DEVELOPMENTAL PHYSIOLOGY

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INTRODUCTION

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DEVELOPMENTAL BIOLOGY

- central to all other areas of biology – unites cell biology, genetics and morphology
- in last 25 years grew into the most exciting and dynamic field of biology…..due to the advances in three main traditions: the experimental embryology, developmental genetics and molecular biology. All animals develop similarly, that including the worm, fly, fish or mammal.
- significant impact on society, in vitro fertilization, teratology, birth defects.
- future impact: functional genomics and functional proteomics, disease therapy, prenatal screening, transplantation, embryonal stem cells, therapeutic cloning.

COMMON FEATURES OF DEVELOPMENT

Developmental Biology: A science that explains how a structure of organism changes with time

- structure or morphology or anatomy: an arrangement of parts (large/small, organs, parts of an organ, cells in a part of an organ)

- many forms of development: embryonic (zygote ⟶ complete organism), postembryonic (larva ⟶ adult organism), regeneration (asexual reproduction through budding, replacement of lost parts)

-….but same problems

1. Regional specification: pattern appearance in uniform mass of cells.

2. Cell differentiation: 200 different cell types in vertebrate body, each differ by particular proteins made through activation of particular genes.

3. Morphogenesis: cell and tissue movements creating the 3D design of organ.

4. Growth: increase in size and control of proportions among the parts.

GENOMIC EQUIVALENCE, CLONING OF ANIMALS

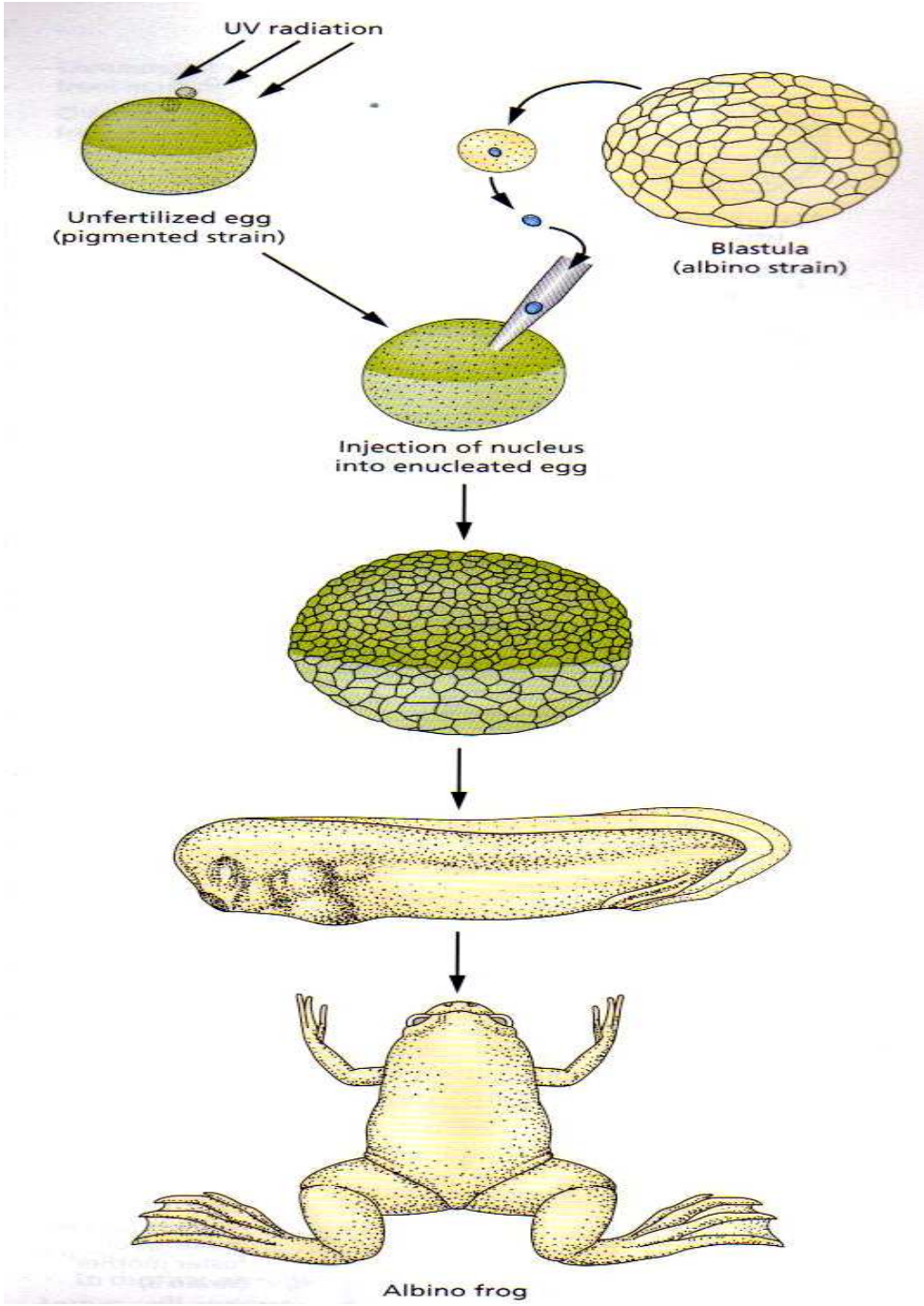
In any animal…..
-sperm, egg and precursors = <u>GERM LINE</u>

-the rest = <u>SOMATIC CELLS</u>

-somatic cell contain full complement of genes because most genes are needed at some stage of development
(with exceptions…..T and B-lymphocytes)

- some cell contain more than 2 total copies of every gene – **Polyploidy** vs. **Polyteny**

- the differences among somatic cells stem from differential gene activation therein



GAMETOGENESIS

- male and female gametes
- fertilized egg or ovum
- each gamete is 1n – a haploid set of chromosomes
- Zygote is 2n – a diploid set of chromosomes

Gametes are formed from germ cells in the embryo

Germ line: cells that can or will become the future gametes.

Somatic cells: all other except the germ line.

Future germ cells decide to be „germ“ early in the embryo development somethimes through inheriting a cytoplasmic element from the egg – a **germ plasm** (polar granules in *C. elegans*).

