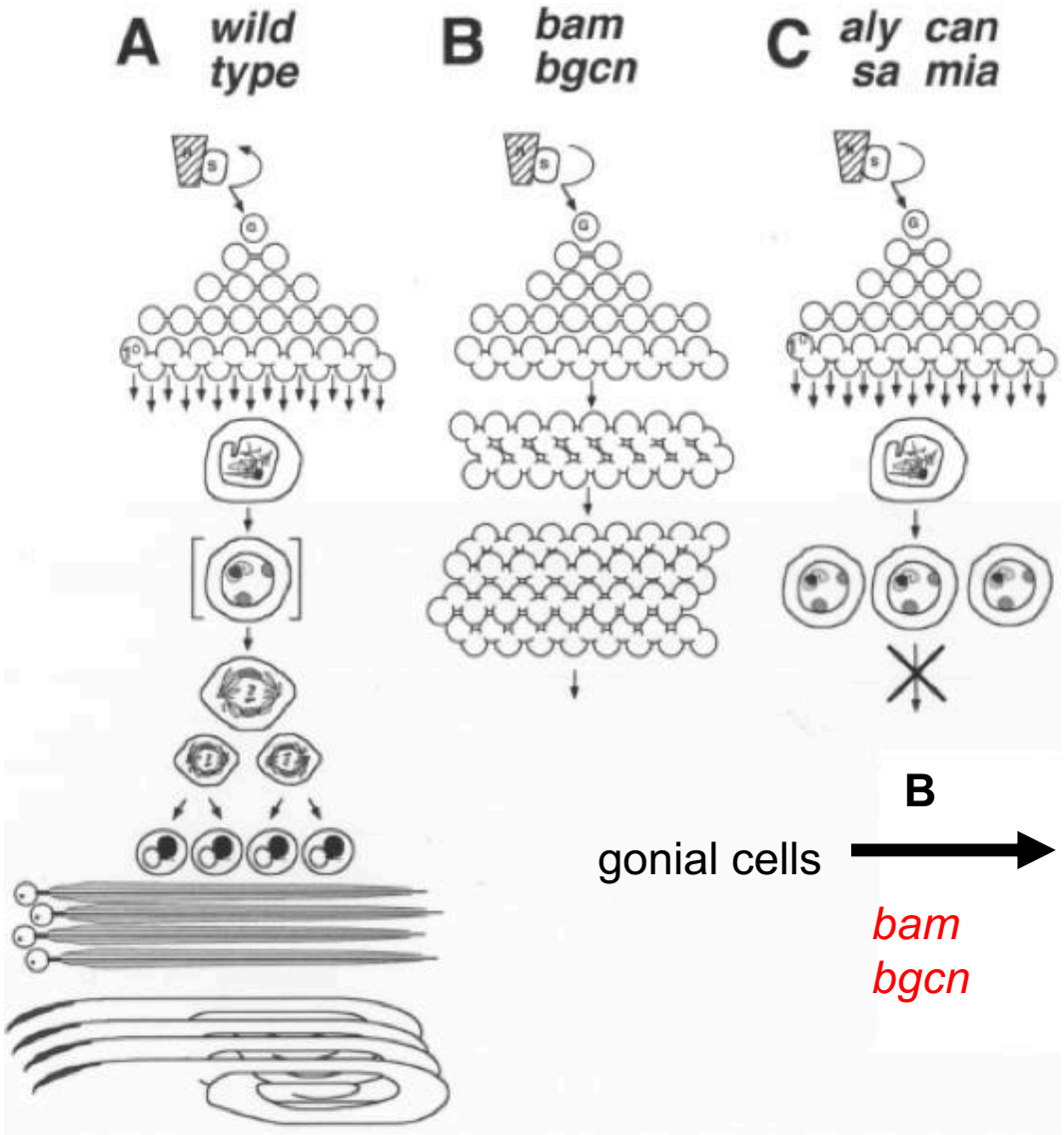
In a biosynthetic/developmental pathway one sees a buildup of substrates or differentiation intermediates




gure 1. Overview of wild type spermatogenesis in *Drophila*, showing three major cell fate transitions. Male



# In a biosynthetic/metabolic pathway the upstream gene is epistatic



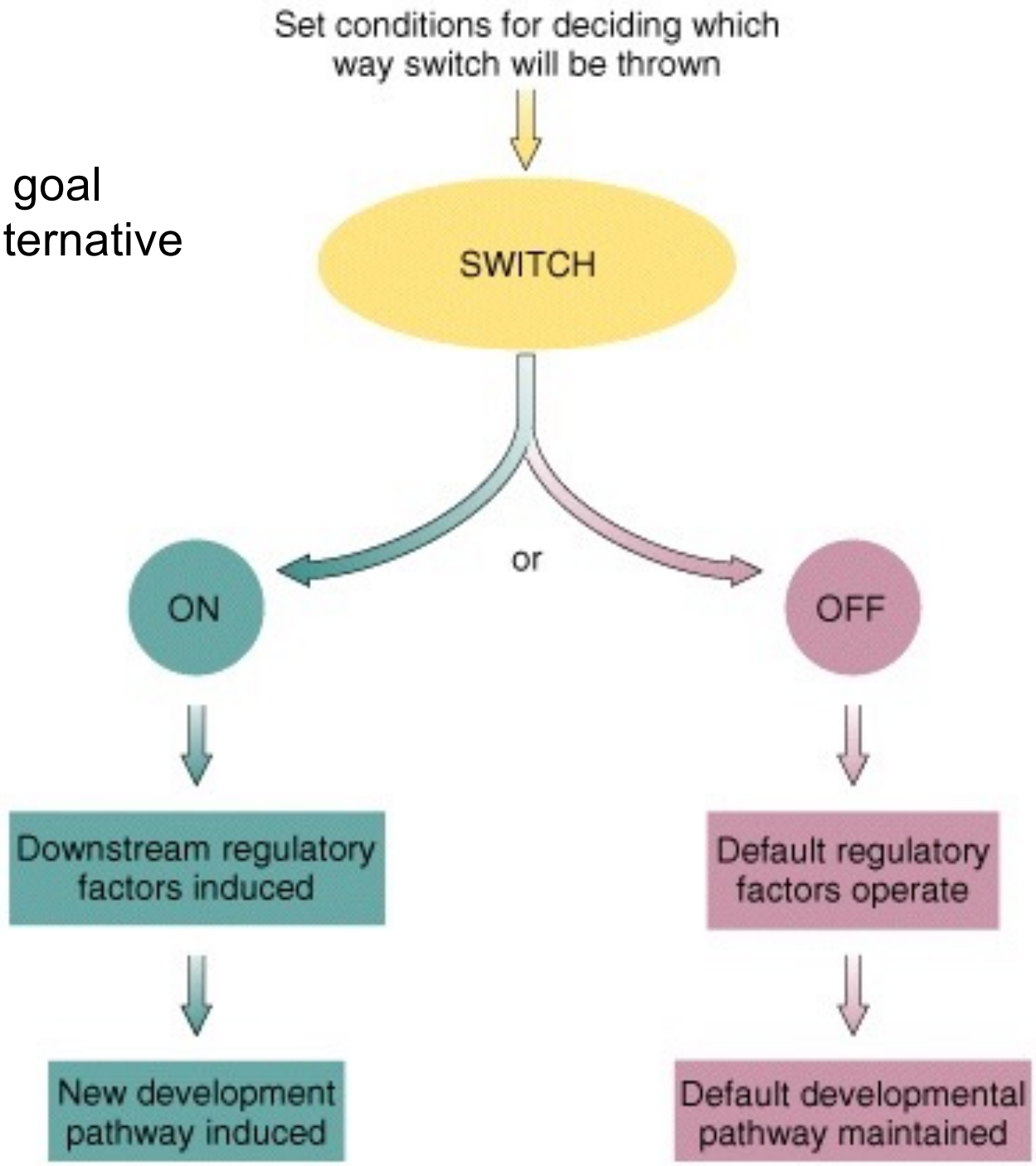

gonial cells —B→ primary spermatid —C→ sperm

*bam*
*bgcn*

*mia*
*aly*
*can*
*sa*

The phenotype of a *b; c* double mutant is extra gonial cells

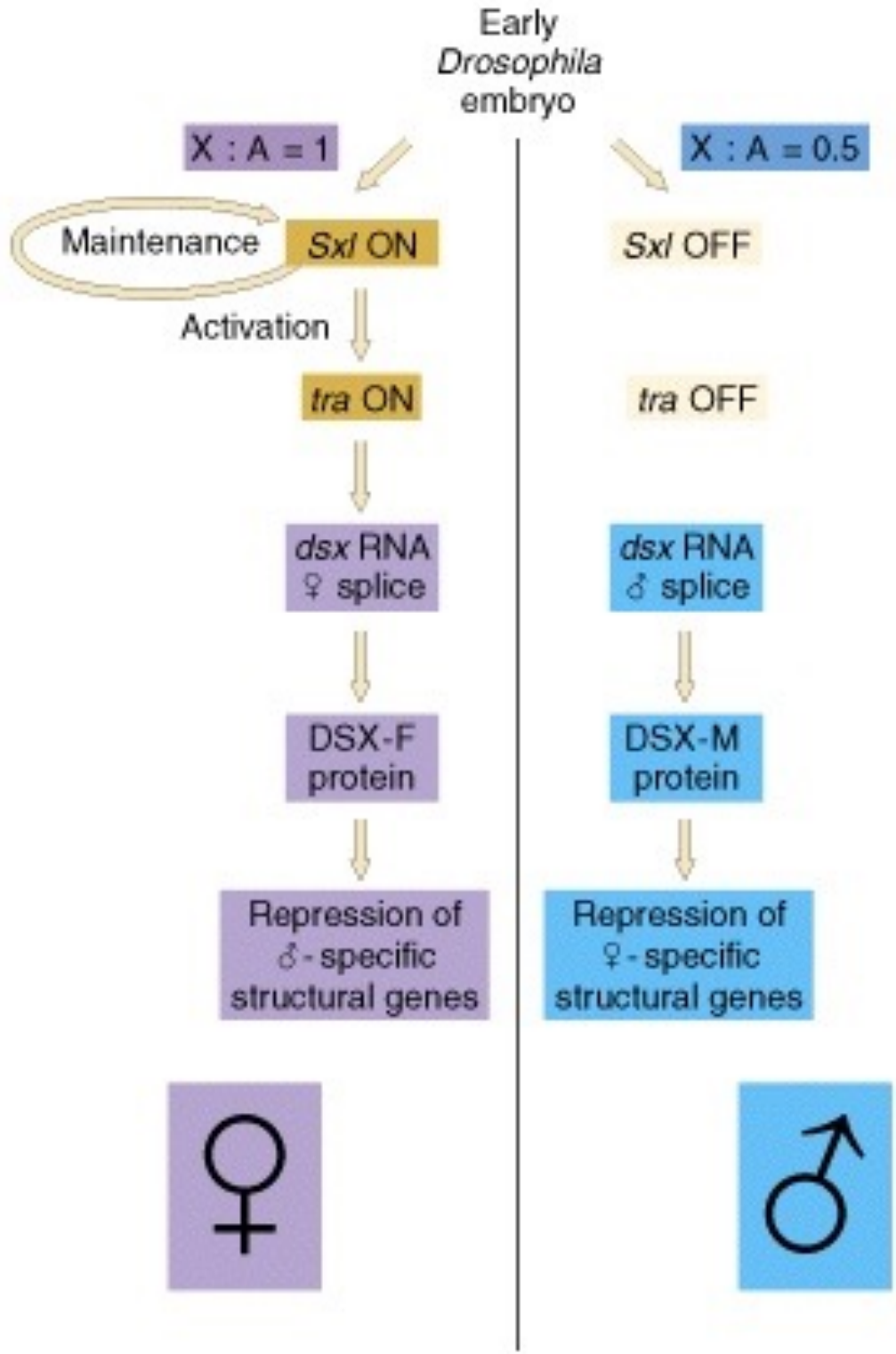
In a switch pathway the goal
is to choose between alternative
cell fates or states



