Imprinting; mutants and transgenics in analysis of development

BIOS 0702

2017

Life starts from one cell

If you are a multicellular organism, you started your life with a cell; so how did you end up with so many kinds of cells?

The logic

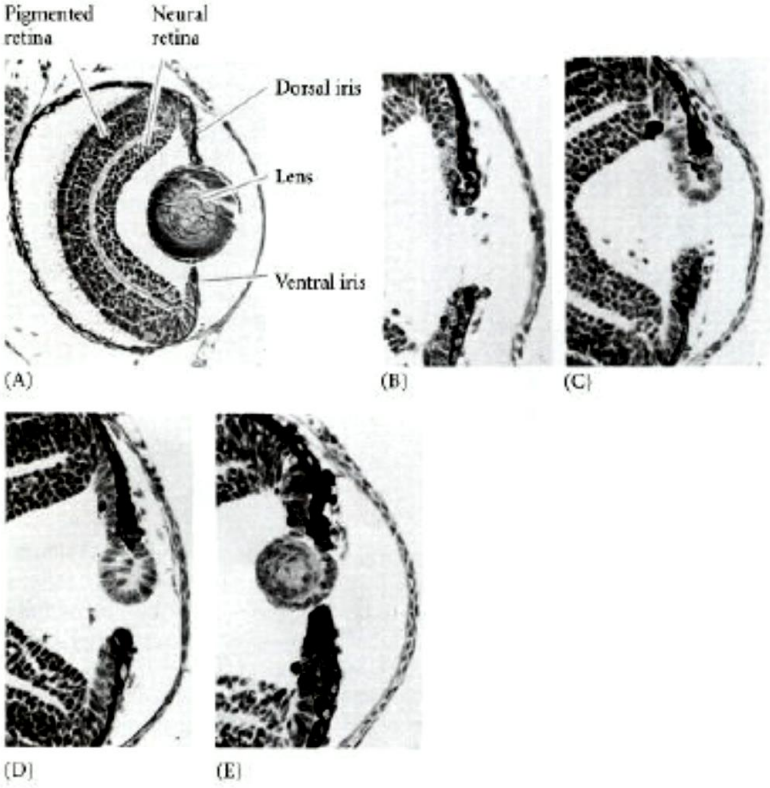
- First cell divisions are mitotic.
- That means daughter cells are same as mother cells.
- So however many divisions you go through, all the cells at the end will be the same.
- So the basic question of developmental biology is, when you start with the same how can you end with variation?
- We know that mitotic divisions are equal
- So all the daughter cells have the same material, right?
  - Almost.
- So lets start with some basics….

Genomic Equivalence

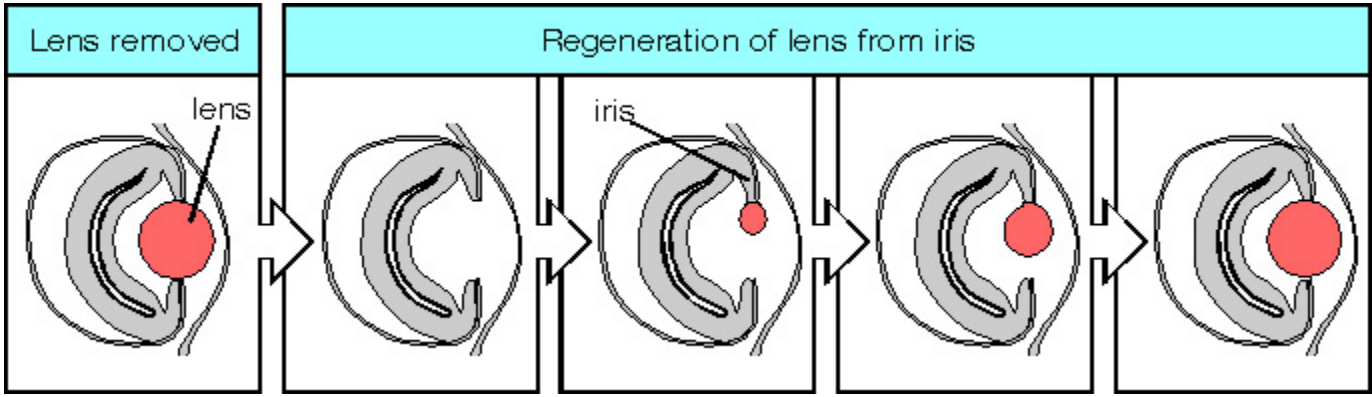
All the daughter cells have the same genetical material as mother

How do we know that?