During embryonic development germ cells multiplicate and migrate to find a **gonad.**

Gonad: mesodermal by origin and composed entirely of somatic cells, when germ cells arrrive they integrate into gonad and in post-embryonal life undergo GAMETOGENESIS

MEIOSIS

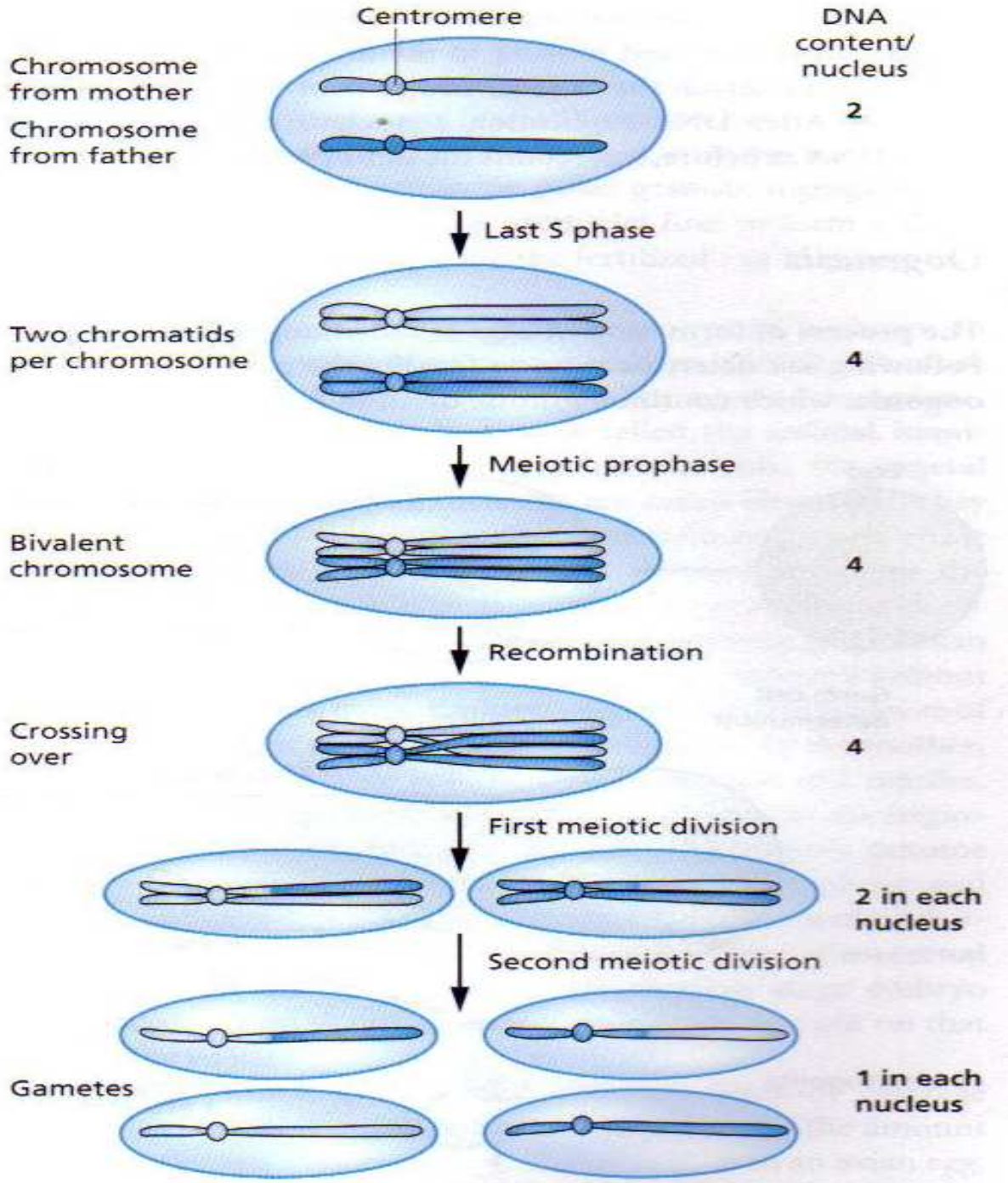
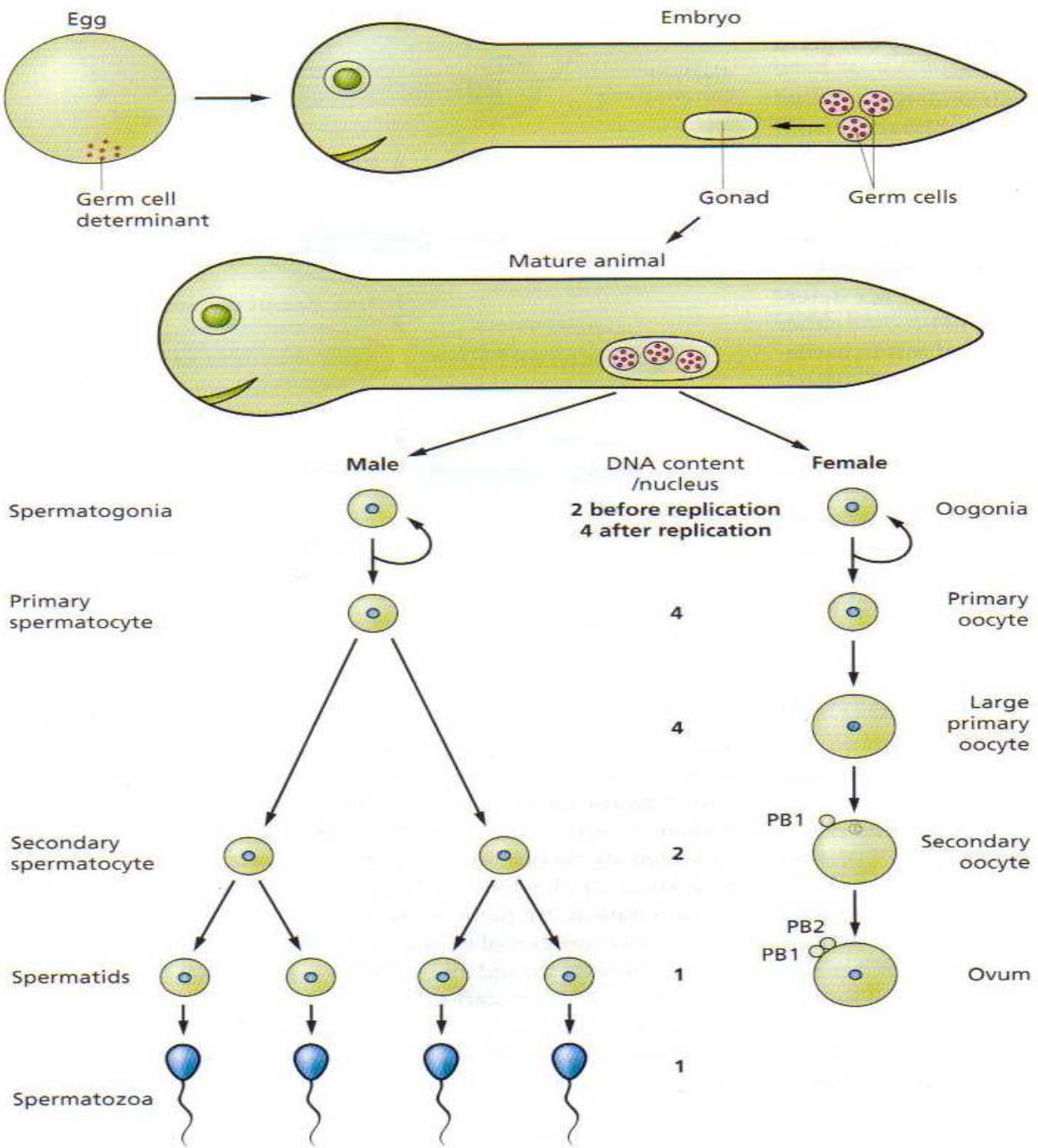