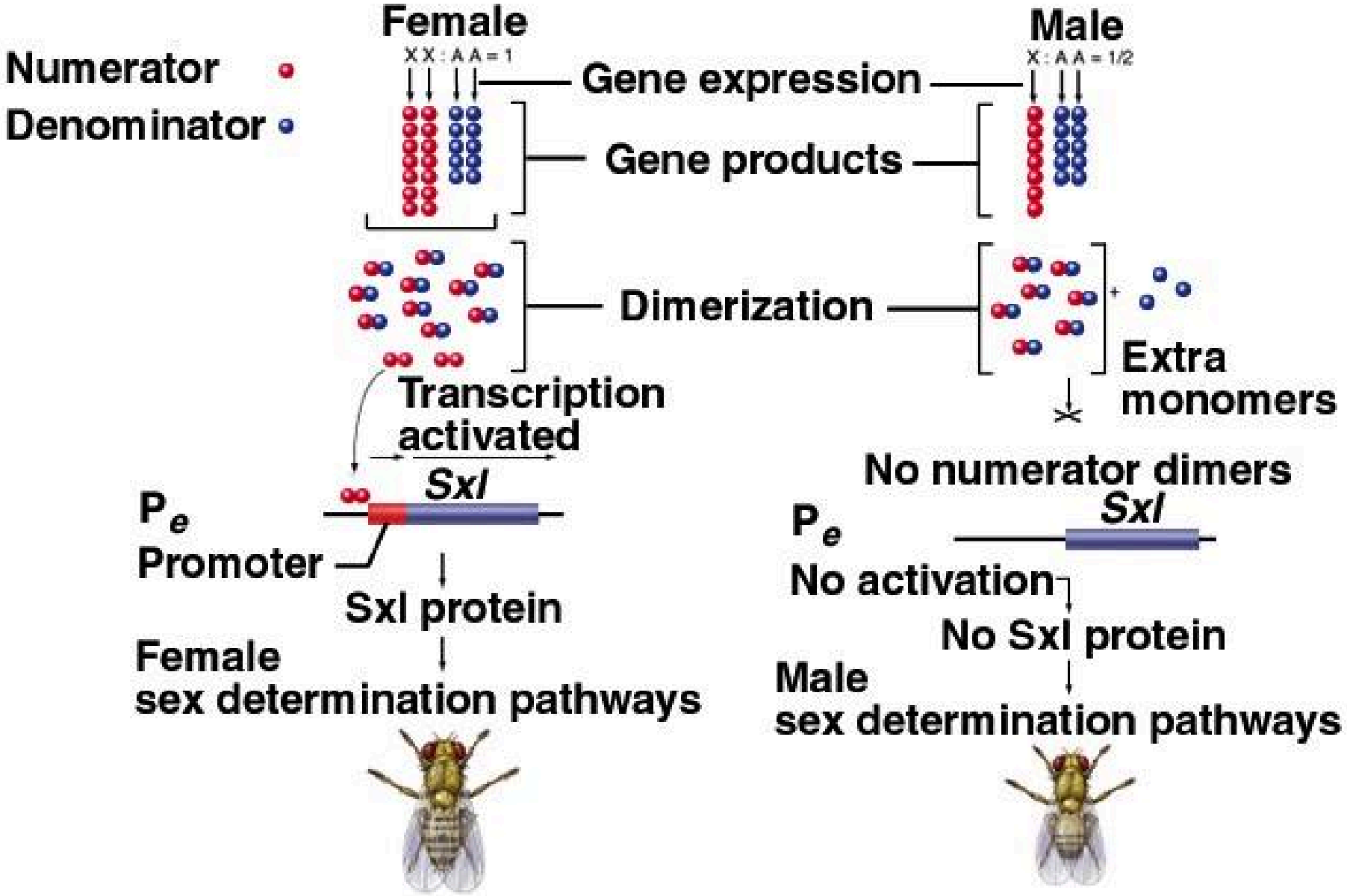
# A simple switch: the downstream gene is epistatic


| | Xgal |
|---|---|
| $lacI^-$ | blue |
| $lacZ^-$ | white |
| $lacI^-$, $lacZ^-$ | white |

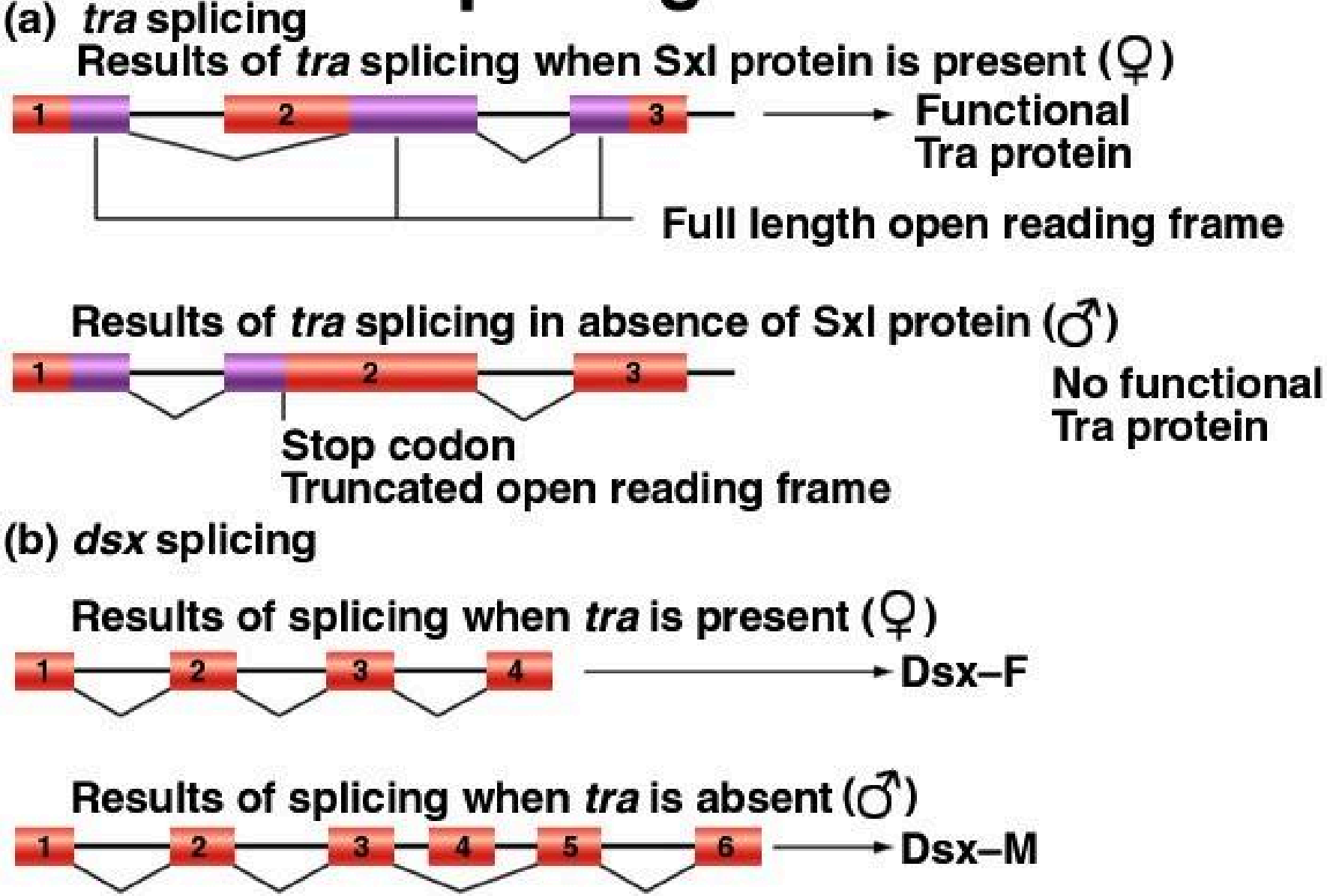
$$lacI \dashv lacZ$$

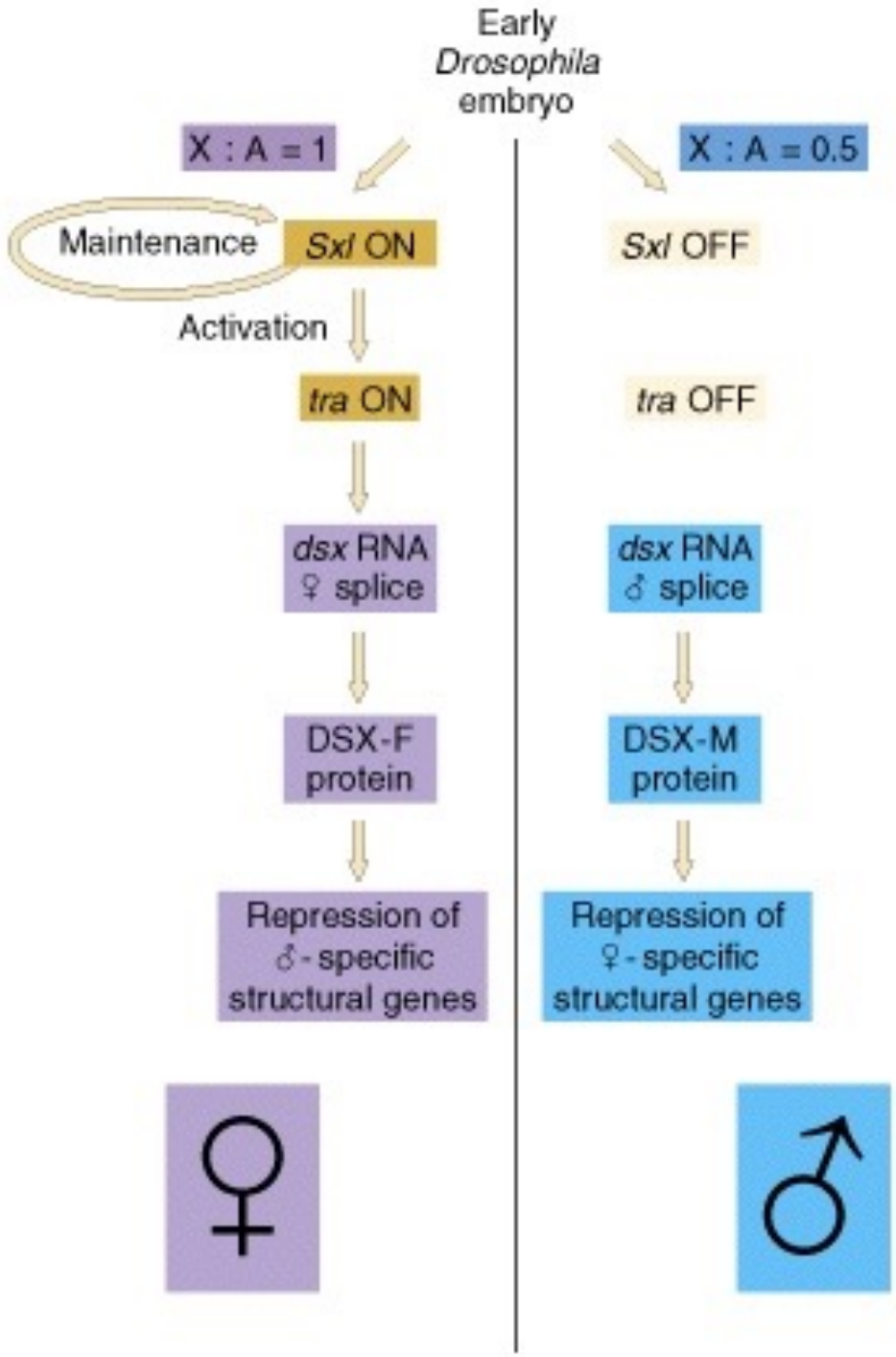

Early
Drosophila
embryo
X : A = 1
X : A = 0.5
Maintenance
Sxl ON
Sxl OFF
Activation
tra ON
tra OFF
dsx RNA
♀ splice
dsx RNA
♂ splice
DSX-F
protein
DSX-M
protein
Repression of
♂ - specific
structural genes
Repression of
♀ - specific
structural genes

