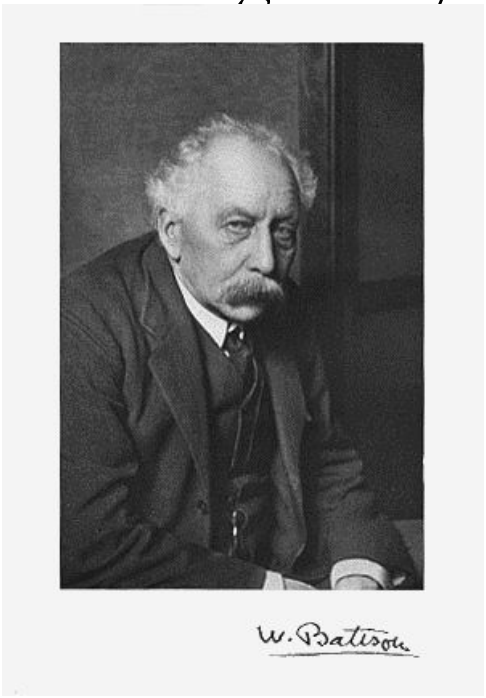
Regeneration, the replacement of lost or damaged tissue(s) or organ(s) of an organism, is a wide spread phenomenon in animals.

It is a process that allows an organism to regain the function of an organ or structure damaged by injury or disease.

(the term having been first used by Bateson in 1905)

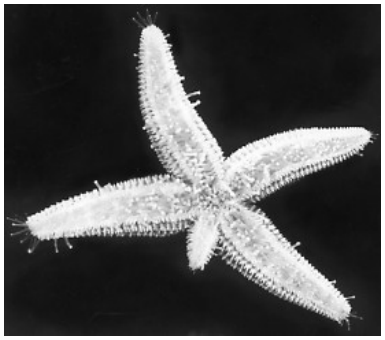


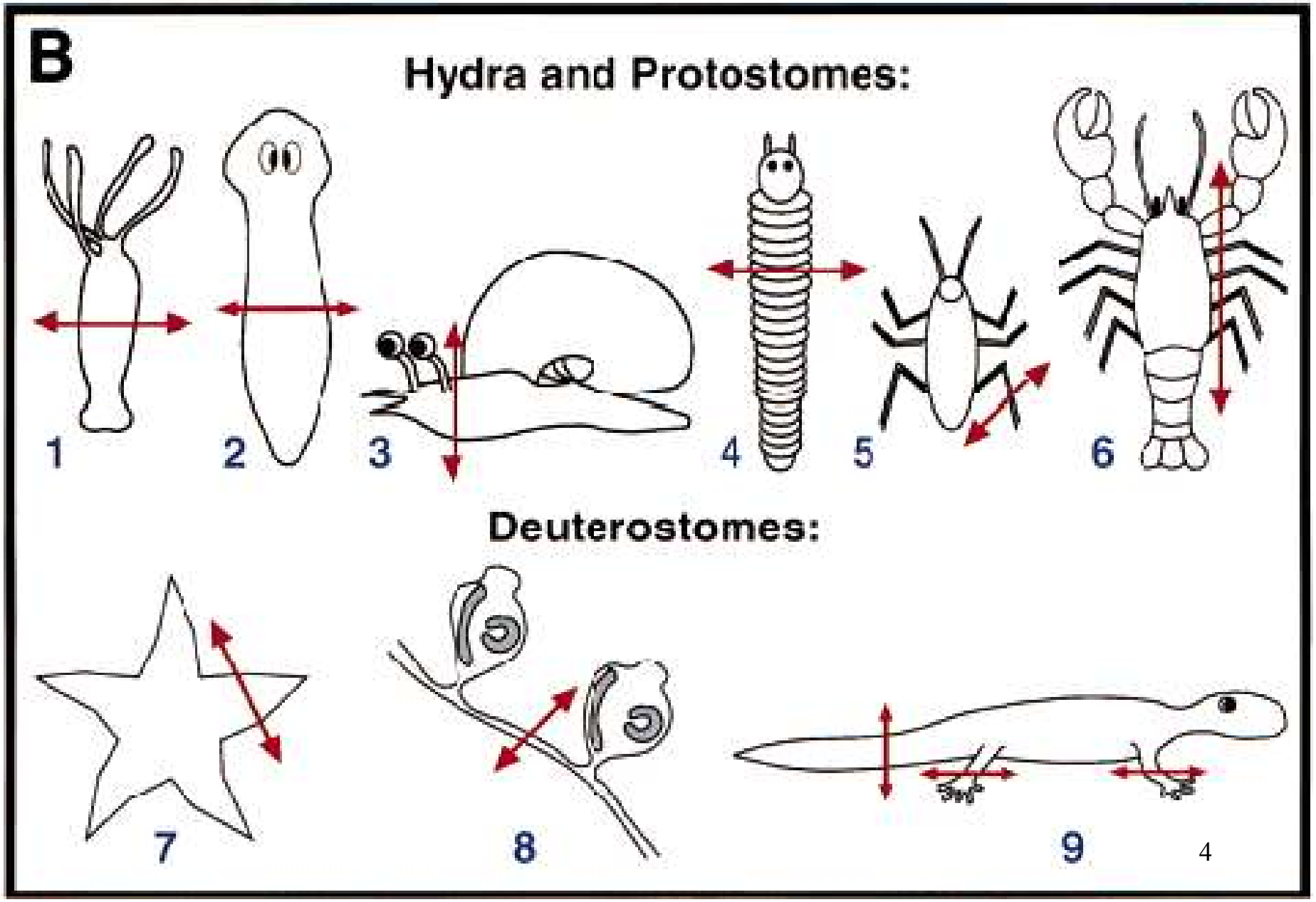
William Bateson (8 August 1861 – 8 February 1926) was an English Biologist