Metaplasia

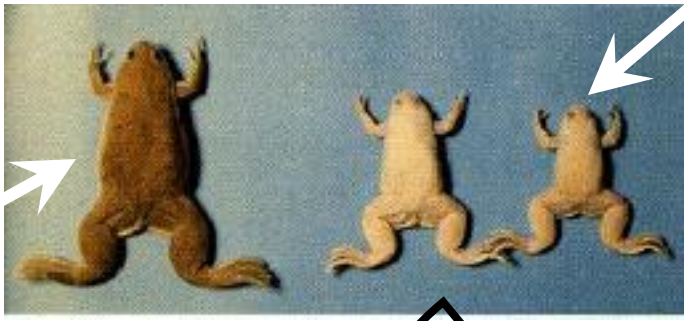


Salamander
eye generation



The pluripotency of somatic cells

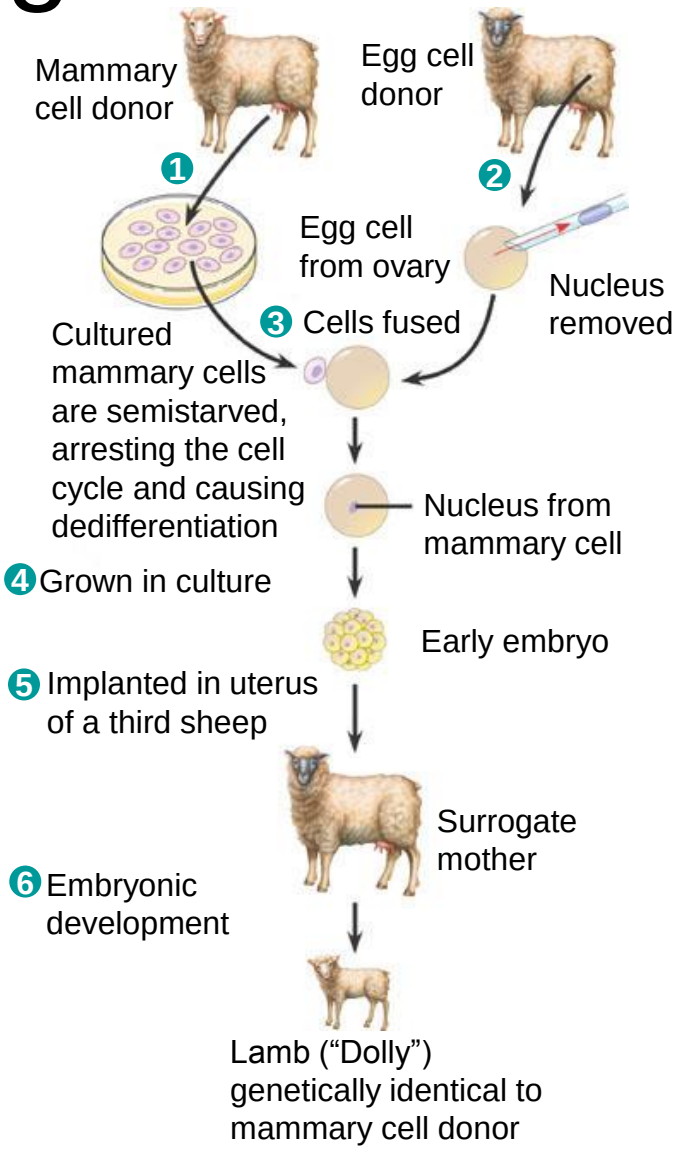
Nucleus donor
Albino



Wild type
Cytoplasm donor

Cloning

Or Dolly



Note: There are exception of this rule. B and T cells.

What do you think?

- All you need is nucleus, right?
- All the daughter cells have the same genetic materials
- But may not have same cytoplasmic material
- How does that impact the developmental process?
- Hopefully we will learn the basic processes involved
- Lets learn some techniques first