# The X-to-autosome ratio

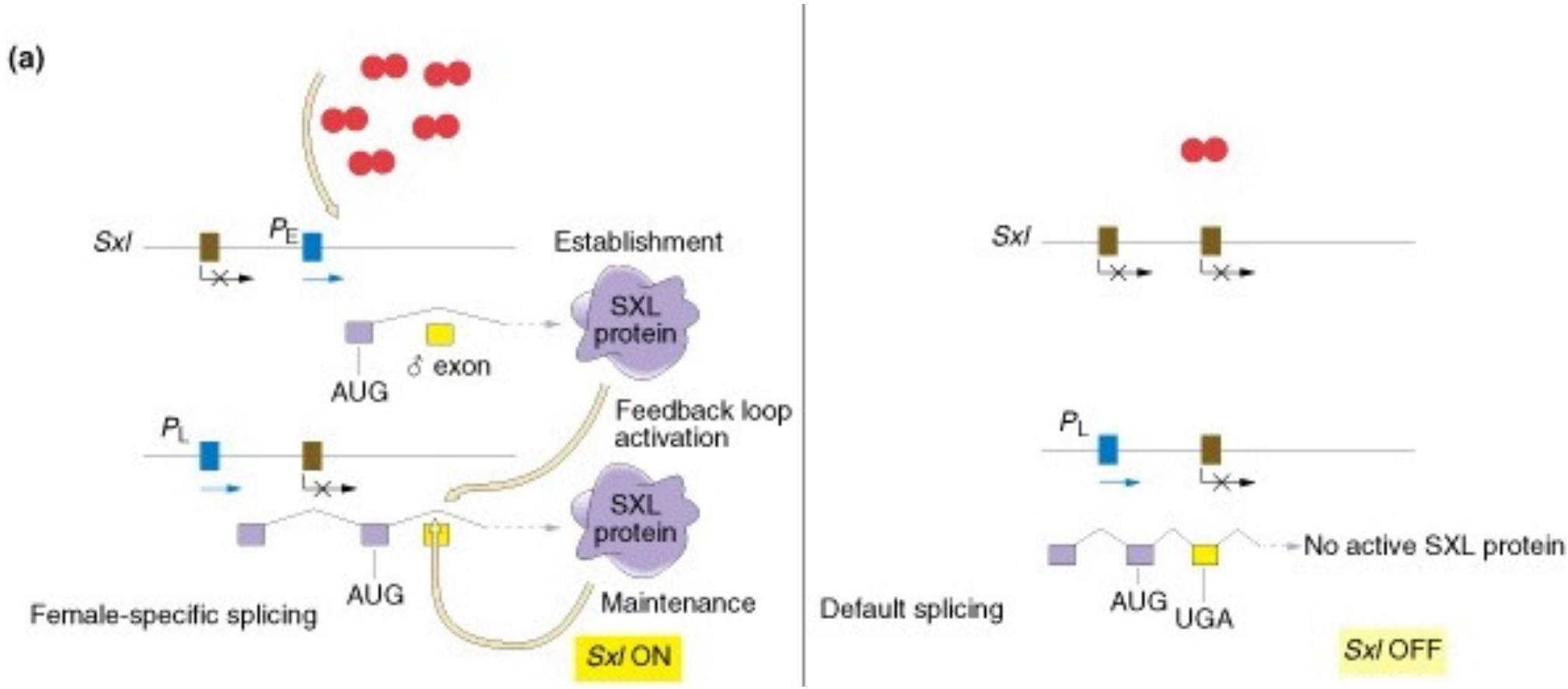


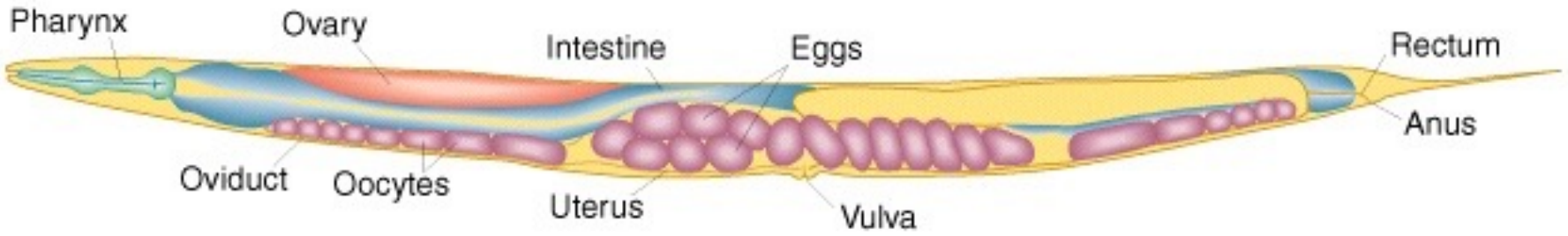
# A splicing cascade

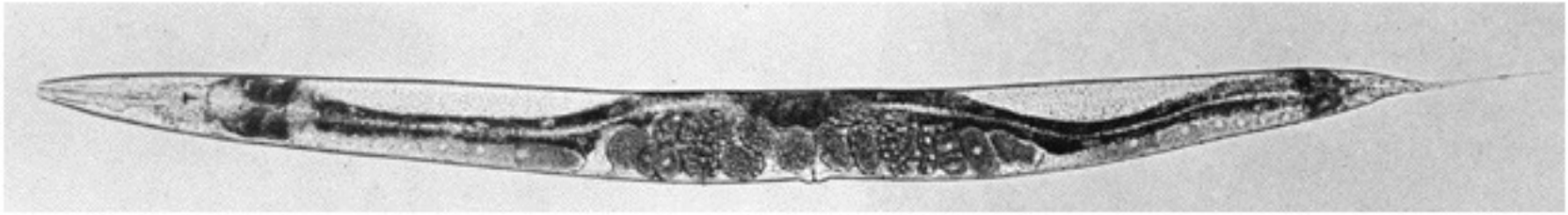



Early
Drosophila
embryo
X : A = 1
X : A = 0.5
Maintenance
Sxl ON
Sxl OFF
Activation
tra ON
tra OFF
dsx RNA
♀ splice
dsx RNA
♂ splice
DSX-F
protein
DSX-M
protein
Repression of
♂ - specific
structural genes
Repression of
♀ - specific
structural genes

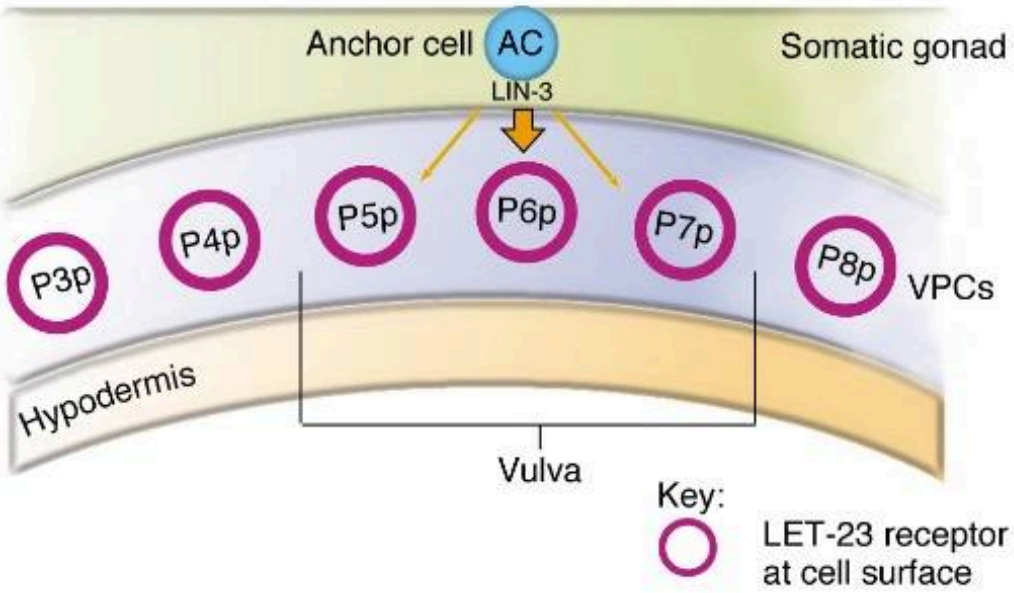
Sex lethal regulates its own splicing such that once its transcription has been induced (in the female embryo) SXL now promotes only female-specific splicing of any future SXL protein.



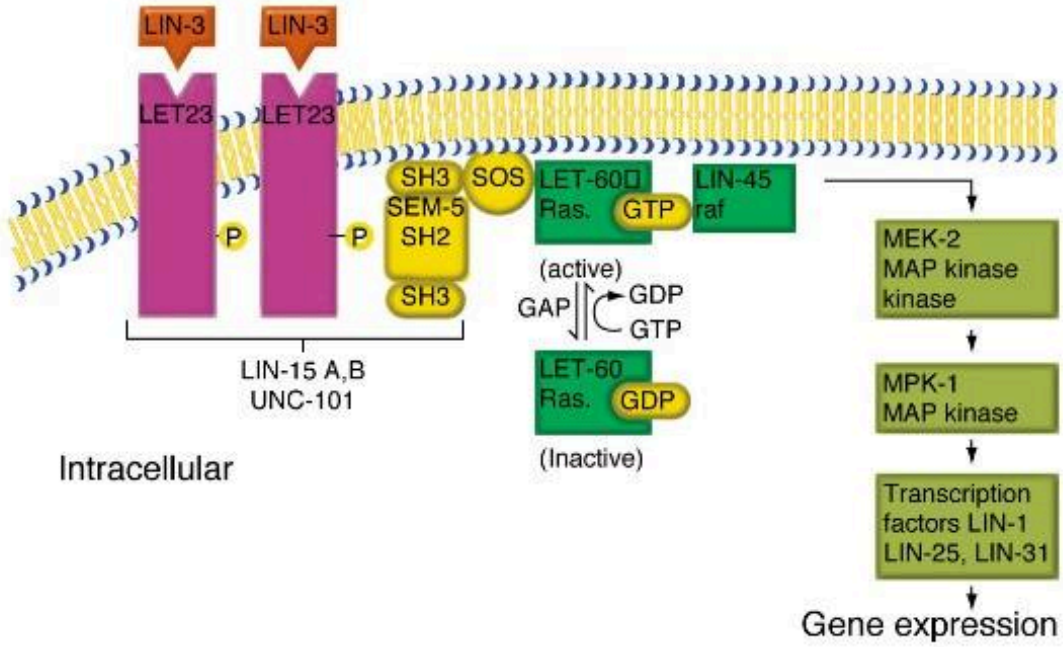

Pharynx
Ovary
Intestine
Eggs
Rectum
Anus
Oviduct
Oocytes
Uterus
Vulva



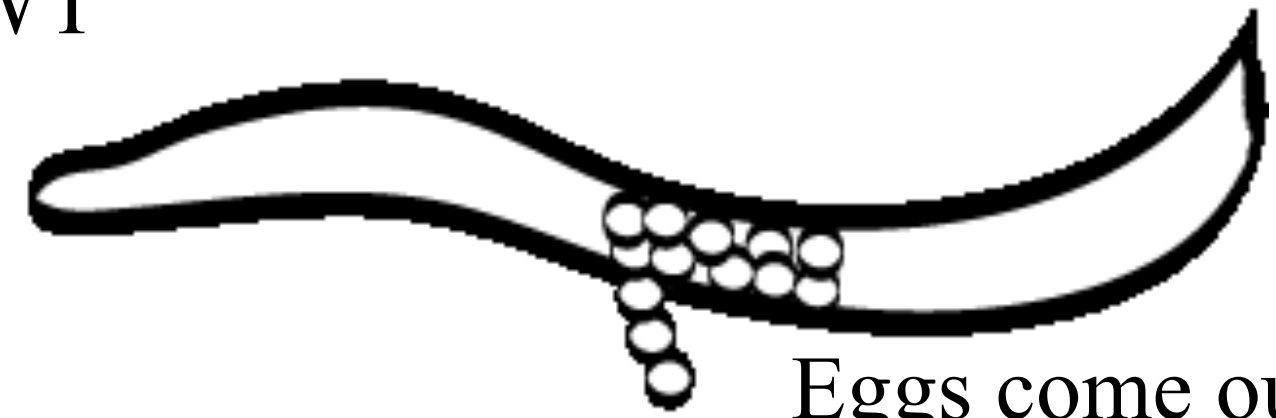
(a)




(c) Extracellular

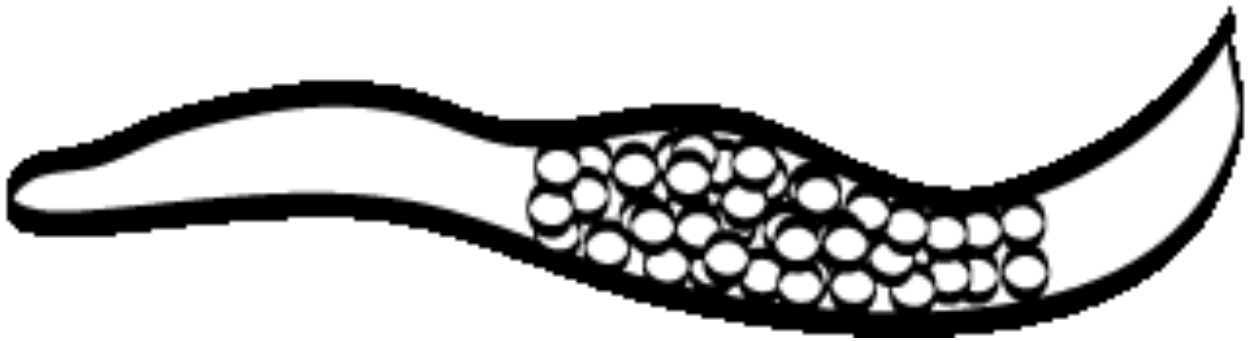


WT



Eggs come out of Vulva

Vulvaless mutant



There is also a multi-vulva phenotype, which allows egg laying and has multiple openings to the environment


vulvaless
phenotype

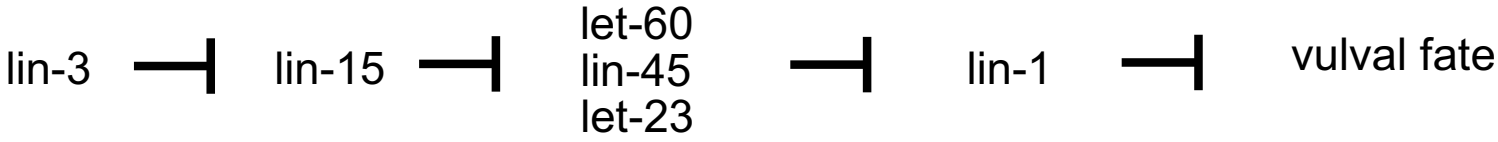
## Constructing an epistasis pathway for the vulva

Vul = let-23
lin-3
let-60
lin-45

Muv = lin-15
lin-1

lin-1 epistatic to let-23
lin-1 epistatic to lin-3
lin-1 epistatic to let-60
lin-1 epistatic to lin-45

let-23 epistatic to lin-15
lin-15 epistatic to lin-3
let-60 epistatic to lin-15
let-45 epistatic to lin-15



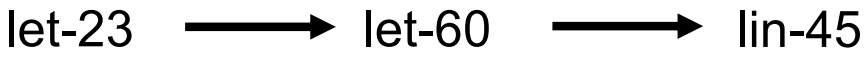

## Dominant mutations and epistasis

Epistasis relationships can only be determined between mutations that have different phenotypes. Dominant alleles of mutations that have a similar LOF phenotype can be used to determine epistasis relationships between these genes.