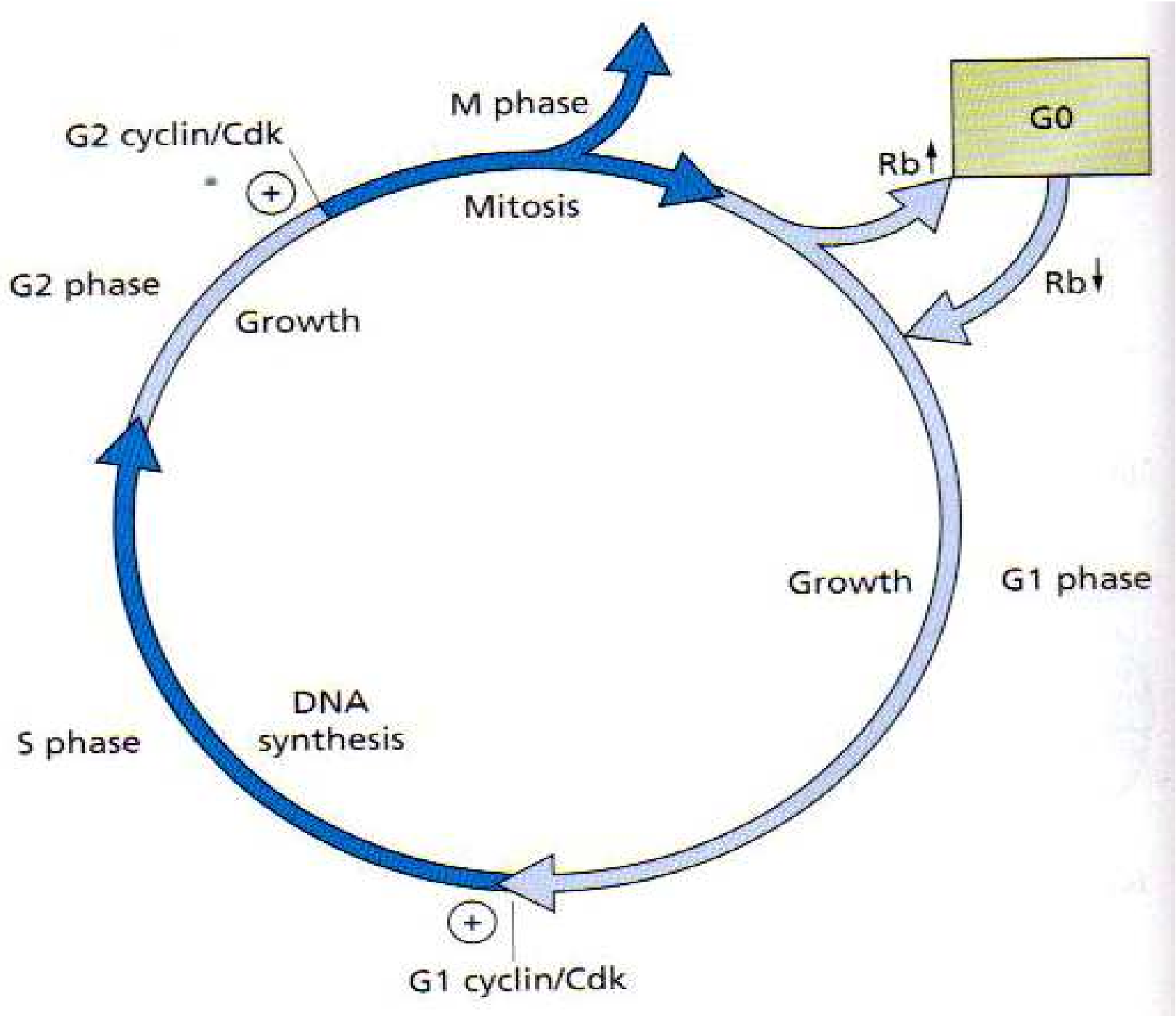
Fig. 2.3 Behavior of chromosomes during meiosis.

OOGENESIS vs. SPERMATOGENESIS

- Formation of eggs or sperm
- **Primary oocytes** – long-lived cells that increases its size during the life-span
- Often depends on accessory cells in its growth
- In mammals all oocytes are produced before birth and remain dormant to the puberty
-**Ovulation:** hormone stimulated resumption of the meiotic divisions and release of oocyte from the ovary. Accompanied by breakdown of oocyte nucleus (germinal vesicle) and migration of the division spindle to the cell periphery. Meiotic divisions produce polar bodies that play no further role in development – **unequal meiosis**.