Techniques

Cloning

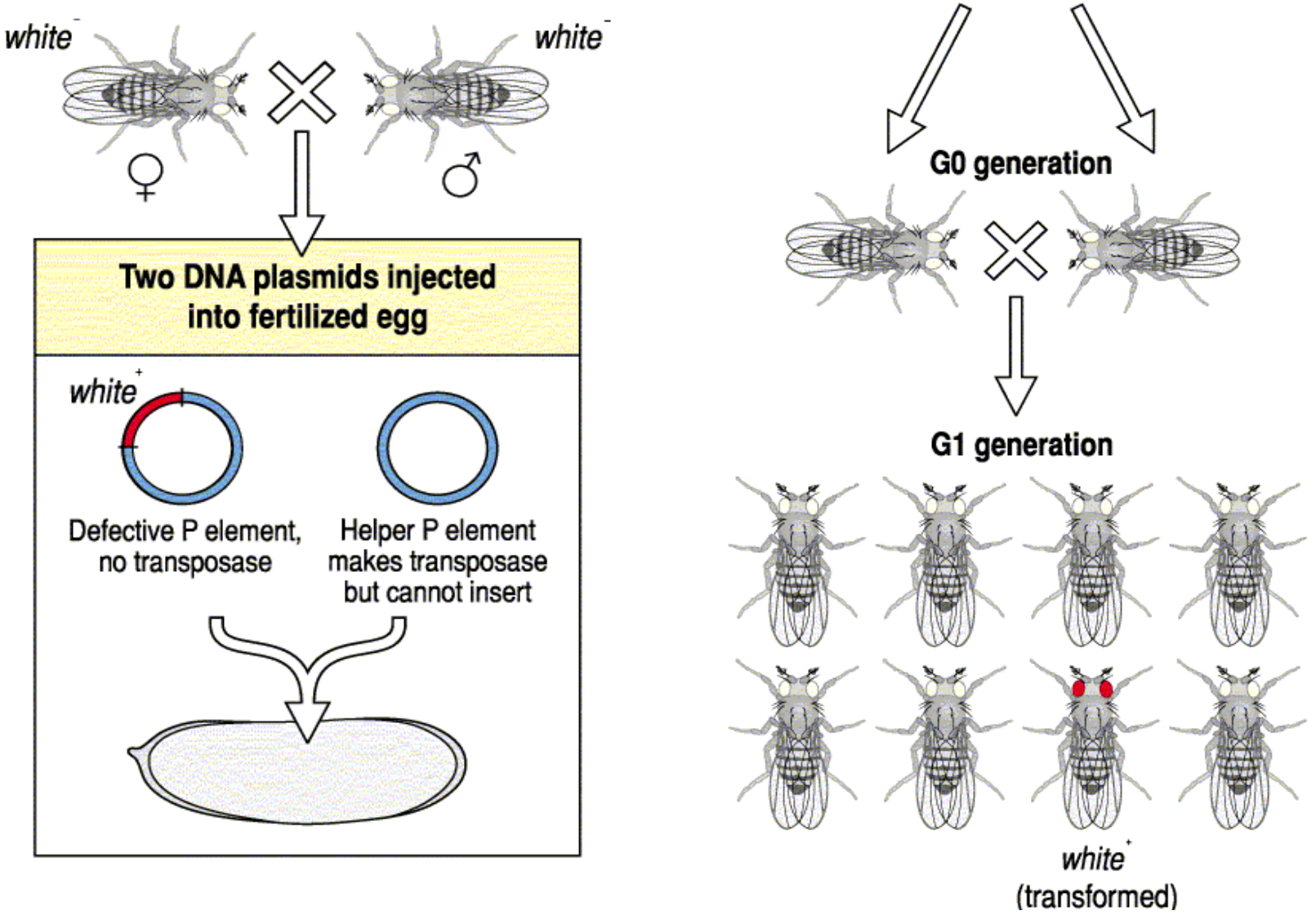
Gene knockout

Gene knockdown

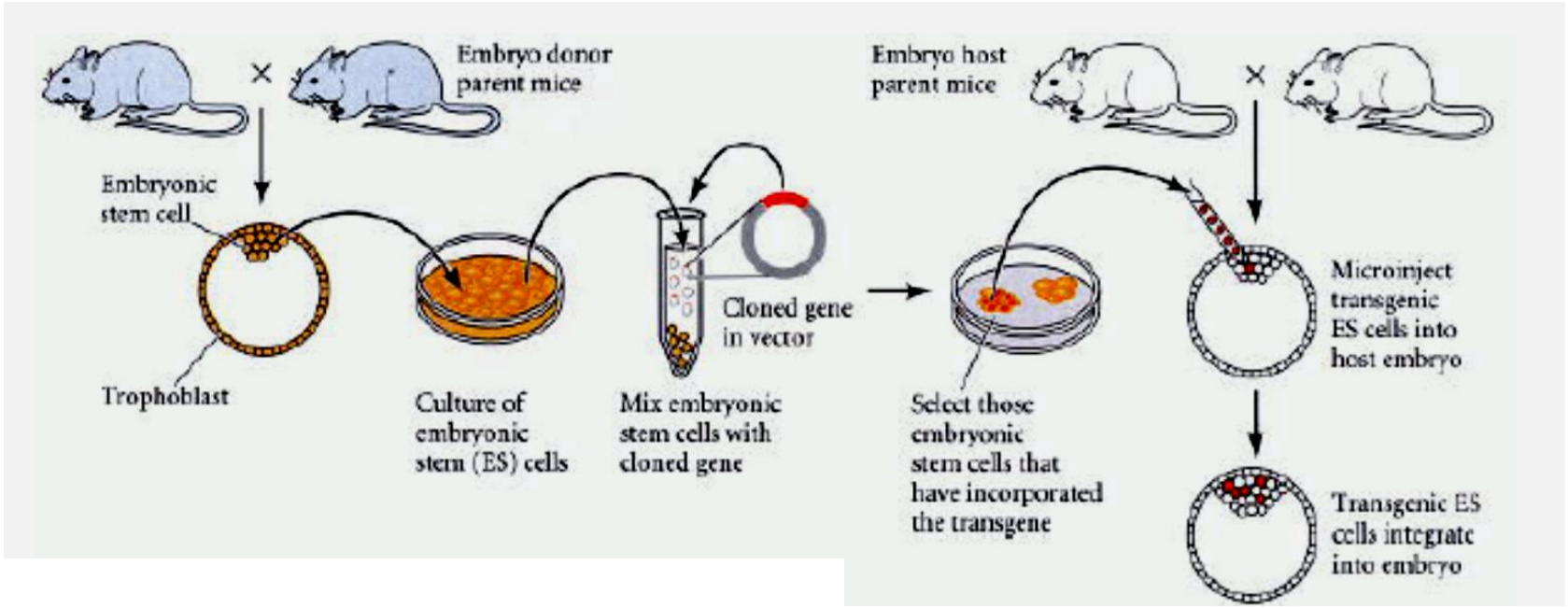
Mutants

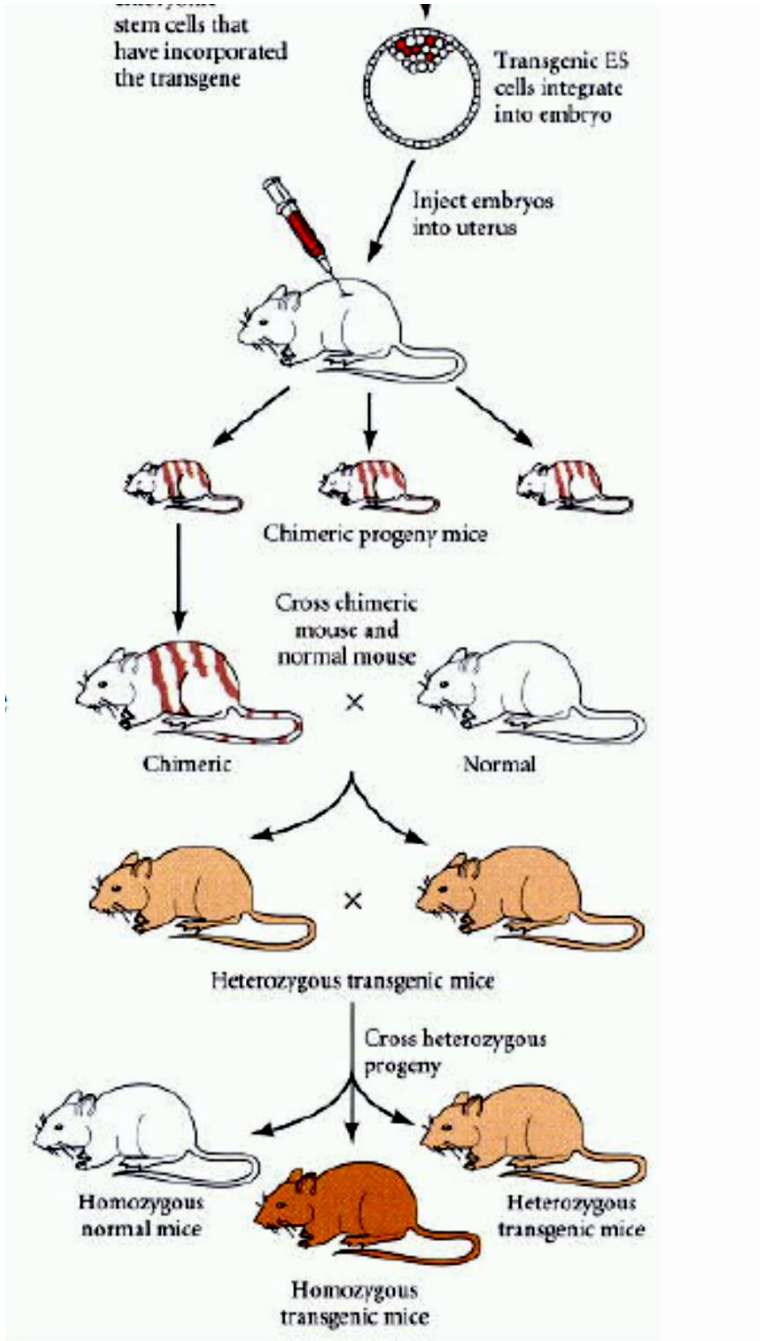
Gene cloning

In flies


white⁻
white⁻
♀
♂
Two DNA plasmids injected into fertilized egg
white⁺
Defective P element, no transposase
Helper P element makes transposase but cannot insert
G0 generation
G1 generation
white⁺
(transformed)

In mouse


Embryo donor parent mice
Embryonic stem cell
Trophoblast
Culture of embryonic stem (ES) cells
Cloned gene in vector
Mix embryonic stem cells with cloned gene
Embryo host parent mice
Select those embryonic stem cells that have incorporated the transgene
Microinject transgenic ES cells into host embryo
Transgenic ES cells integrate into embryo


stem cells that
have incorporated
the transgene
Transgenic ES
cells integrate
into embryo
Inject embryos
into uterus
Chimeric progeny mice
Cross chimeric
mouse and
normal mouse
×
Chimeric
Normal
×
Heterozygous transgenic mice
Cross heterozygous
progeny
Homozygous
normal mice
Heterozygous
transgenic mice
Homozygous
transgenic mice