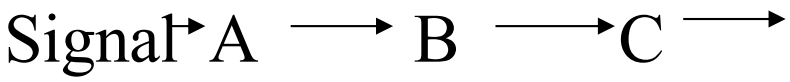
ras = Let-60 GOF = Muv

lin-45; let-60 GOF = Vul

let-23; let-60 GOF = Muv



## Ordering genes with the same loss-of-function phenotype




A- = B- = C- = off

How to order genes A, B and C?

Method 1: Constitutive mutations: Gene is on regardless of upstream regulation

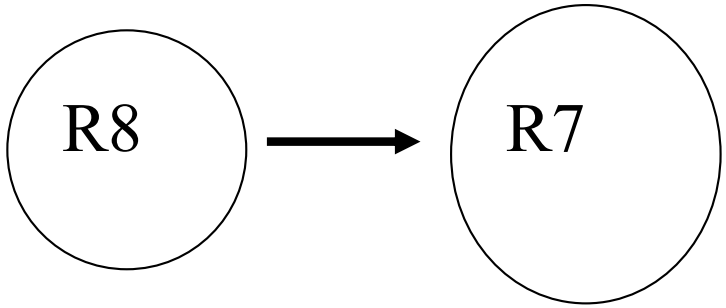
$B^c$ = On

A- $B^c$ = On

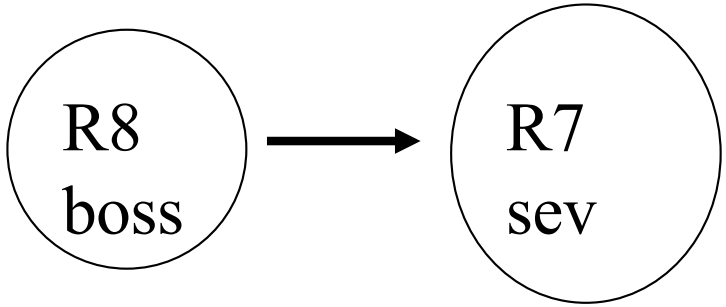
$B^c$ C- = Off

## Ordering genes with the same loss-of-function phenotype

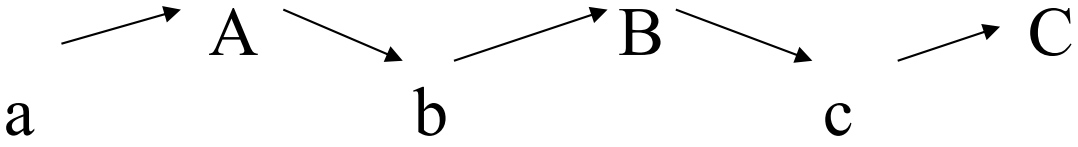
Method 2: site of action in cell signaling




Boss acts in R8
Sev acts in R7



# Ordering genes with the same loss-of-function phenotype




Method 3: Molecular epistasis

a- B expression off C expression off
a regulates b and c

b- A expression on C expression off
b regulates c, but not a

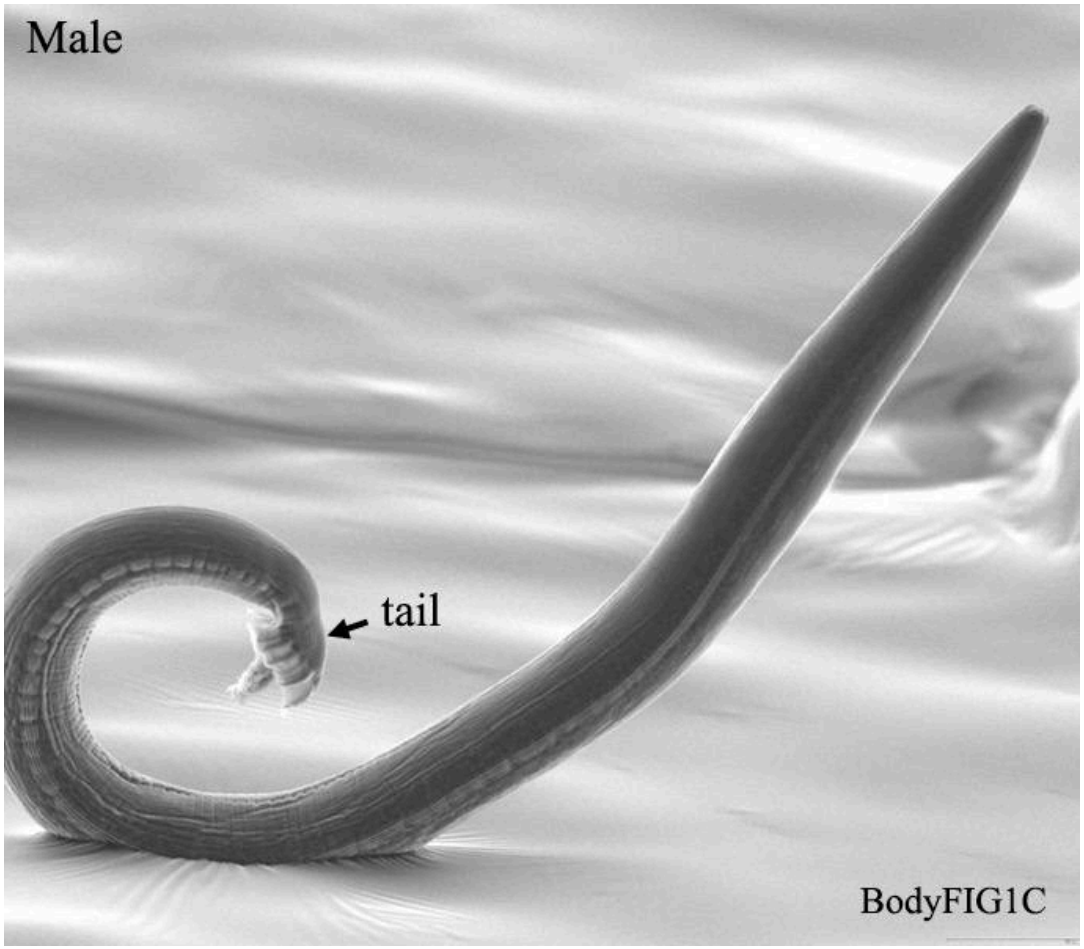
c- A expression on B expression on
c does not regulate a or b

Can also use molecular epistasis to order other types of regulatory pathways, such as splicing or phosphorylation

# Direct screens for cell death muta

# *C. elegans*


Hermaphrodite
BodyFIG1A
HEAD
TAIL
Image source: Juergen Berger & Ralph Sommer, Max-Planck Institute for Developmental Biology


Male
tail
BodyFIG1C

# Worm Genetics

XX = hermaphrodite
Makes both eggs and sperm;
Is able to self fertilize internally

XO = male
Able to mate with
and fertilize
hermaphrodites

XX

self progeny
XX

XX x XO



self progeny
XX

cross progeny
1/2 XX; 1/2 XO

# So how do you tell the difference between self and cross progeny?

Dumpy (*dpy-5* I) hermaphrodite x wt male



self progeny

Dpy hermaphrodite

cross progeny

1/2 nonDpy hermaphrodite; 1/2 nonDpy male

# Apoptosis plays many important roles

- Histogenic cell death: up to a half of the neurons normally die during development of parts of the brain.

- Phylogenic cell death: the loss of the vertebrate tail during human fetal development.

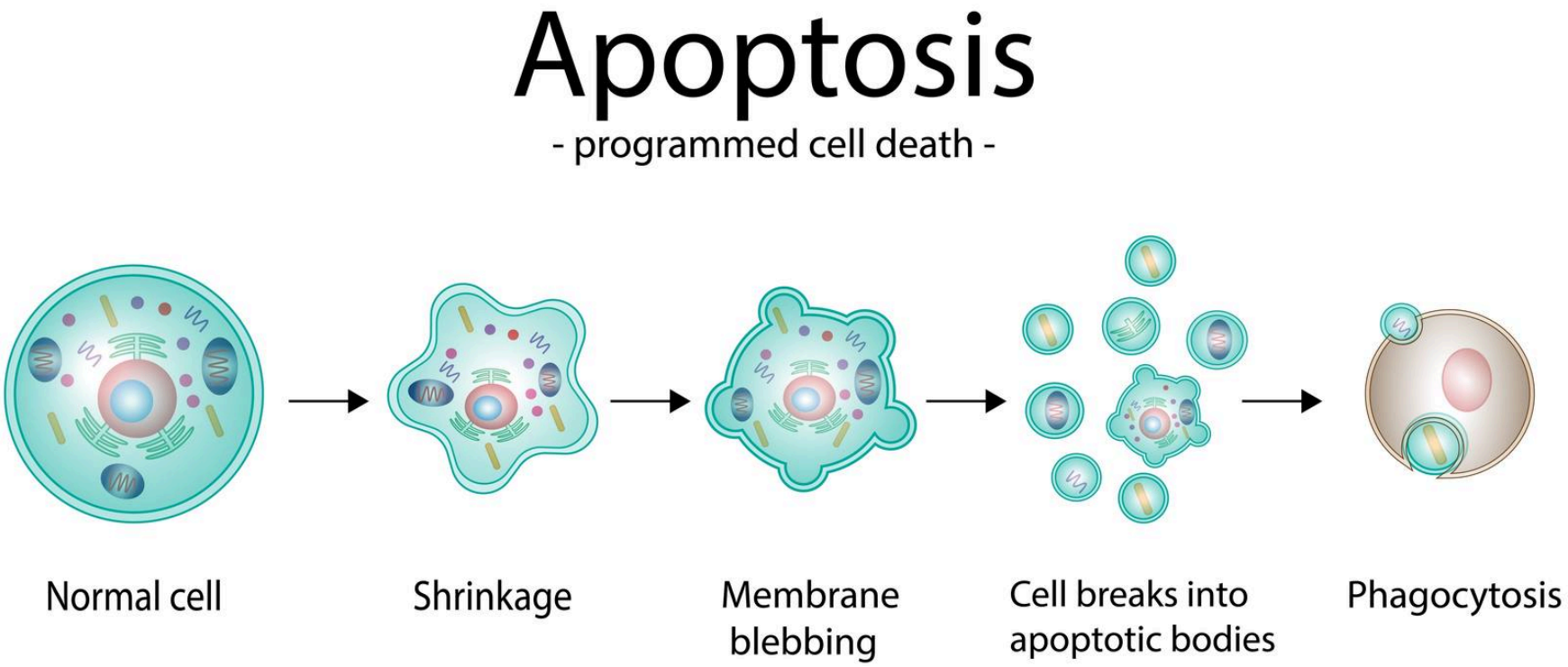
- Morphogenic cell death: the loss of mesenchyme between the digit.