GROWTH AND DEATH

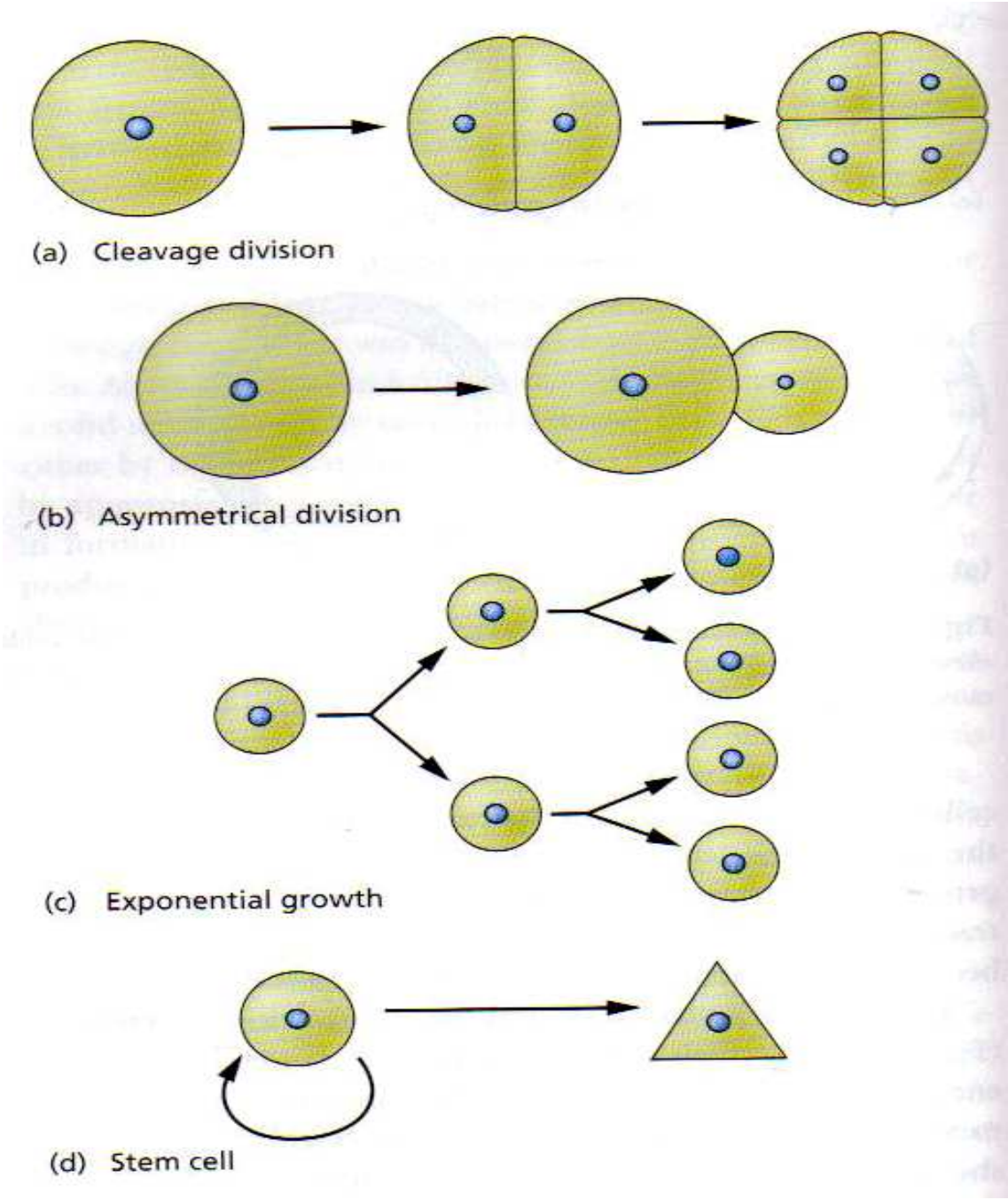


GROWTH AND DEATH

Growth = cell division + increase of cell size + deposition of extracellular matrix.

Differentiation leads to cessation of division. In postembryonic life most cells that divide are either **stem cells** or their immediated progeny called **transient amplifying cells**.

Differentiated cells are ultimately removed through **apoptosis** (programmed cell death). Apoptosis: a way of removing cells without spilling their bioactive content into the surroundings. In developement, apoptosis is important in limb development where digits form through removal of tissue between by apoptosis.



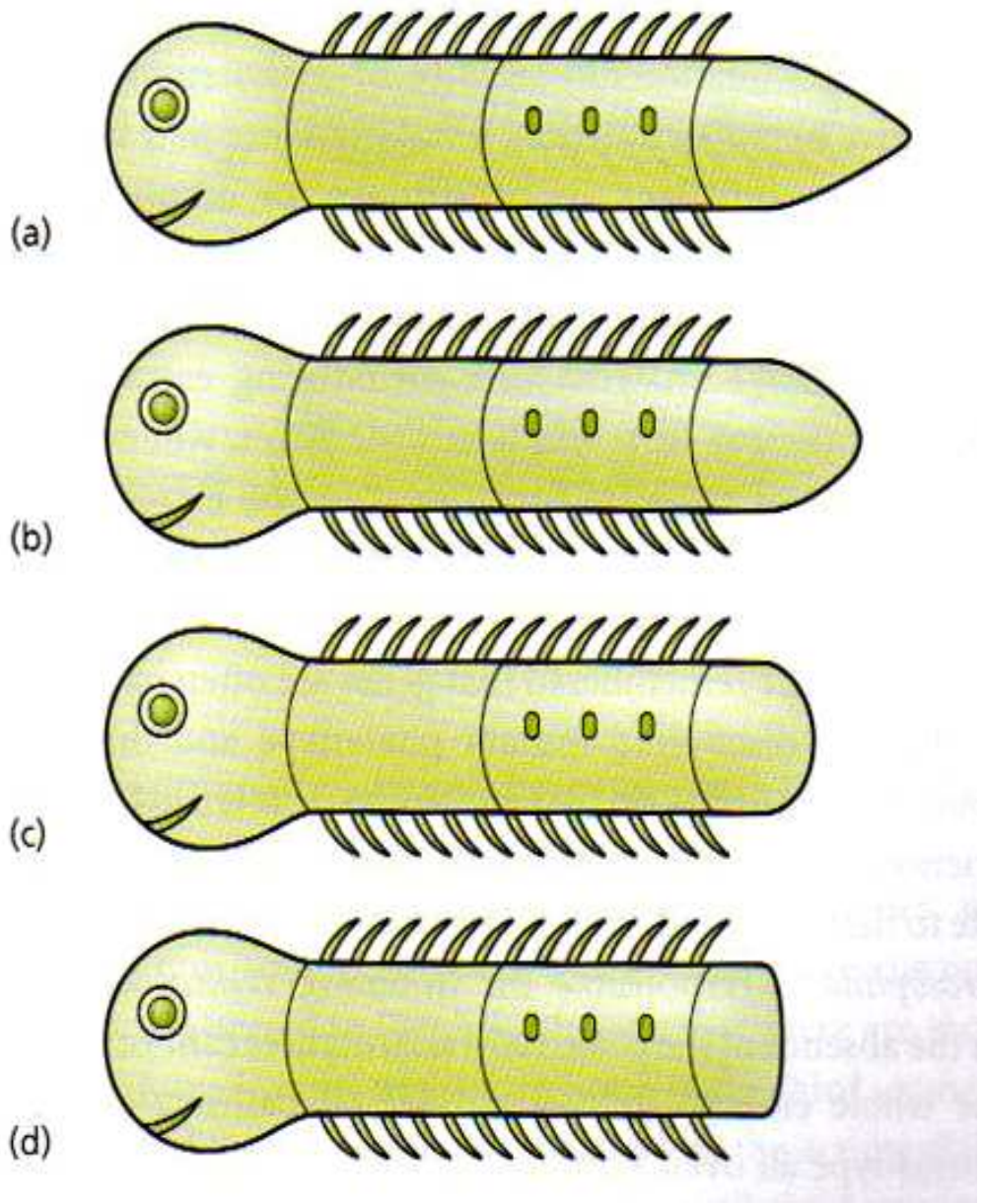
DEVELOPMENTAL GENETICS

DEVELOPMENTAL MUTANTS

Mutants represent important tool for developmental genetics. Mutation may be spontaneous or induced by the experimentator.

MUTATION TYPES: **Loss/gain-of-function** – protein product of a gene is more/less active then wild type. **Null** – complete lack of the active gene product.