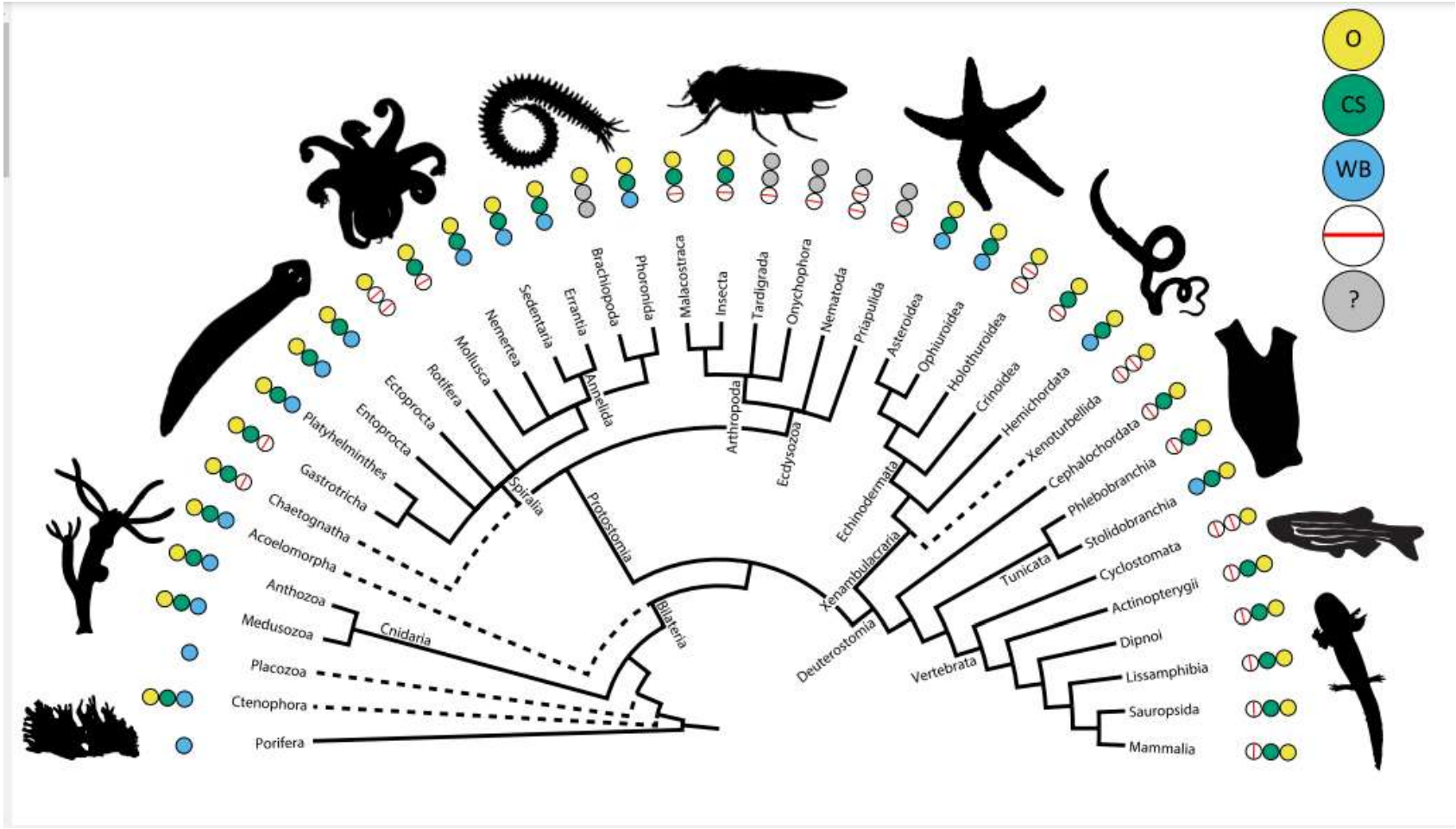
Invertebrates



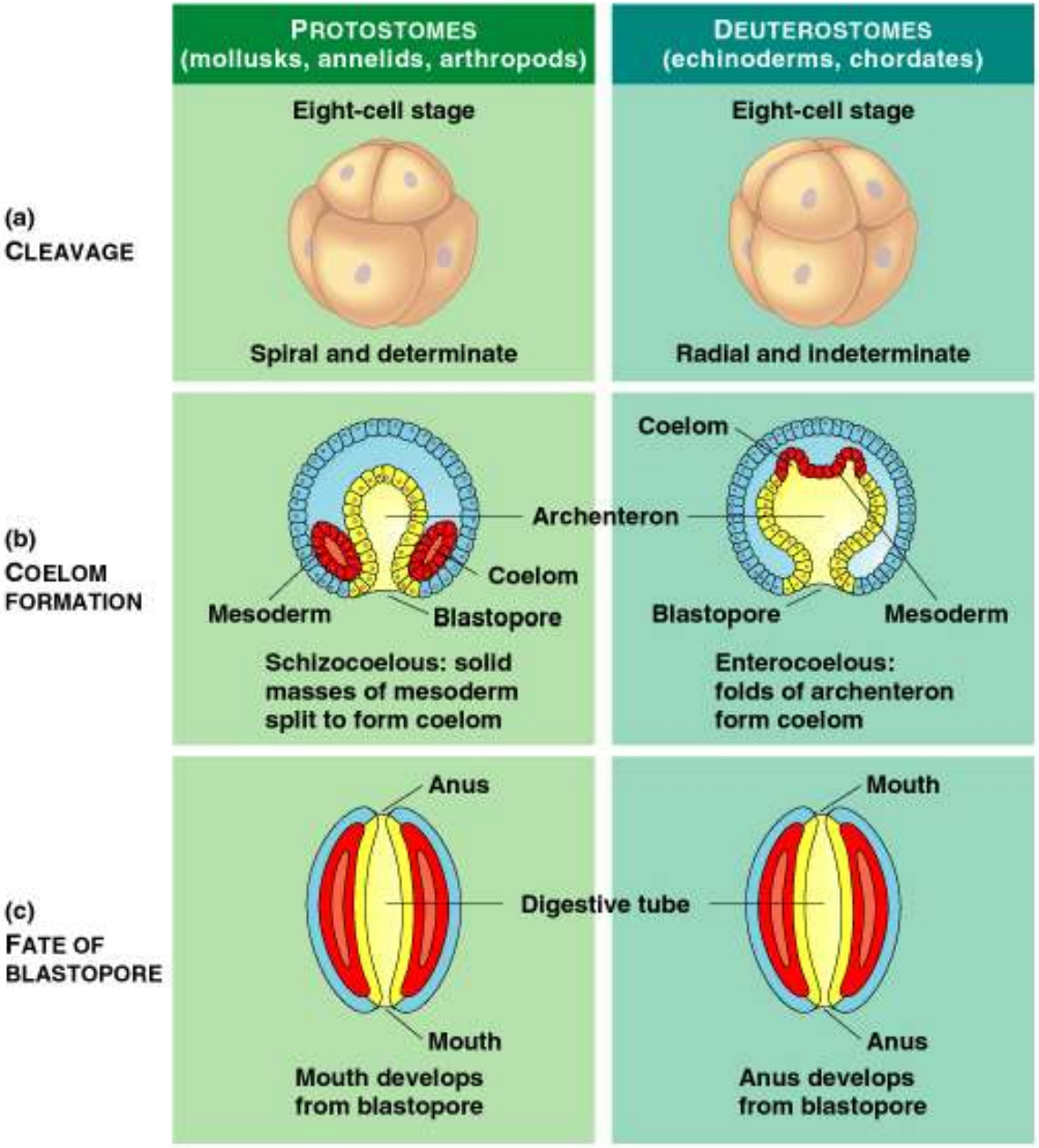


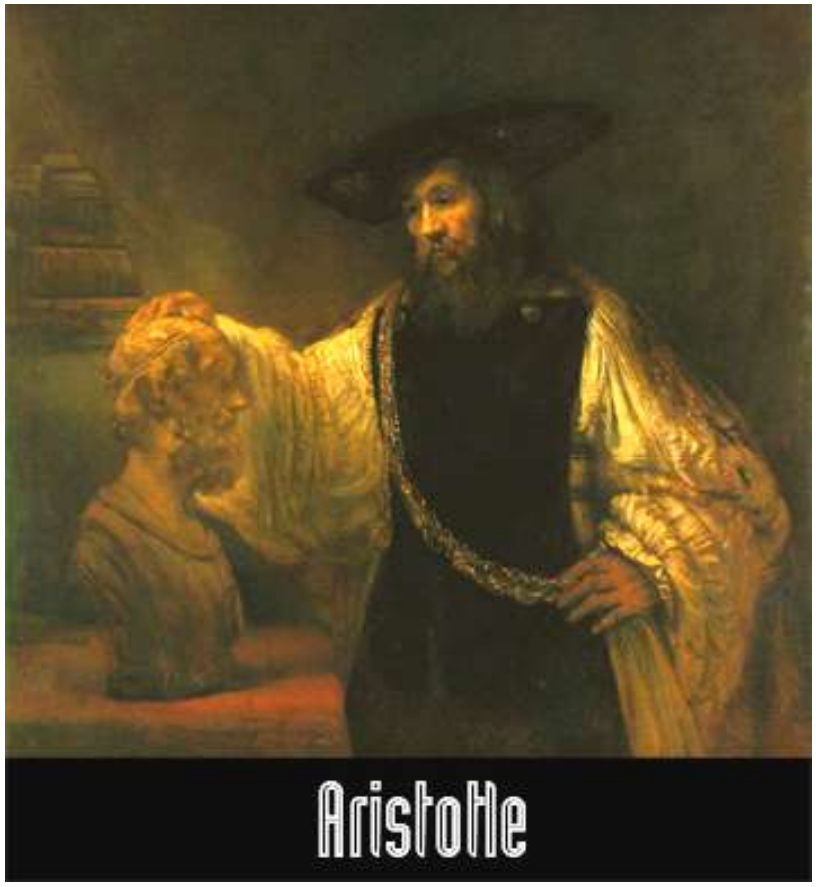




B
Hydra and Protostomes:
1
2
3
4
5
6
Deuterostomes:
7
8
9



Mapping regeneration types on the metazoan phylogenetic tree. Most metazoan phyla include representative species with documented abilities to regenerate **organs** (O, yellow circles) and **complex structures** (CS, green circles). While absent (struck-through circles) in all vertebrates, ecdysozoans, and some other scattered lineages, **whole-body regeneration** (WB, blue circles) is a widespread phenomenon among Metazoa. **Lack of substantiated data** (gray circles) is mostly observed in Brachiopoda and Ecdysozoa. Phylogenetic tree topology is derived from recent metazoans' phylogenies, with still-controversial lineage positioning depicted with dotted lines.