- Cancer: damaged precancerous cells are removed by programmed cell death

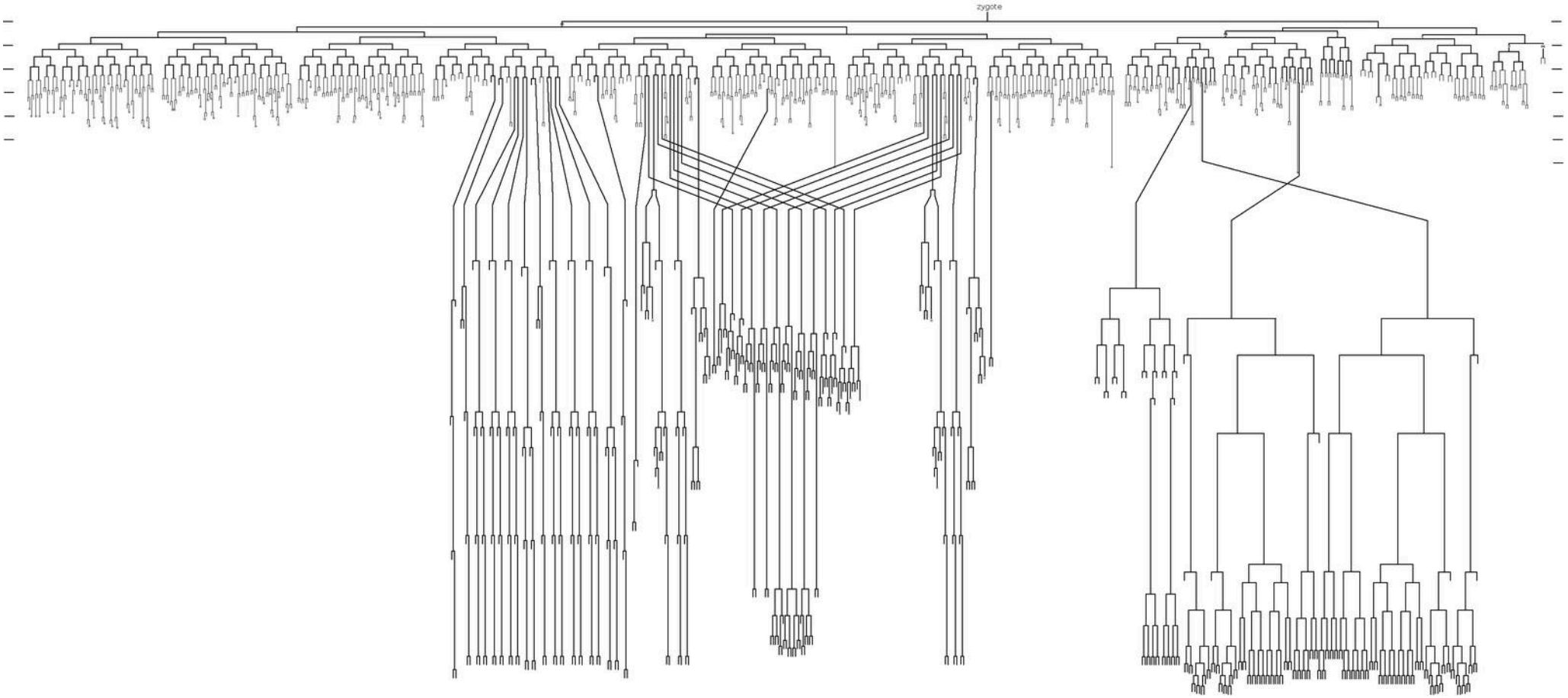
- Programmed cell death in *C. elegans*: more than 10% of the cells produced during development die.

# Hallmarks of apoptosis

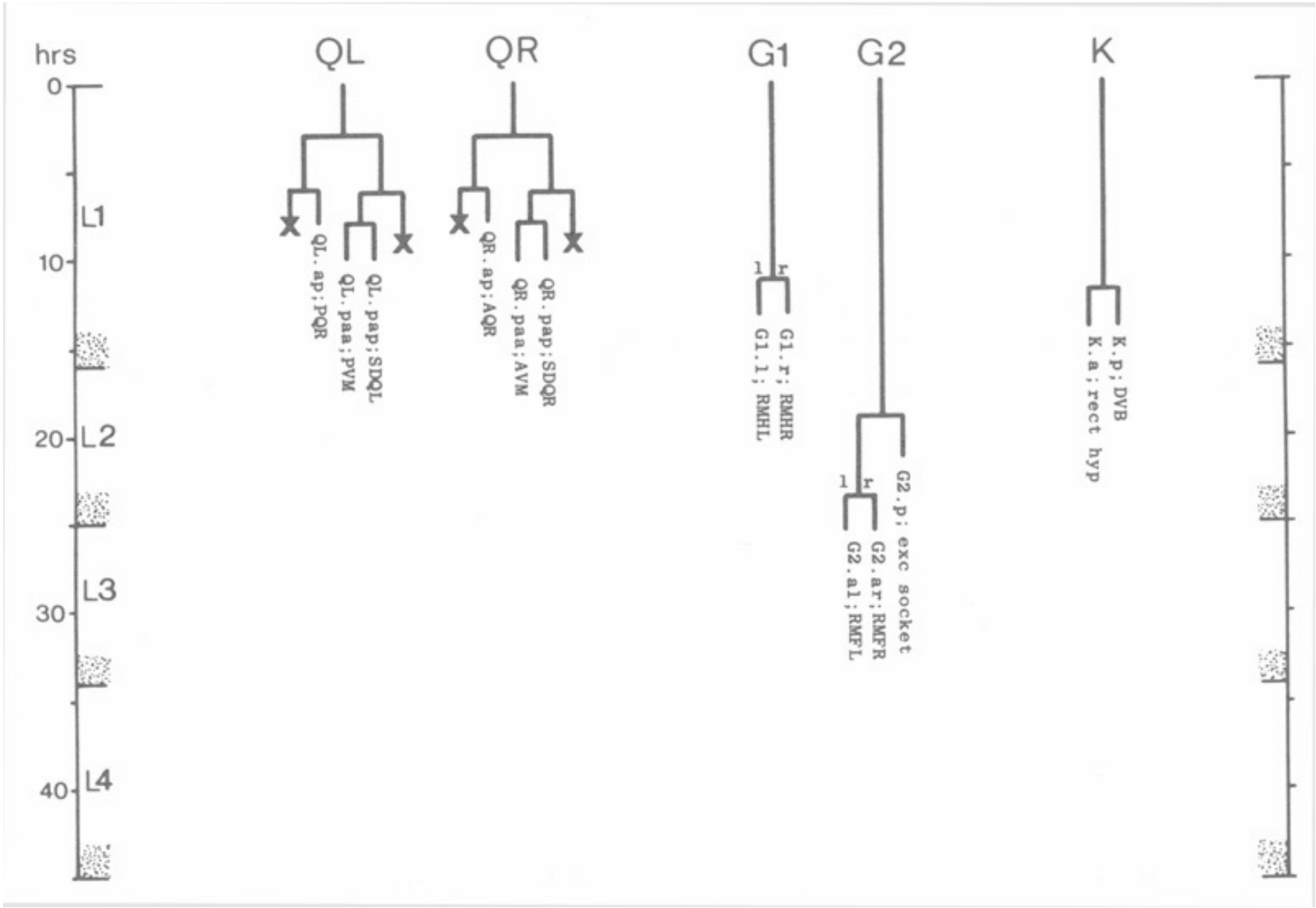
- Nucleus condensation.
- DNA fragmentation.
- Phagocytosis



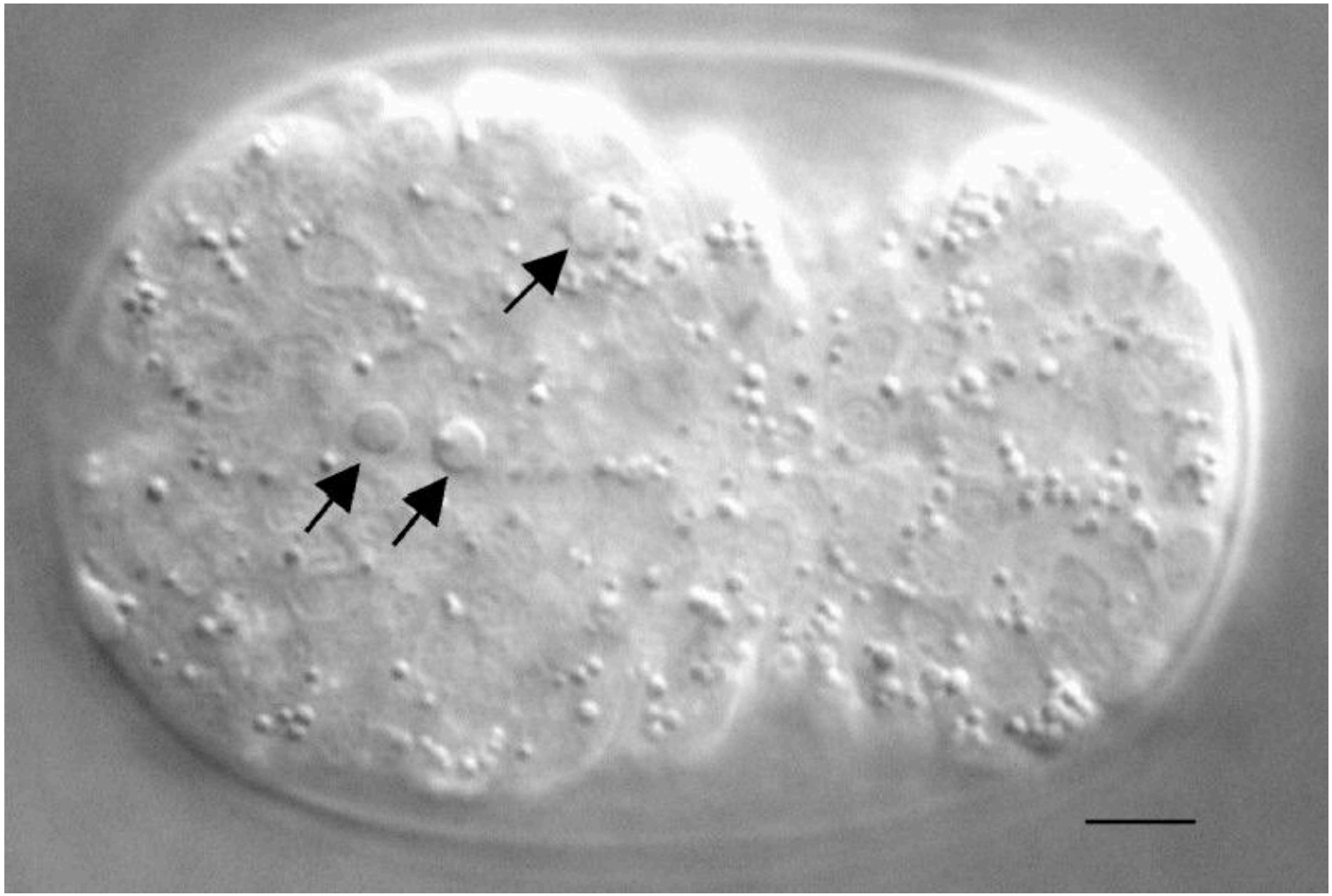
# *C. elegans* develops from an invariant lineage



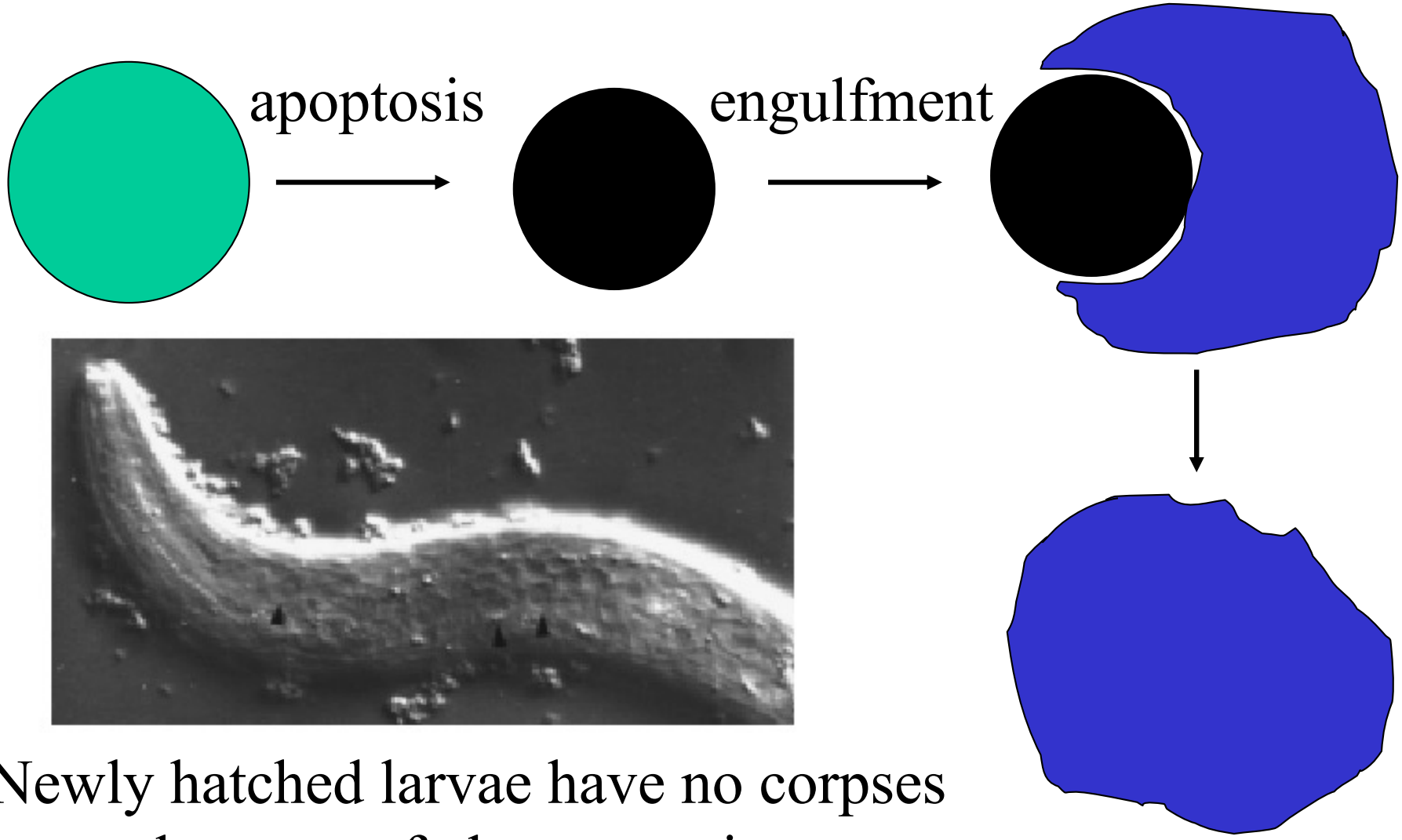
131 cells undergo apoptosis or programmed cell death in the *C. elegans* hemaphrodite.
The cell deaths are indicated by Xs in the lineage.