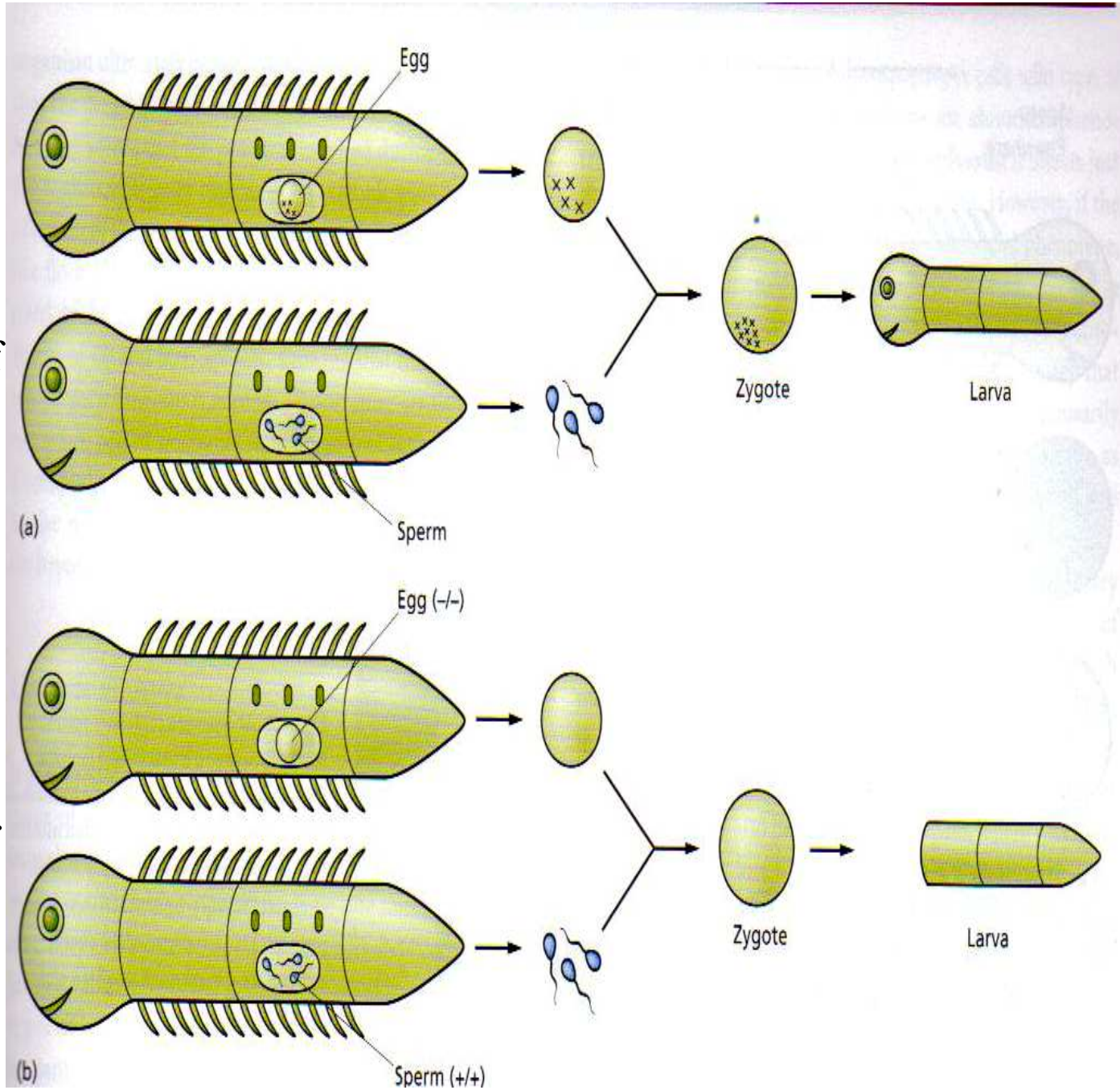
Alleles – different forms of gene. One normal (wild type), possibly many different mutated. **Allelic series** - different degrees of loss-of-funtion, ordered by the severity of the phenotype.



MATERNAL AND ZYGOTIC

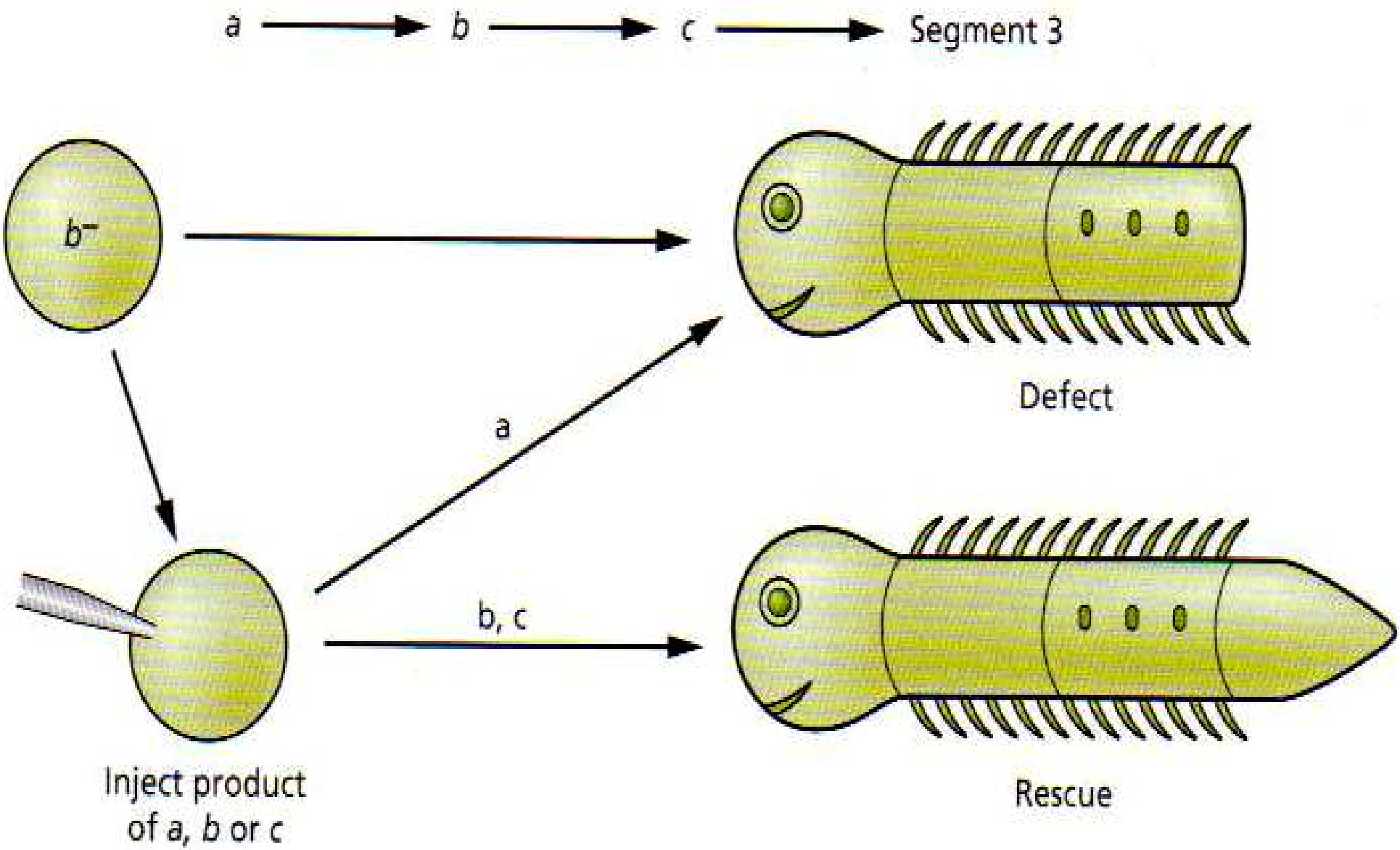
In early development the phenotype of an individual not always corresponds to its genotype. This is because some early developmental events depend on the situation in mother rather then embryo itself (**maternal-effect gene**).