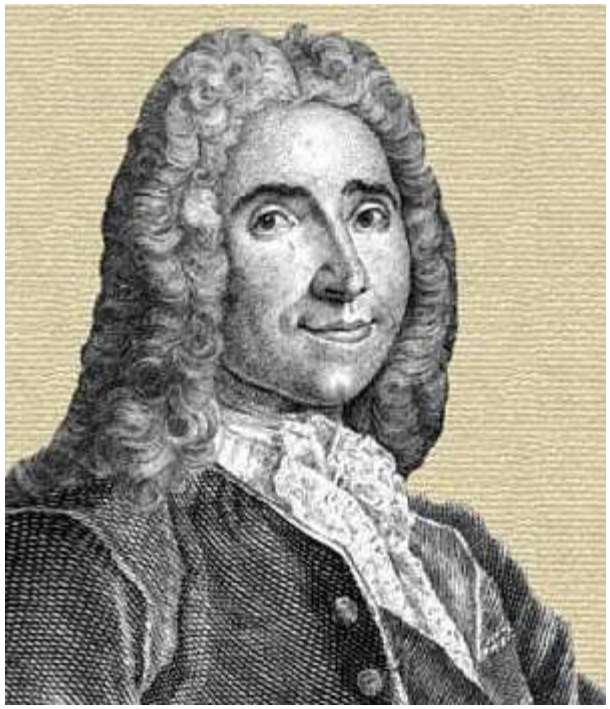


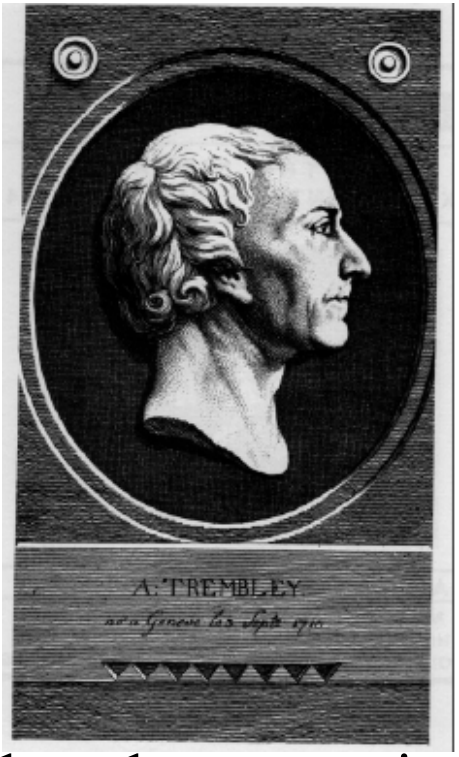
Aristotle 384-322 BC

Lizards could shed and regrow a lost tail

Rene-Antoine Ferchault de Reaumur (1683-1757)
Reaumur appears to be the first scientist to perform a serious study of regeneration. In 1712, he presented his paper on regeneration of **crayfish**

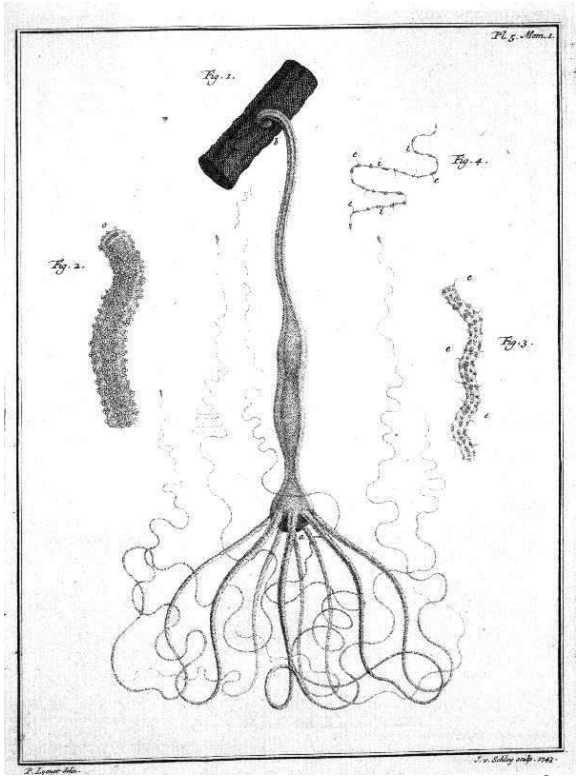
limbs and claws to the **French Academy**.





Abraham Trembley 1744
was the original **hydra biologist**.
His experiments are described in his "Memoires Concerning the Natural History of a Type of Freshwater Polyp

with Arms Shaped Like Horns."