# Cell corpses can be observed by Nomarski optics



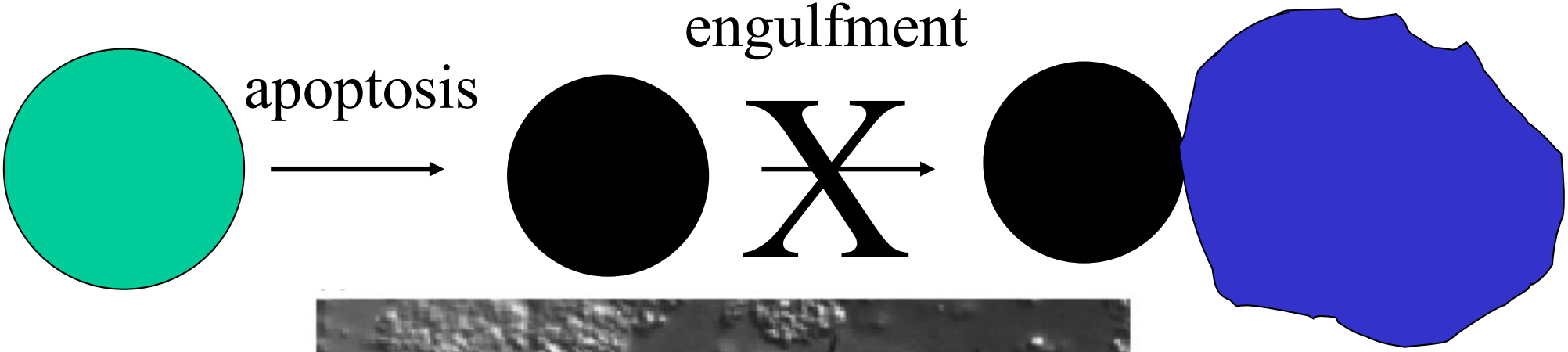
# Cell death mutants defective in different stages

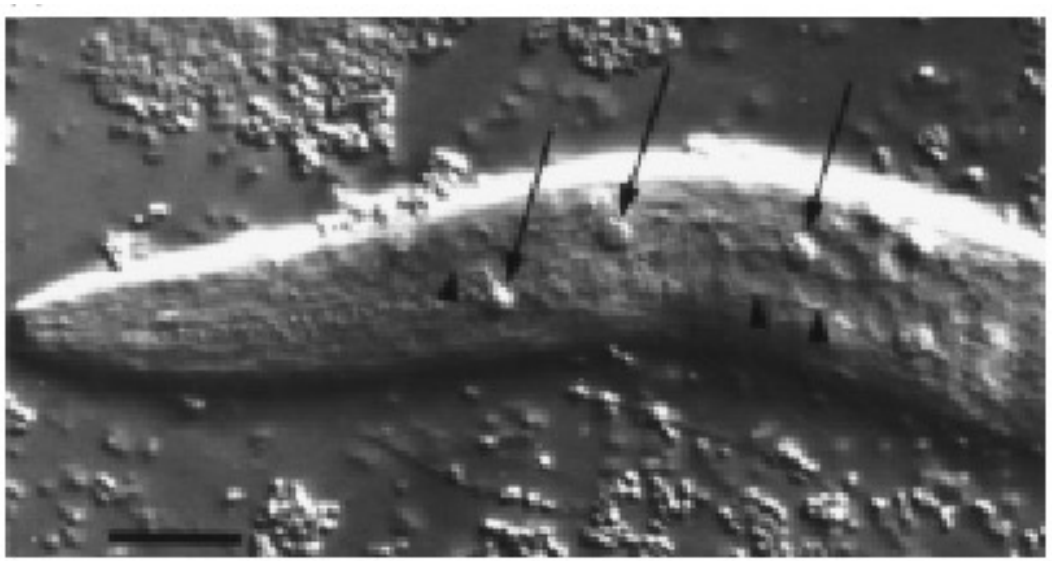





Newly hatched larvae have no corpses because of phagocytosis.

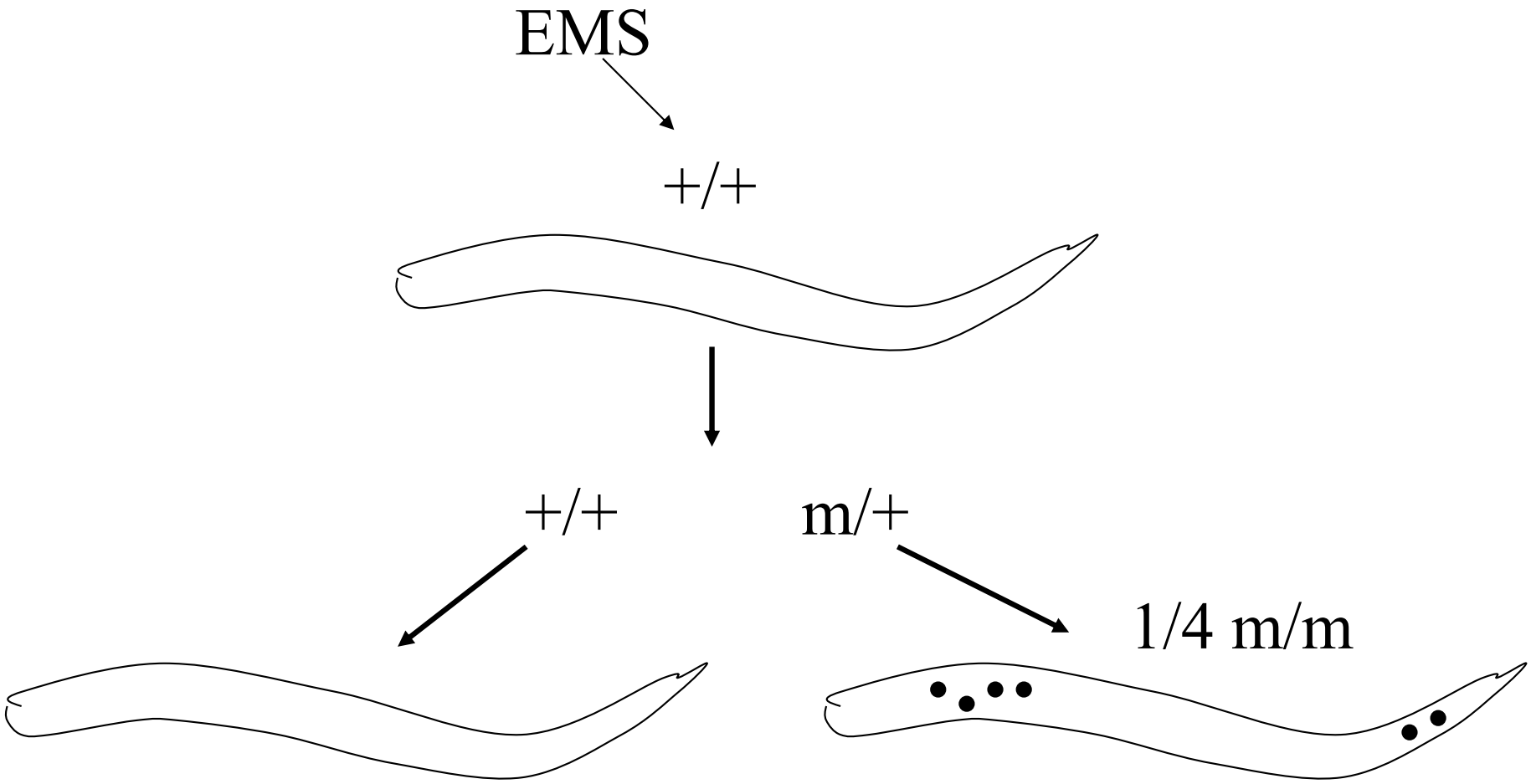
# Disruption of engulfment genes results in persistent corpses.



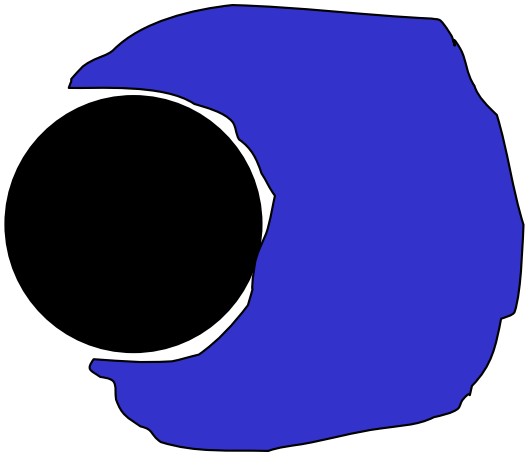



Newly hatched larvae have persistent corpses if there is a defect in phagocytosis.

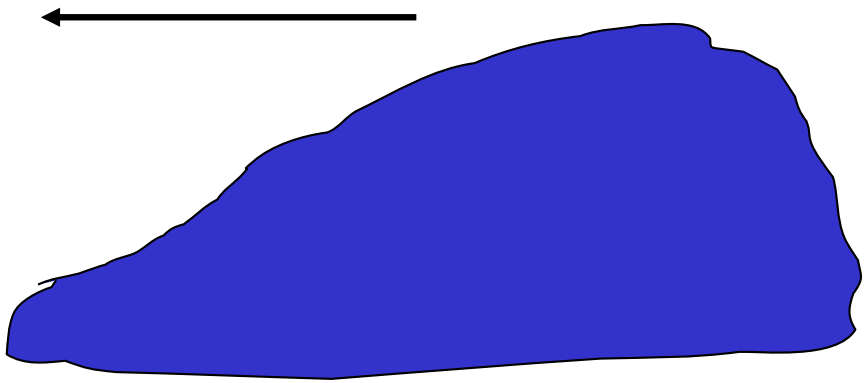
In screens for engulfment mutants Ed Hedgecock identified the *ced-1* and *ced-2* genes.
Additional screens by the Horvitz lab identified the *ced-5, ced-6, ced-7, ced-8, ced-10,* and *ced-12* genes.



The engulfment *ced* genes function in phagocytosis and often cell migration. Regulate cell movement.