Gene knockout

Formation of ES cells carrying a knockout mutation

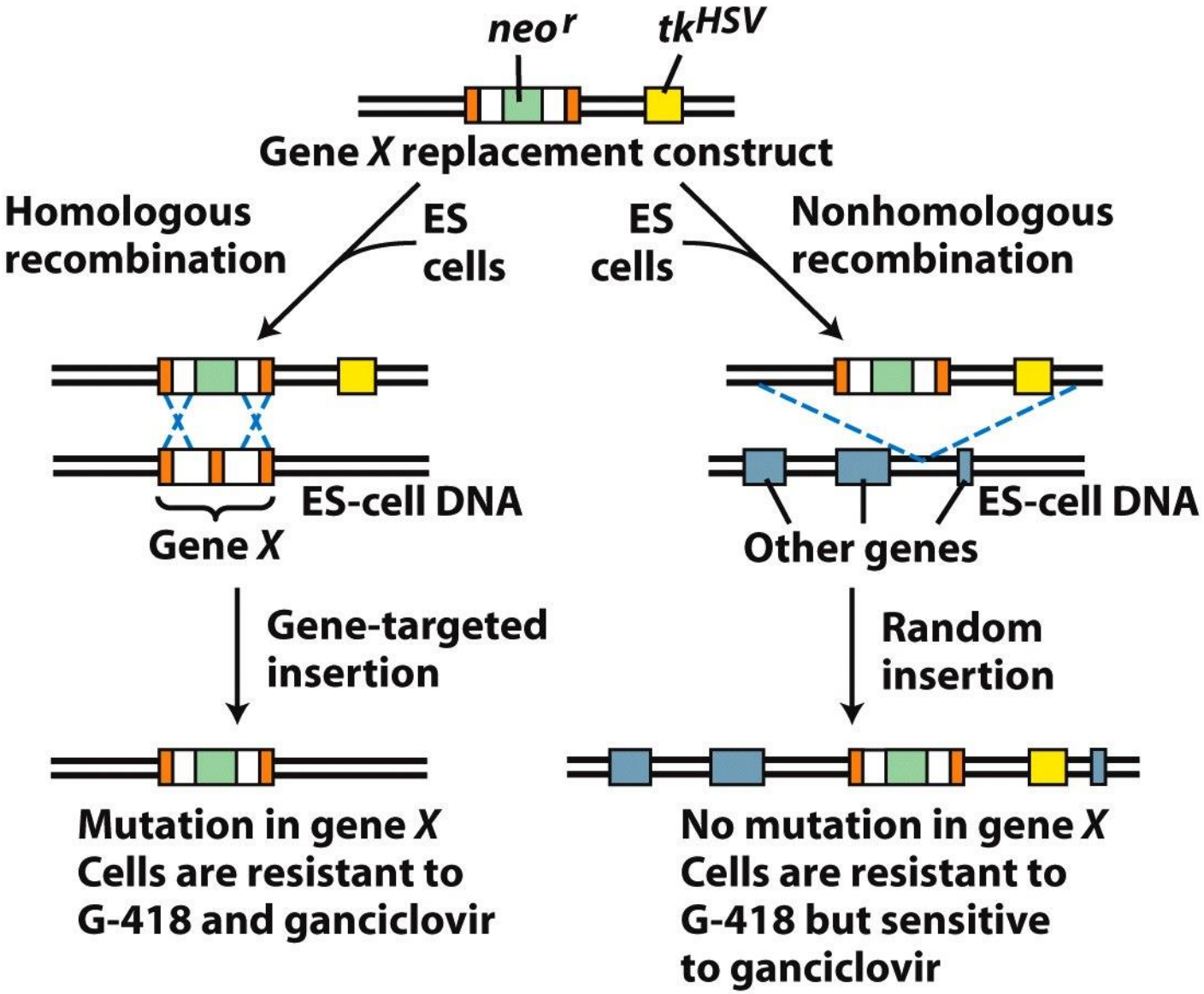


Figure 5-40a
Molecular Cell Biology, Sixth Edition

Positive and negative selection of recombinant ES cells