The maternal control of development continues after fertilization because in most animals the embryo‘s own genome, called the **zygotic genome**, remains inactive during the early cleavage stages. The zygotic genome may be activated at different stages in animal, ranging from early cleavage to late blastula.

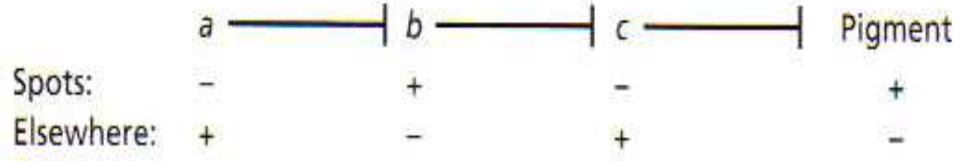


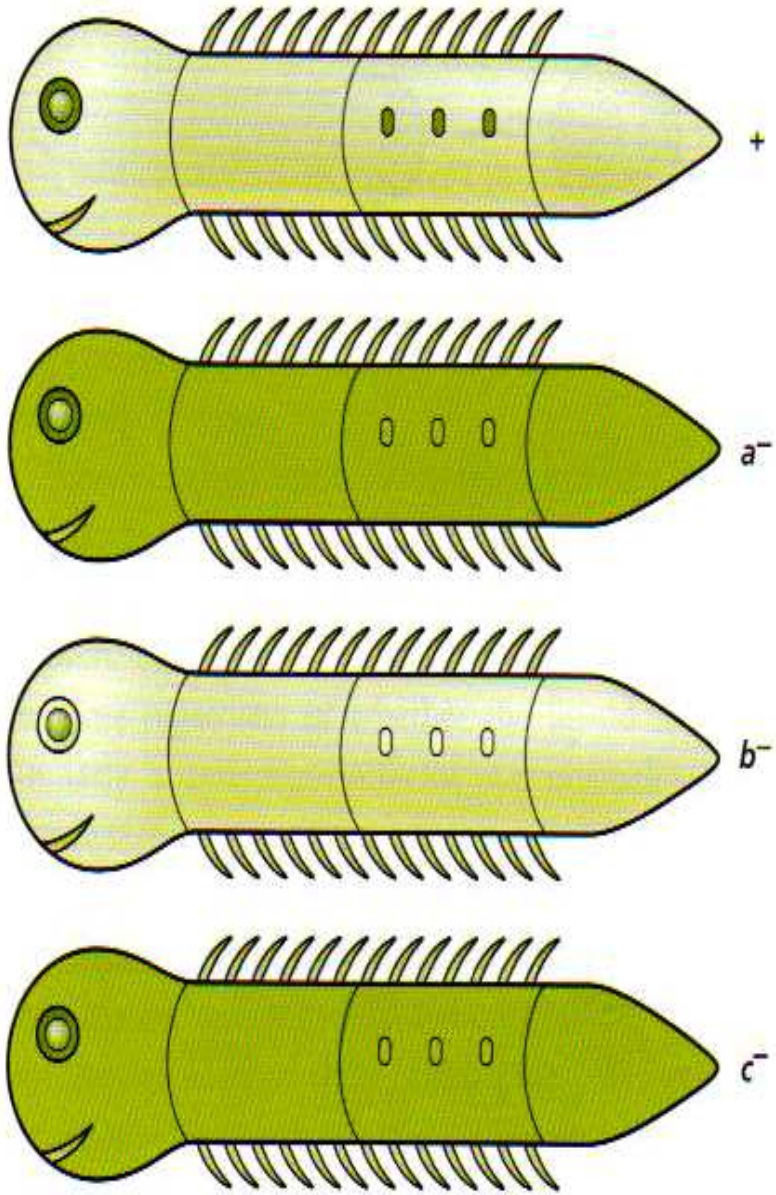
GENETIC PATHWAYS

Genetic data can be used to delineate a pathway, a set of genes regulating specific process.



Repressive pathways:
Normally gene A is inactivated in spots (segment 2) only resulting in formation of pigment. In a loss-of-function mutant of A or C the whole organism is pigmented. In a loss-of-function mutant of B there is no pigment. The mutant phenotypes show that B must act after A and before C. An example of **epistasis analysis** – the gene that represses another gene is **epistatic** to it.