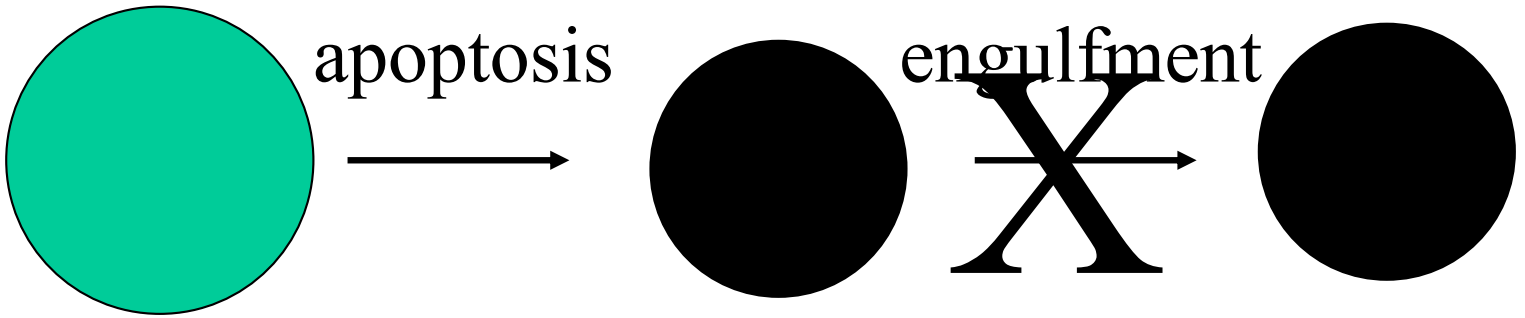
phagocytosis



cell migration

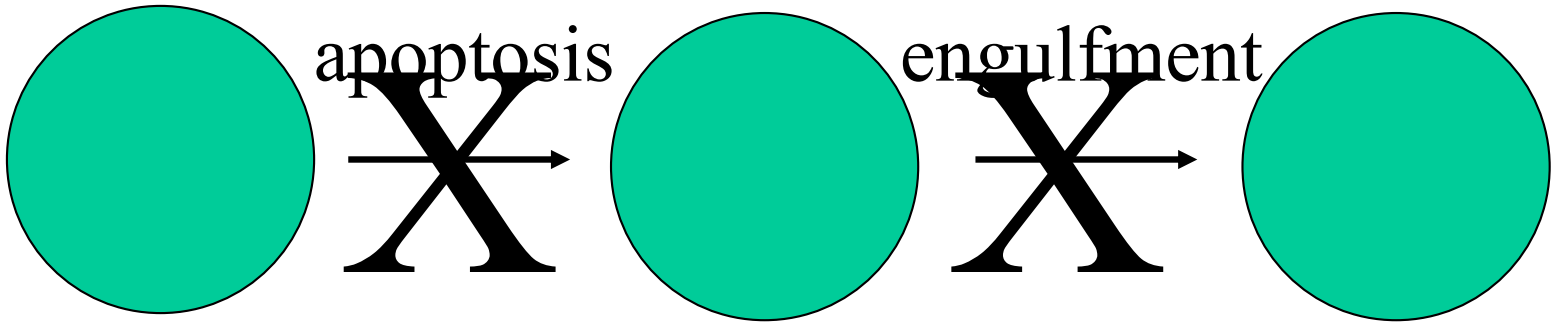
Screens for apoptosis mutants took advantage of the persistent corpses in *ced-1* mutants.

Engulfment mutant




persistent corpse

Engulfment; apoptosis double mutant

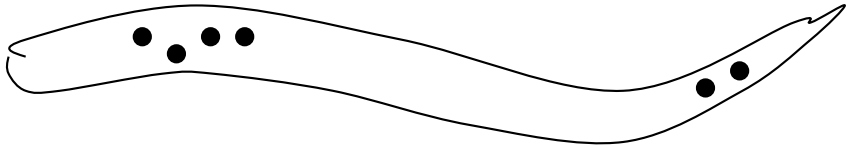



no persistent corpse

Screens for the loss of cell corpses in *ced-1* mutants identified genes involved in apoptosis.

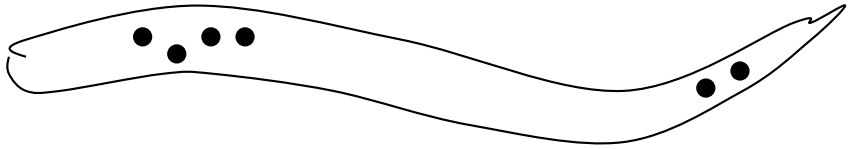
EMS

*ced-1/ced-1*

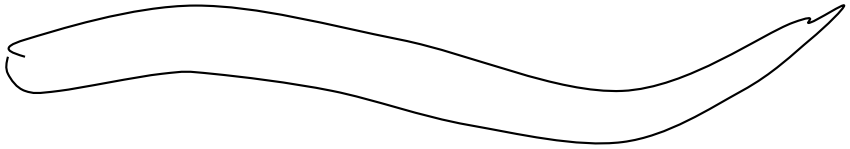


*ced-1/ced-1*

*ced-1/ced-1; m/+*



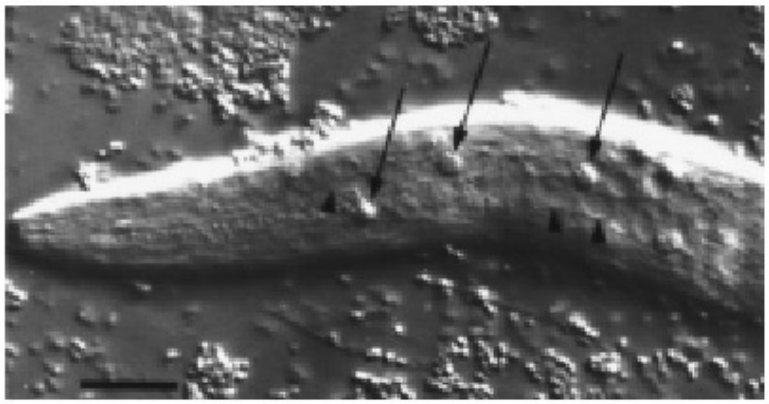
1/4 *ced-1/ced-1; m/m*



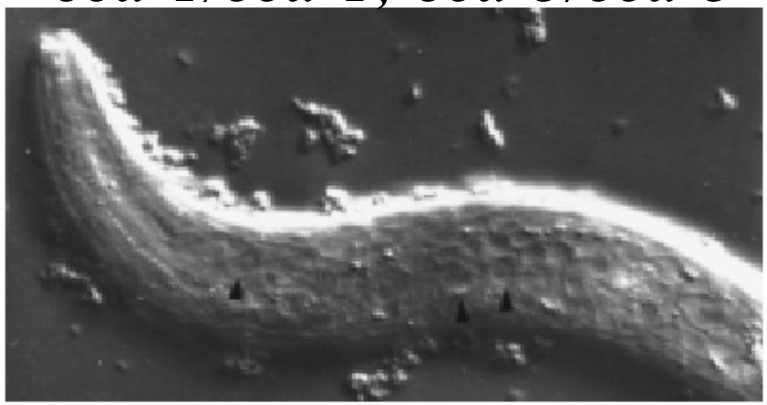
Screen for newly hatched larvae lacking cell corpses

In *ced-3(lf), ced-4(lf), ced-9(gf)* and *egl-1(lf)* mutants all 131 cells that normally die survive.

*ced-1/ced-1*

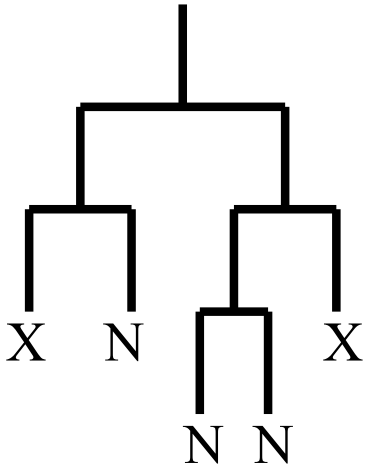


*ced-1/ced-1; ced-3/ced-3*



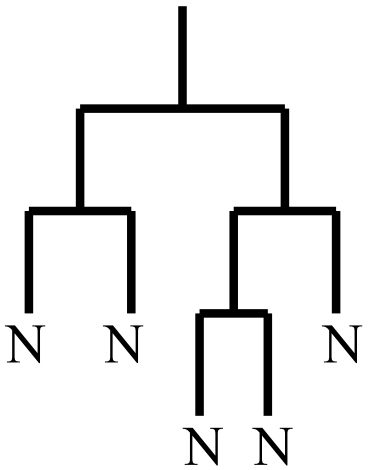
+/+

Q neuroblast



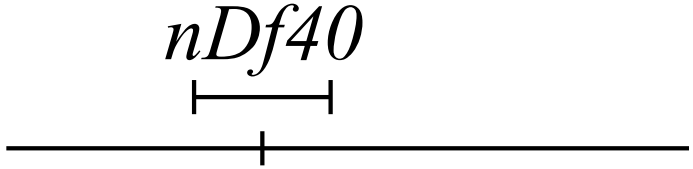

*ced-3/ced-3*

Q neuroblast




Arrowheads indicate extra cells

*n1950* is a dominant mutation in *ced-9*



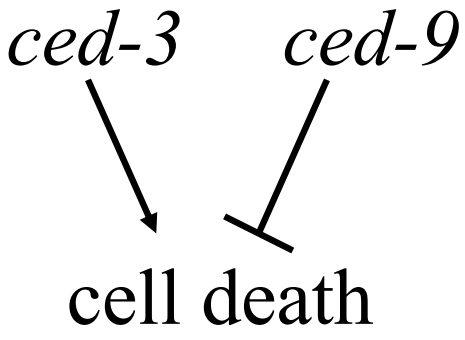

*nDf40*/+ wild type
*n1950*/+ many cells that should die survive
Therefore gain-of-function mutation

Loss of function and gain-of-function alleles of *ced-9* have opposite phenotypes.

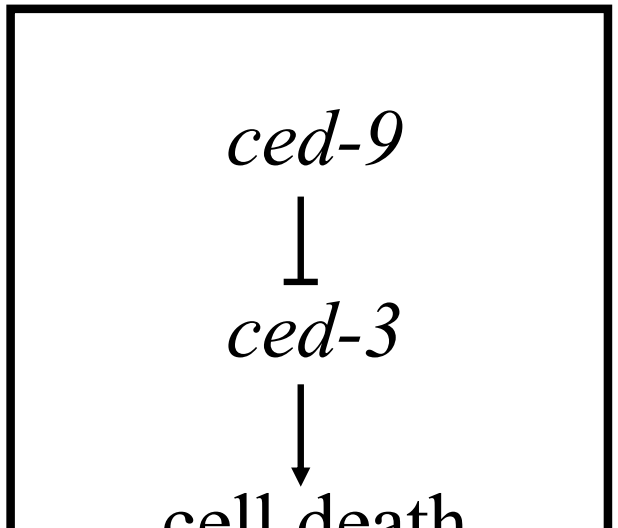
*ced-9(gf)* disrupts apoptosis
*ced-9(lf)* is recessive lethal
because of widespread cell death

# *ced-3* promotes apoptosis
# *ced-9* inhibits apoptosis

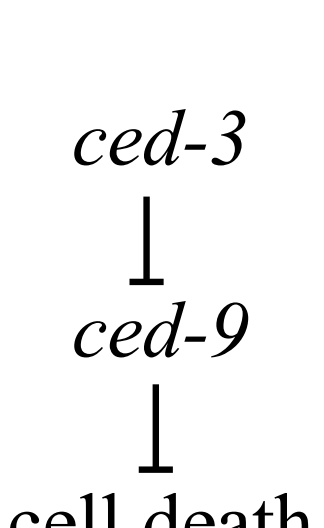
## Models




Prediction: *ced-3(lf); ced-9(lf)* intermediate phenotype




Prediction: *ced-3(lf); ced-9(lf)* animals survive and have no apoptosis




Prediction: *ced-3(lf); ced-9(lf)* animals die because of extensive apoptosis

# Cell death: a switch pathway

**The downstream gene is epistatic in double (null) mutants in a switch pathway. This in contrast to the situation in a biosynthetic pathway, in which the upstream gene is epistatic in terms of the phenotype produced.**

**egl-1 ⊣ ced-9 ⊣ ced-4 → ced-3 → cell death**

| | | | | |
|---|---|---|---|---|
| **ON** | **OFF** | **ON** | **ON** | **cell death** |
| **OFF** | **ON** | **OFF** | **OFF** | **cell survival** |