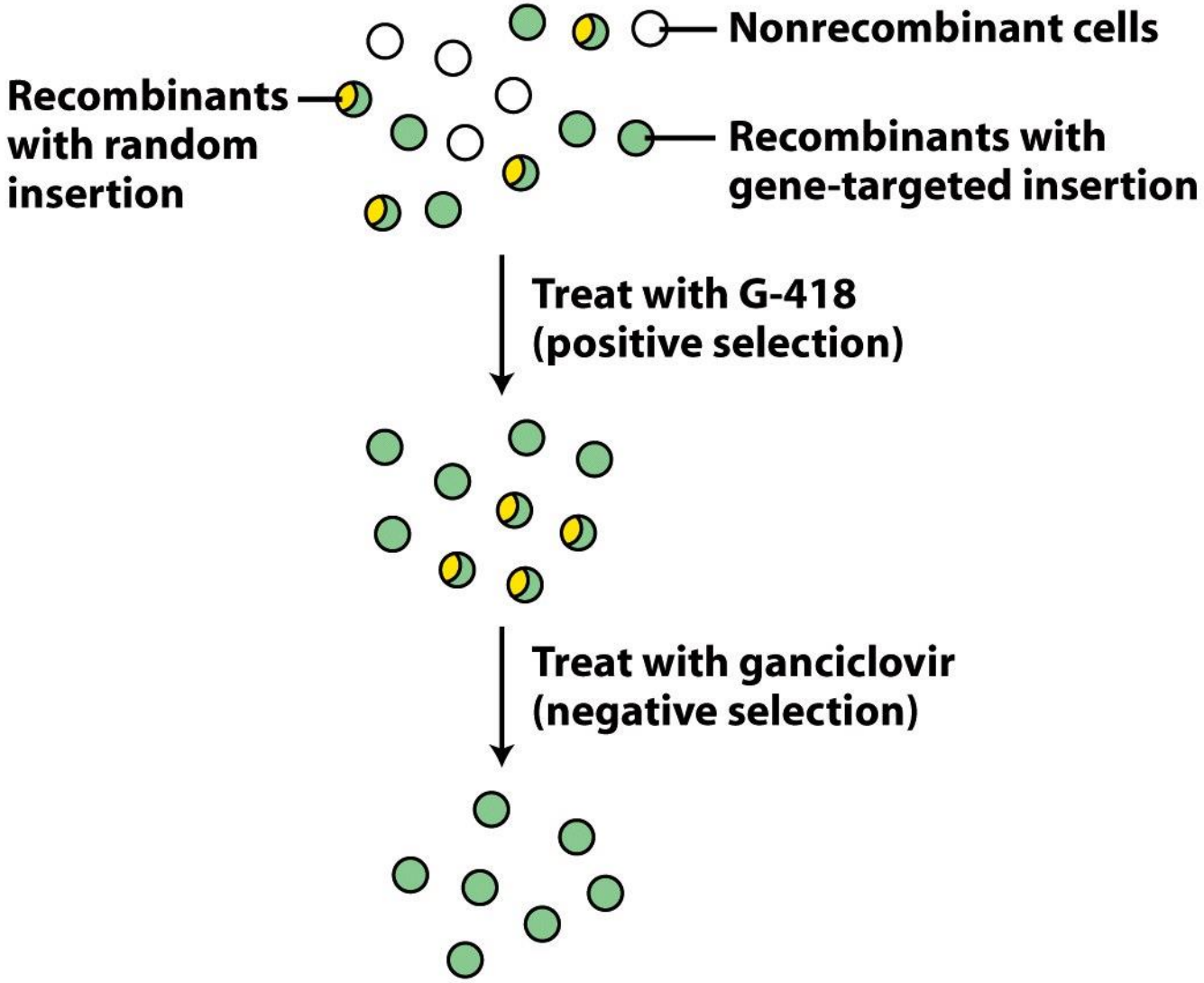


Figure 5-40b
Molecular Cell Biology, Sixth Edition

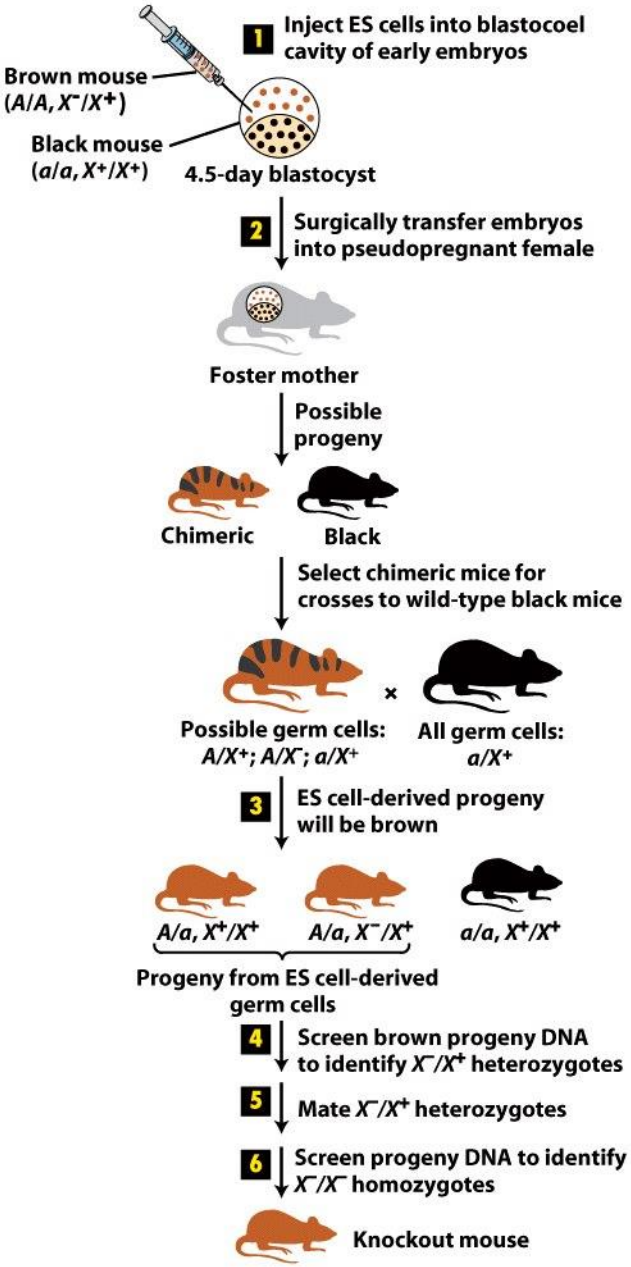
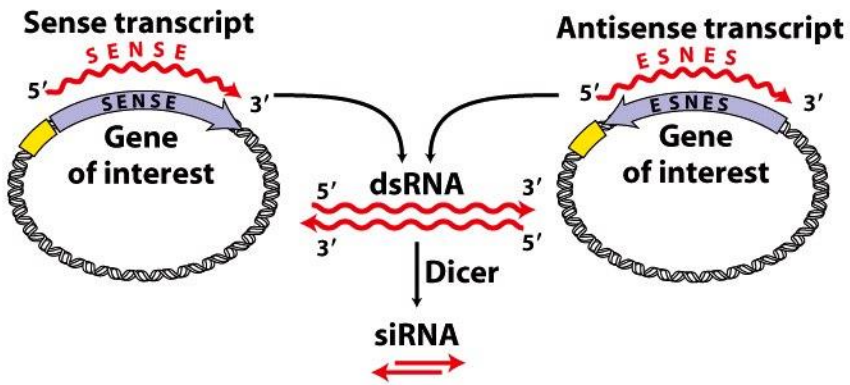
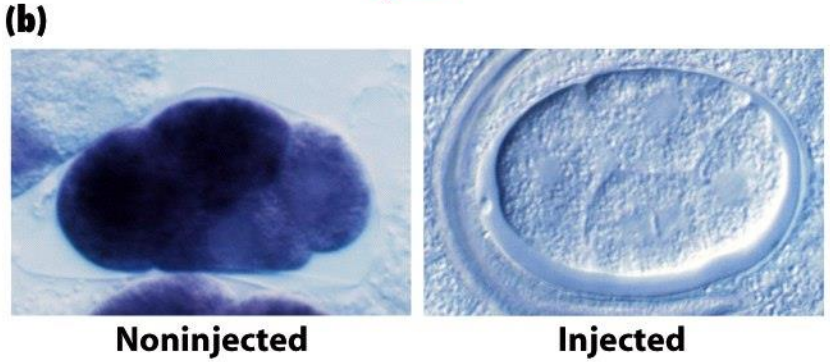
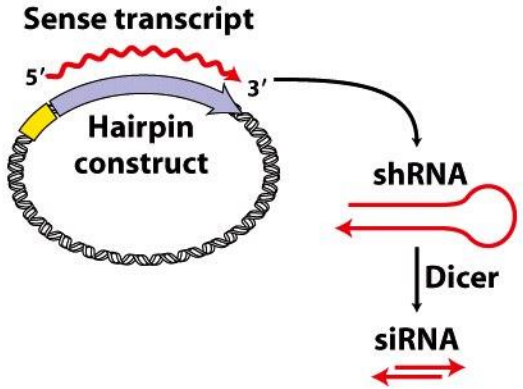