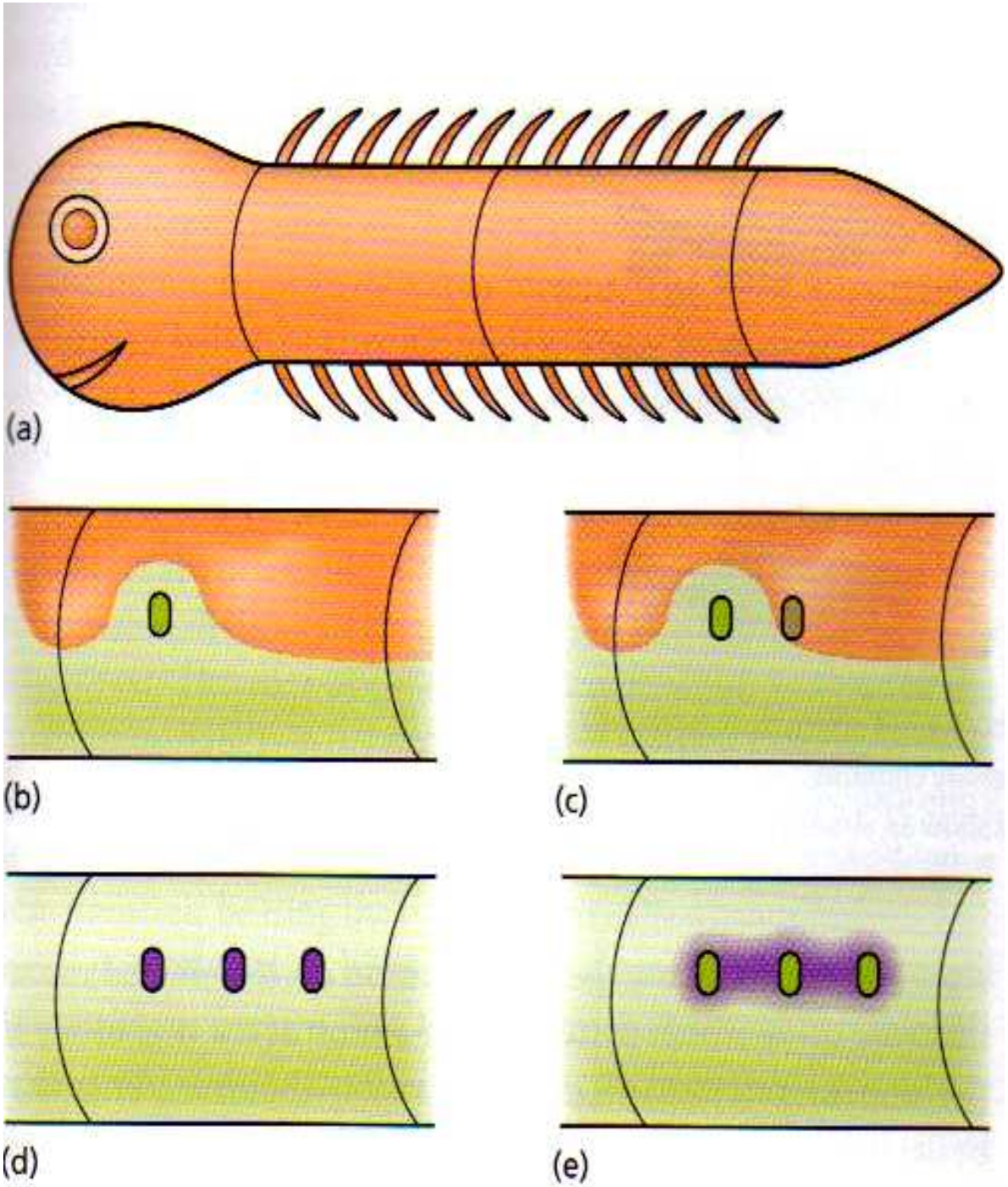
Double mutant phenotypes

	a^-	b^-	c^-
a^-		White	Pigmented
b^-	White		Pigmented
c^-	Pigmented	Pigmented	

GENETIC MOSAICS

Genetic mosaic – an organism that consists of cells of different genotypes. In mammals occurs naturally when X chromosome is inactivated (**X inactivation mosaic**).

Use of genetic mosaic. (a) mutant of a gene in which the three spots (segment 2) are missing. In (b) and (c) the genetic mosaics are made in which the red tissue is null mutant and green is wild-type. In (b) the spots appear in the wt zone so the gene must have **autonomous function** (it affects just the region of its expression), corresponding the wt expression pattern (d). In (c) a spot appears in the zone of mutant tissue so the gene must have a **nonautonomous function** (affects structure outside of the domain of its expression) (e).



SCREENING FOR MUTANTS

Forward genetics – an investigation that starts with the discovery of a mutant phenotype. **Reverse genetics -** a functional investigation on a known gene.