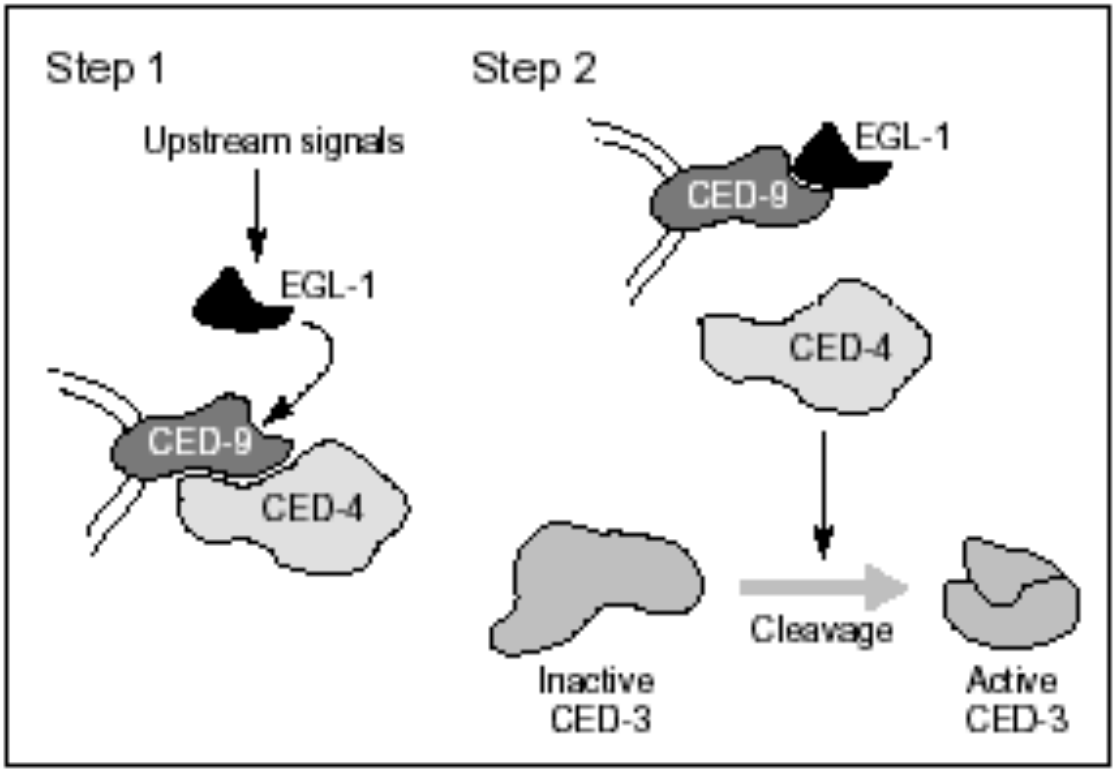
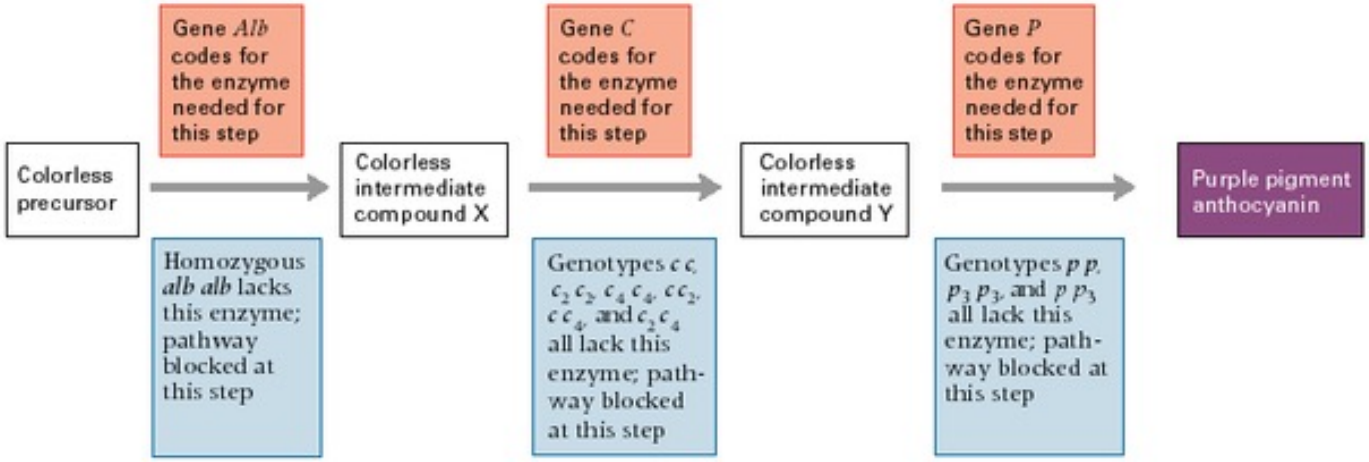
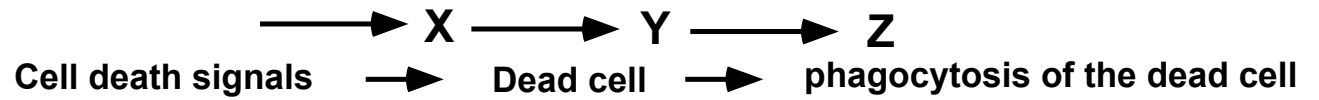



FIGURE 3. A model for the activation of programmed cell death in *Caenorhabditis elegans*. (a) Step 1: upstream signals lead to the production or activation of the EGL-1 protein. EGL-1 binds to CED-9, shown localized to cell membranes, leading to the release of CED-4 from CED-9. Although shown as a monomer, CED-9 likely functions as a dimer, as do other Bcl-2 family proteins[7]. (b) Step 2: free CED-4 then promotes the proteolytic cleavage of the pro-enzyme form of CED-3; p17 and p13 subunits derived from this processing assemble into the active form[27] (active caspases are thought to be tetramers, consisting of dimers of such a heterodimer; reviewed in Ref. 65). It is possible that CED-3 is complexed with CED-4 before EGL-1 mediates the release of CED-4 from CED-9.

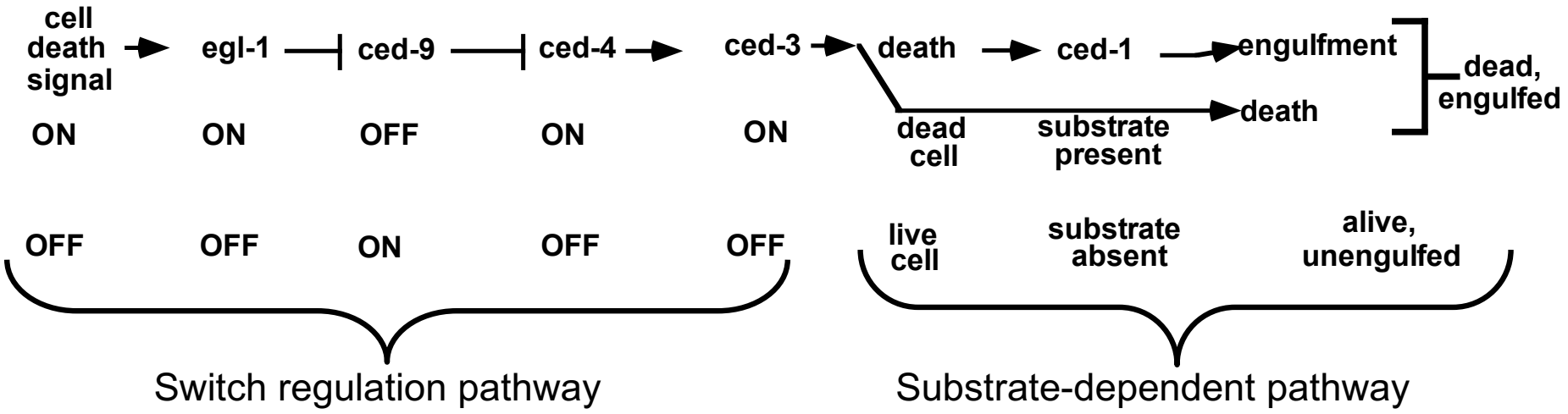



# Assembly-metabolic and switch genetic pathways

**1) Assembly-metabolic pathways are substrate-dependent. An obligate series of sequential steps is required to generate a final outcome. Each step generates a product that is acted on by the next step in the pathway.**




**The dead cell acts as a substrate for the action of genes involved in pahgocytosis. So in an epistasis experiement in which a mutation that blocks cell death is combined with one that blocks phagocytosis the phenotype is that of the upstream mutation, which in this case is cell survival.**



(a)

| Genotype | Phenotype | |
|---|---|---|
| | Viable? | Programmed cell death |
| Wild-type | Yes | Normal |
| *ced-9*(lf) | No | Excess |
| *ced-4*(lf) | Yes | Reduced |
| *ced-3*(lf) | Yes | Reduced |
| *egl-1*(lf) | Yes | Reduced |
| *ced-4*(lf);*ced-9*(lf) | Yes | Reduced |
| *ced-9*(lf);*ced-3*(lf) | Yes | Reduced |
| *ced-9*(lf);*egl-1*(lf) | No | Excess |

*egl-1* ⊣ *ced-9* ⊣ *ced-4* *ced-3* → Programmed cell death

(b)

| Overexpressed gene | Genotype | Cell killing |
|---|---|---|
| *ced-3* | *ced-4*(+) | +++ |
| *ced-4* | *ced-3*(+) | +++ |
| *ced-3* | *ced-4*(lf) | ++ |
| *ced-4* | *ced-3*(lf) | +/– |

*ced-4* → *ced-3* → Programmed cell death

(c)

| Genotype | | | |
|---|---|---|---|
| *ced-4* | *ced-9* | *ced-3*-induced killing | |
| + | lf | ++++ | *ced-9* protection |
| + | + | +++ | |
| lf | lf | ++ | No *ced-9* protection |
| lf | + | ++ | |

*ced-9* ⊣ *ced-4* → *ced-3*

(d)

| Genotype | *egl-1*-induced killing |
|---|---|
| Wild-type | +++ |
| *ced-3*(lf) | – |
| *ced-4*(lf) | – |
| *ced-9*(gf) | – |

*egl-1* ⊣ *ced-9* ⊣ *ced-4* → *ced-3*

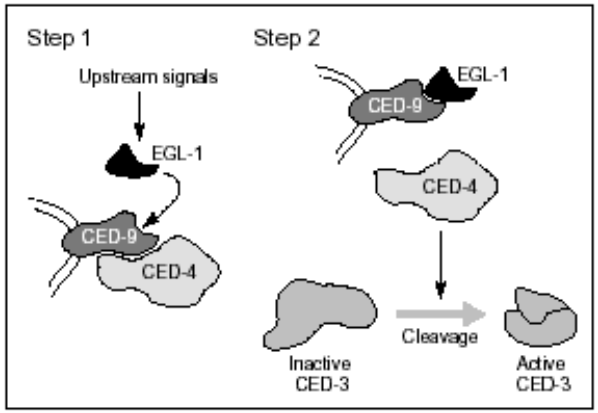



FIGURE 2. Interactions among *egl-1*, *ced-9*, *ced-4* and *ced-3*. Genetic epistasis experiments and studies in which *egl-1*, *ced-9*, *ced-4* and *ced-3* have been overexpressed to kill cells in different genetic backgrounds have suggested an order of action of these genes[10,15,29]. The relative efficiency of cell killing is represented by the number of plus (+) signs, as interpreted from data in Refs 10, 29. (a) Loss-of-function mutations in *ced-4* or *ced-3* suppress the lethality caused by loss of *ced-9* function, suggesting that *ced-9* functions upstream of *ced-4* and *ced-3*. By contrast, a loss of function mutation in *egl-1* does not suppress *ced-9* (lf) lethality, suggesting that *egl-1* functions upstream of *ced-9*. (b) The overexpression of *ced-3* or *ced-4* can kill cells in wild-type *Caenorhabditis elegans*. In the absence of *ced-4* activity, overexpressed *ced-3* can still kill, albeit at a slightly reduced efficiency. By contrast, in the absence of *ced-3* activity, *ced-4* is almost incapable of killing cells. These results suggest *ced-4* acts upstream of *ced-3* to activate programmed cell death. (c) Endogenous *ced-9* activity protects cells from programmed cell death induced by overexpression of *ced-3*, because killing is more efficient in a *ced-9*(lf) background than in a *ced-9*(+) background. While elimination of *ced-4* function reduces the killing activity of *ced-3*, this residual *ced-3* activity is no longer inhibited by *ced-9*, suggesting that *ced-9* functions to protect against programmed cell death, at least in part, by negatively regulating *ced-4*. (d) The overexpression of *egl-1* can also kill cells. This killing is blocked by loss-of-function mutations in *ced-4* or *ced-3* or by a gain-of-function mutation in *ced-9*, suggesting that *egl-1* acts upstream of these genes.

| Genotype | Phenotype | |
|---|---|---|
| | Viable? | Programmed cell death |
| Wild-type | Yes | Normal |
| *ced-9*(lf) | No | Excess |
| *ced-4*(lf) | Yes | Reduced |
| *ced-3*(lf) | Yes | Reduced |
| *egl-1*(lf) | Yes | Reduced |
| *ced-4*(lf);*ced-9*(lf) | Yes | Reduced |
| *ced-9*(lf);*ced-3*(lf) | Yes | Reduced |
| *ced-9*(lf);*egl-1*(lf) | No | Excess |

ced-4 promotes ced-3 activity

| Overexpressed gene | Genotype | Cell killing |
|---|---|---|
| *ced-3* | *ced-4*(+) | +++ |
| *ced-4* | *ced-3*(+) | +++ |
| *ced-3* | *ced-4*(lf) | ++ |
| *ced-4* | *ced-3*(lf) | +/– |

*ced-4* → *ced-3* → Programmed cell death

ced-9 protects, but only in the presence of ced-4.

| Genotype | | | |
|---|---|---|---|
| *ced-4* | *ced-9* | *ced-3*-induced killing | |
| + | lf | ++++ | *ced-9* protection |
| + | + | +++ | |
| lf | lf | ++ | No *ced-9* protection |
| lf | + | ++ | |

*ced-9* ⊣ *ced-4* → *ced-3*