Figure 5-41
Molecular Cell Biology, Sixth Edition

Gene knockdown







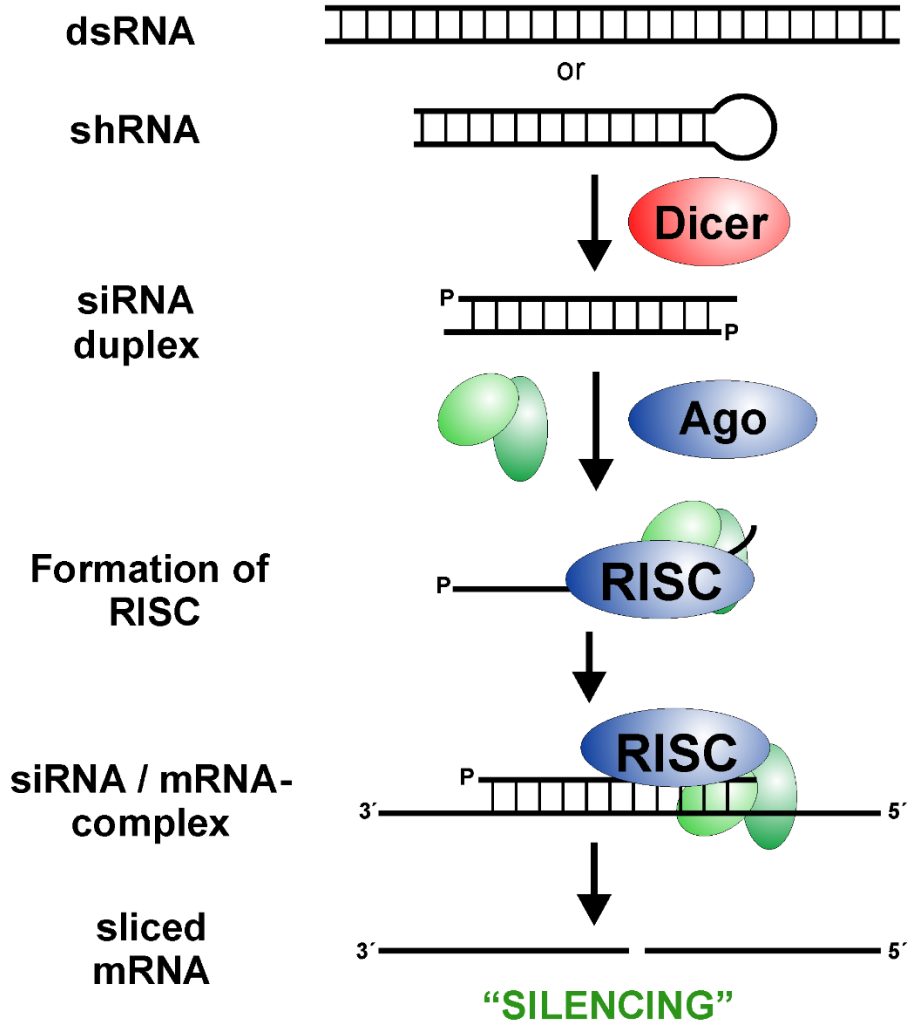


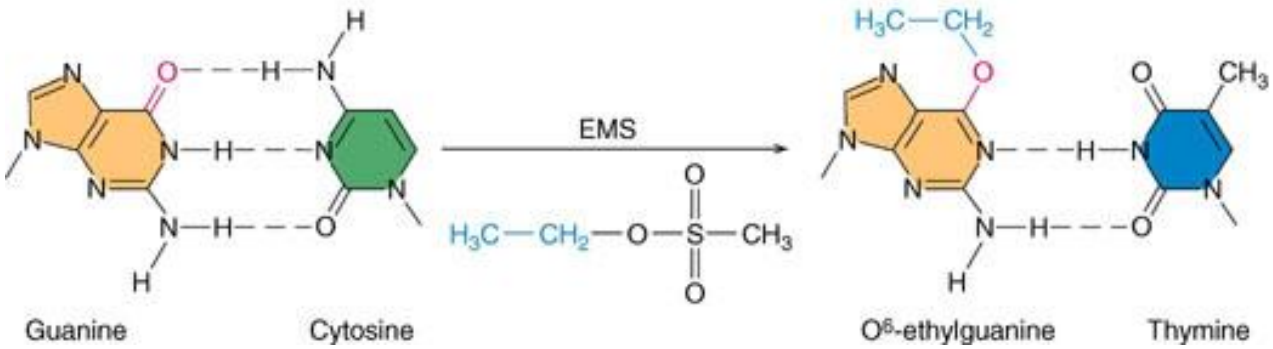
Figure 5-45
Molecular Cell Biology, Sixth Edition