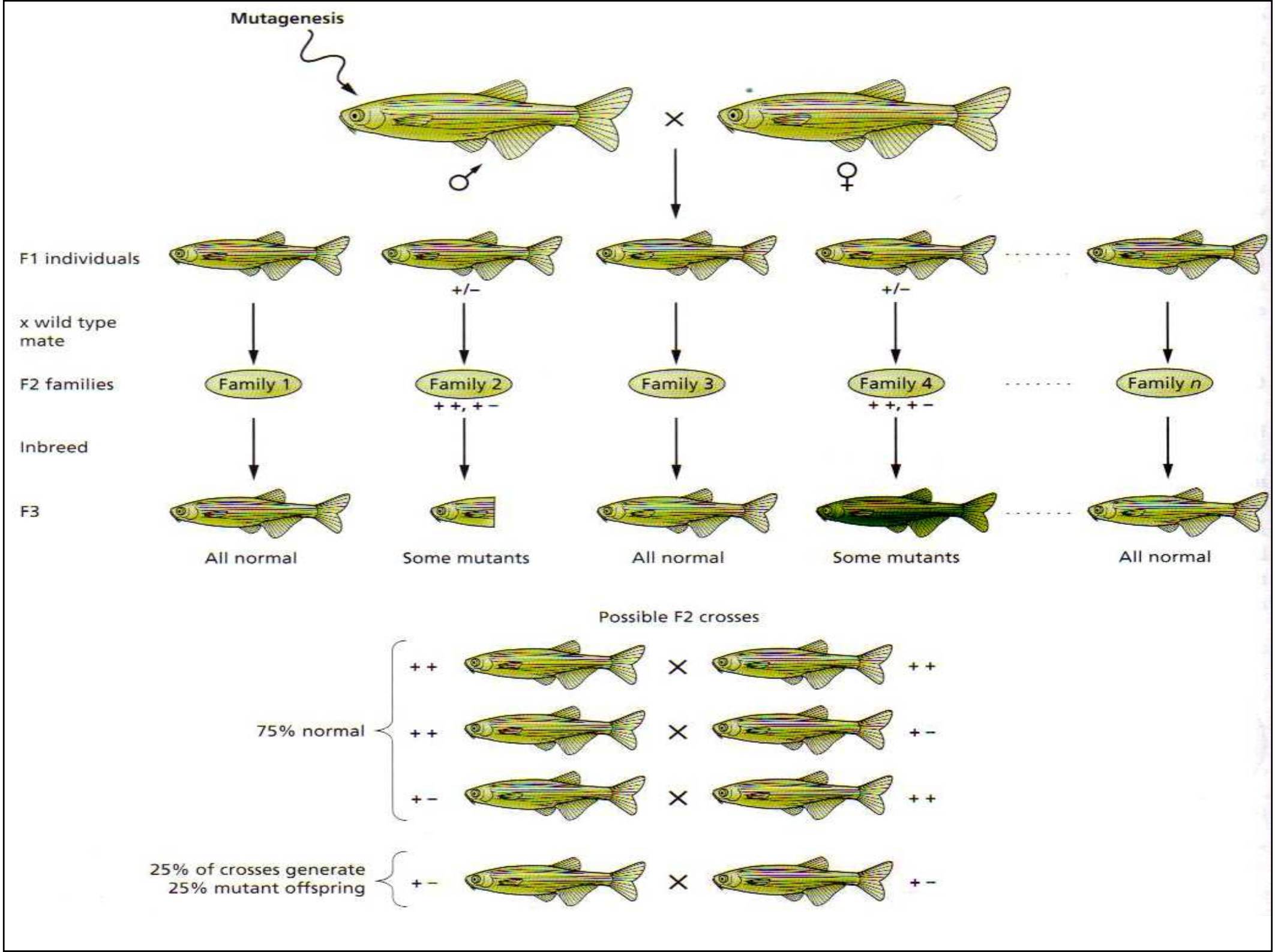
SCREENING FOR MUTANTS

1. A group of males is treated by chemical mutagene to induce mutations on spermatogonial cells.

2. Males mated with females to create F1. Each of F1 individuals carries the mutation in heterozygous form.

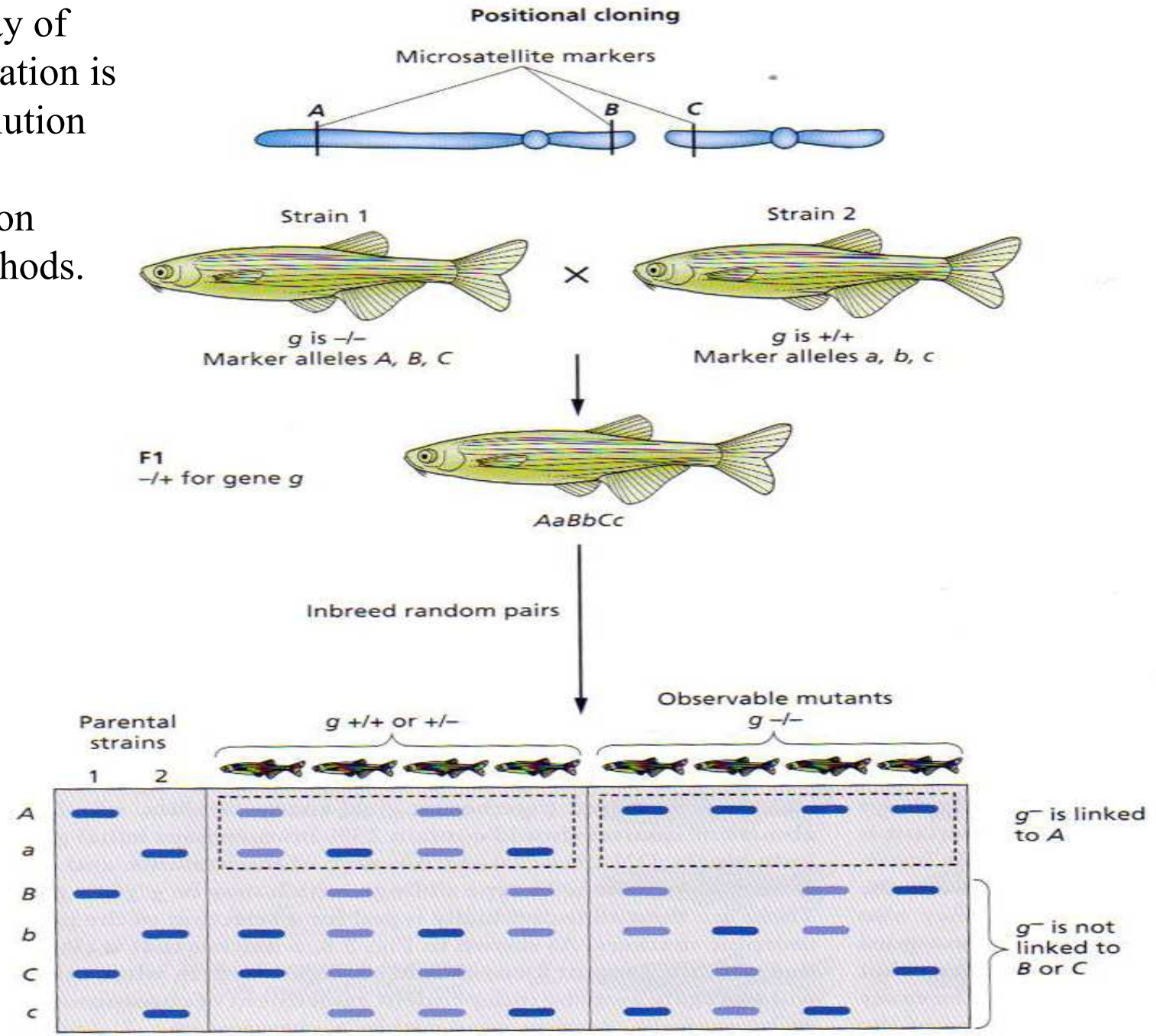
3. Each F1 individual is bred with wt animals to create a family of F2, half of F2 is heterozygous for mutation.

4. A test matings are carried-out between pairs of F2 family to produce F3 that carries 25% of homogygous mutants.


Mutagenesis
♂
×
♀
F1 individuals
+/–
+/–
x wild type mate
F2 families
Family 1
Family 2
+ +, + –
Family 3
Family 4
+ +, + –
Family n
Inbreed
F3
All normal
Some mutants
All normal
Some mutants
All normal
Possible F2 crosses
75% normal
+ +
+ +
+ +
+ –
+ –
+ +
25% of crosses generate 25% mutant offspring
+ –
+ –

CLONING OF GENES

Positional cloning – a way of gene cloning where a mutation is mapped to very high resolution using microsatellite polymorphism or restriction length polymorphism methods.



TRANSGENESIS

Transgenesis – an introduction of extra gene into the genome.
1. In mouse and zebrafish, the DNA is injected into the fertilized egg.
2. In *Xenopus,* the DNA is incorporated to the sperm.
3. In *Drosophila* and *C. elegans*, the transgene is incorporated into the transposable element that is injected into the germ cells of the embryo.

Transgenes are usually regulated by inserted **promotor**, in order to escape the natural gene regulatory processes in cells. In some cases the transgene is designed to probe the local genomic environment – **enhancer trap** – consists of **reporter** gene coupled to **minimal promotor**. This is only active when transgene integrates within the range of enhancer.

TARGETED MUTAGENESIS: A way to introduce specific mutations at a desired site in the genome – used in mouse exclusively. A way how to produce **knockouts** or **loss-of-function** mutations in particular genes. Targeted mutagenesis depends on **homologous recombination** in which is the gene directly replaced by a modified version made *in vitro*.

OTHER WAYS TO INHIBIT GENE ACTIVITY:
1. ANTISENSE REAGENTS:**Morpholinos –** stable oligonucleotide analogs that are resistant to nucleases and can hybridize with DNA. **RNA interference (RNAi)** – introduction of (double-stranded) **dsRNA** of the same sequence as target mRNA triggers antiviral response that destroys the targeted mRNA.
2. NEUTRALISING ANTIBODY directed against the given protein.