Mutants

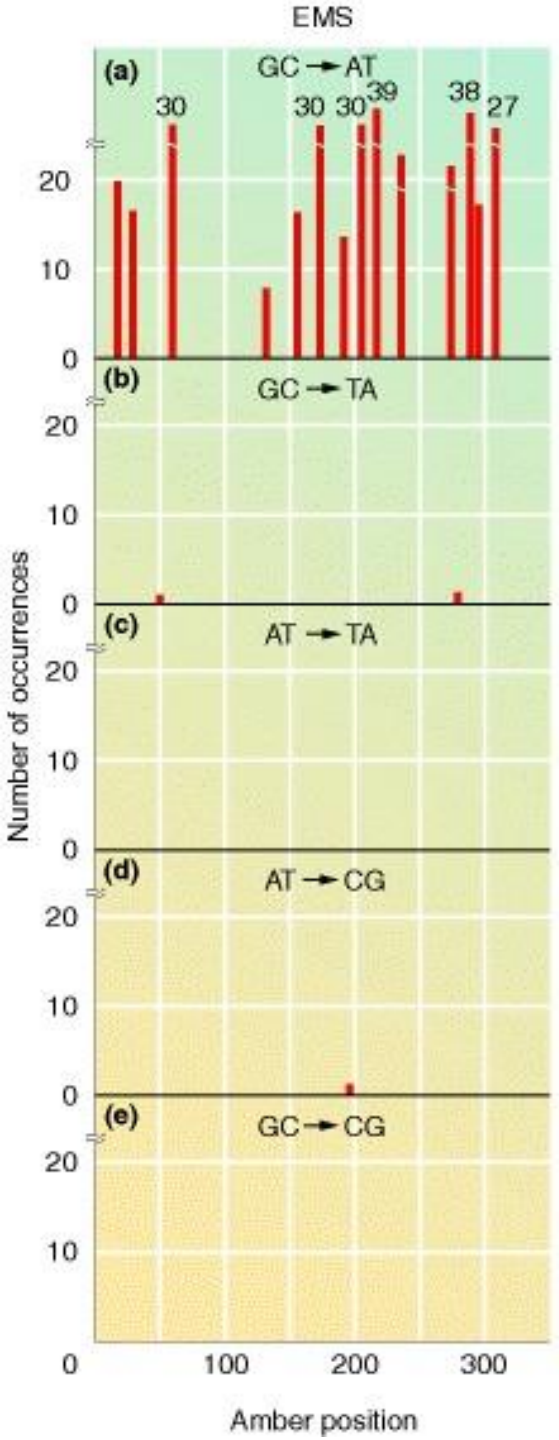
The publication of a single paper in Nature in 1980, entitled "Mutations Affecting Segment Number and Polarity in Drosophila," revolutionized the field of developmental genetics. Prior to this, most developmental biologists assumed that development was so complex that a genetic approach would not be fruitful. The authors, Christiane Nüsslein-Volhard and Eric Wieschaus, realizing that it would be worthwhile to do a systematic genetic screen for developmental mutants, searched for mutant genes that affect the formation of segments in the Drosophila embryo.

Mutagen

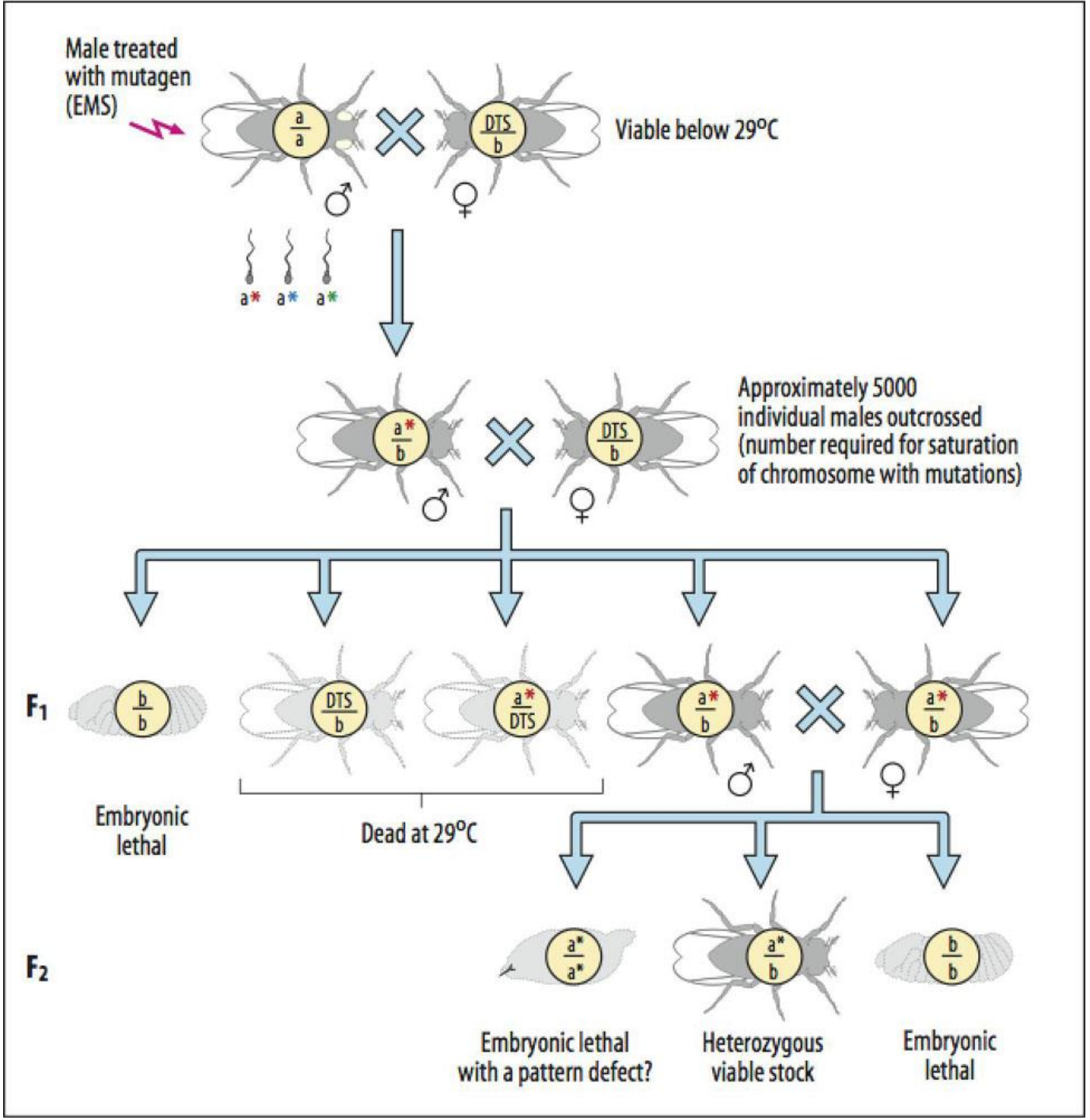
Ethyl methanesulfonate or EMS



- This addition of oxygen at 6 position of guanine result in mispairing with thymine instead of cytosine
  - GC → AT transition



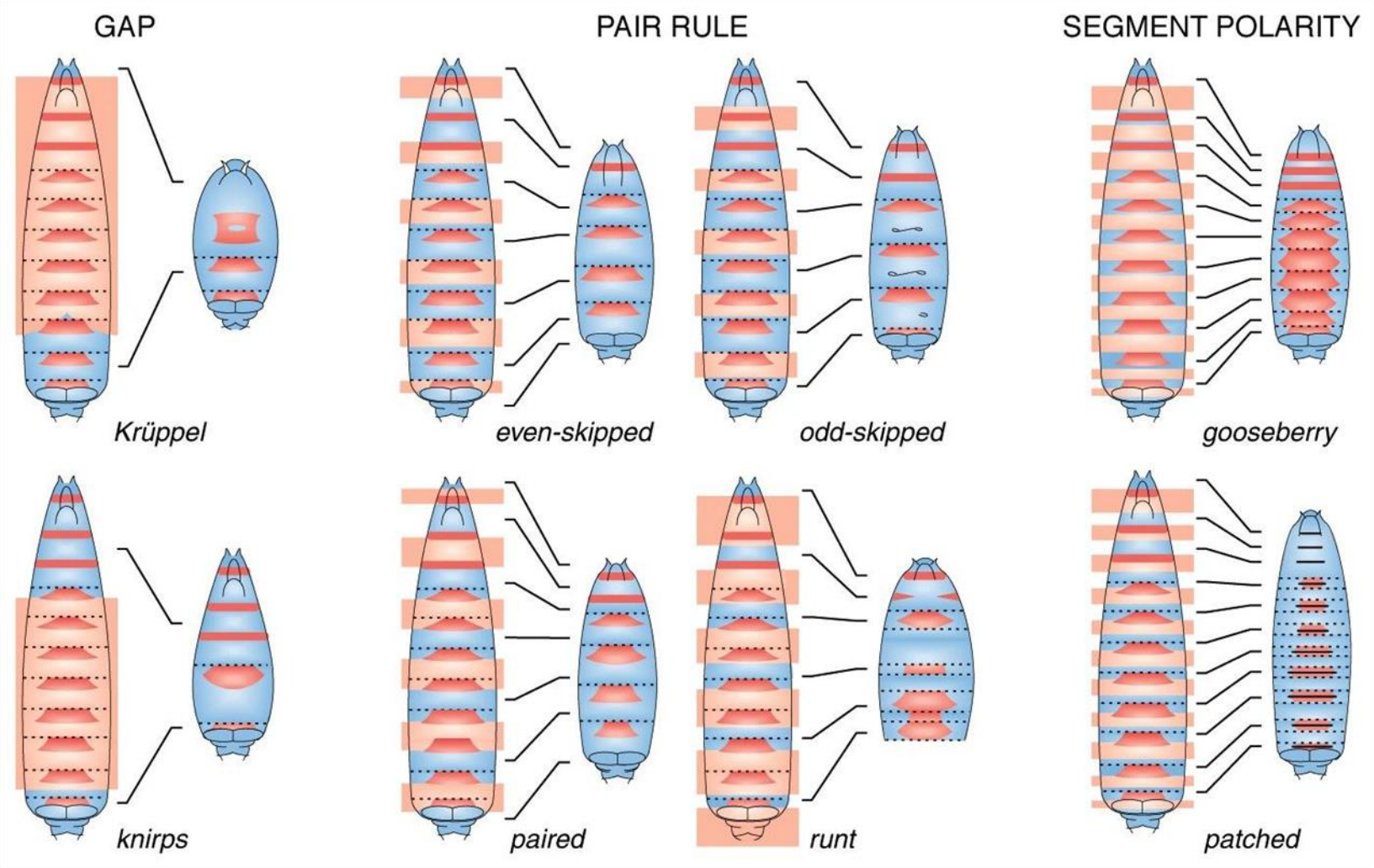
The Scheme


Male treated with mutagen (EMS)
Viable below 29°C
Approximately 5000 individual males outcrossed (number required for saturation of chromosome with mutations)
F1
Embryonic lethal
Dead at 29°C
F2
Embryonic lethal with a pattern defect?
Heterozygous viable stock
Embryonic lethal

Results

- Total lines established and tested 26978
- Lethal mutations 18136
- Mutations causing embryonic lethality 4332
- Mutations with embryonic phenotype 580
- Complementation groups (genes) 139

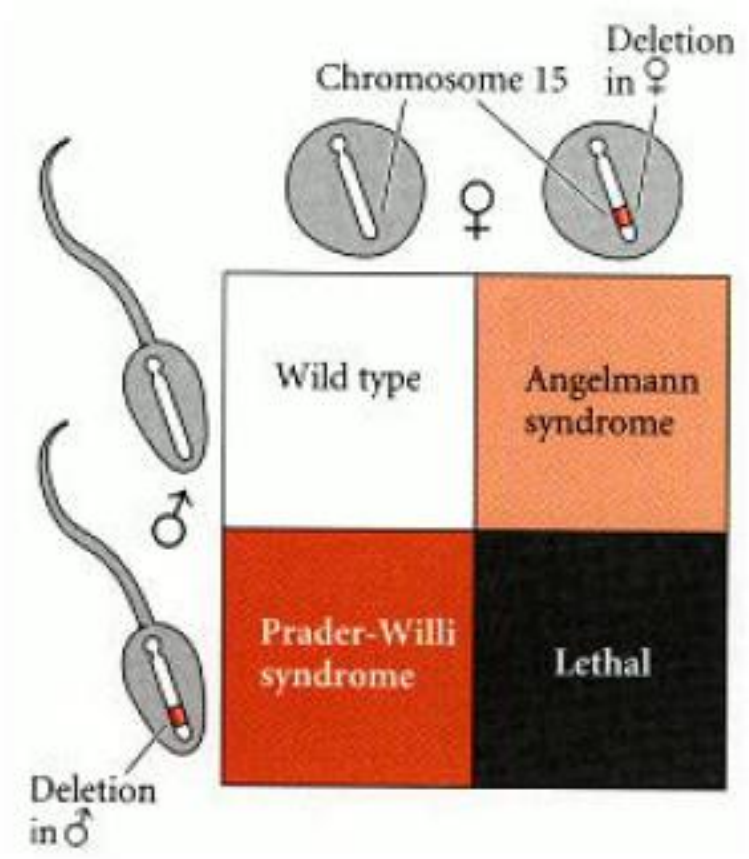
Functional groups found



Genomic imprinting

- Does it matter who provides what gene?
- Yes, sometimes.
- A mouse gets insulin-like growth factor II (*Igf-2*) from father and *Igf-2r* from mother.
- *Igf-2r* controls *Igf-2* in embryo.
- If a mouse gets a mutant *Igf-2r* from father, the mouse is ok
- But if a mouse gets a mutant *Igf-2r* from mother, it dies.

In human



- Prader-Willi syndrome, a disease associated with mild mental retardation, obesity, small gonads, and short stature
- Angelman syndrome, characterized by severe mental retardation, seizures, lack of speech, and inappropriate laughter