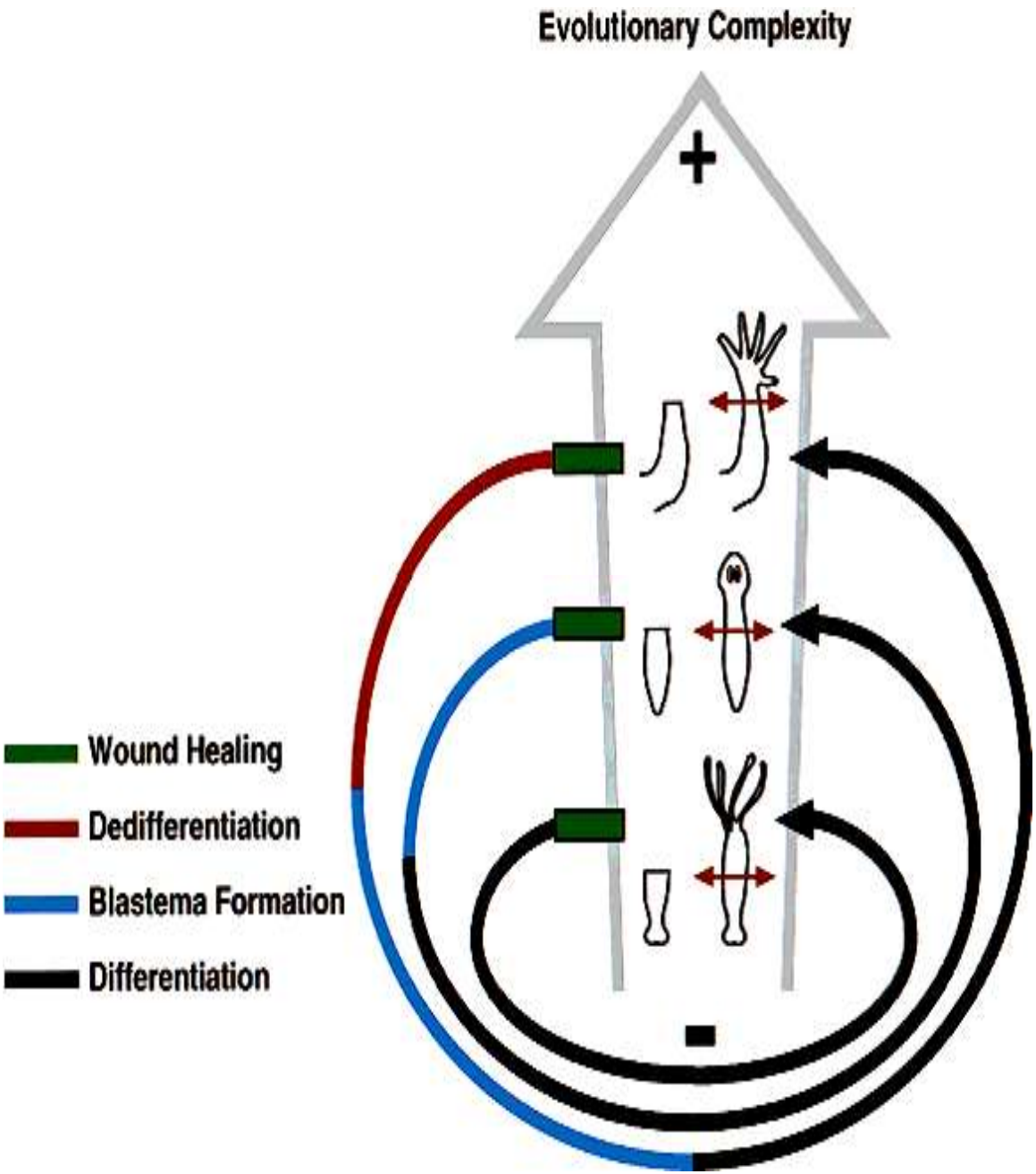


The demonstration of the ability to regenerate missing parts after amputation in an animal by Trembley is the earliest record of this phenomenon. He called this animal **Hydra**, after a **Greek mythological serpent** which can regenerate multiple heads upon decapitation.

- **Charles Bonnet (1720-1793)**
- The **Swiss scientist**, Charles Bonnet, was greatly influenced by the work of his cousin, Abraham Trembley, and Rene-Antoine Reaumur. He began studying regeneration in **earthworms** and published his work in 1744.

Lazzaro Spallanzani

1768
Tadpoles could regenerate a new tail



The gray arrow points in the direction of lower (minus sign) to higher (plus sign) evolutionary complexity .

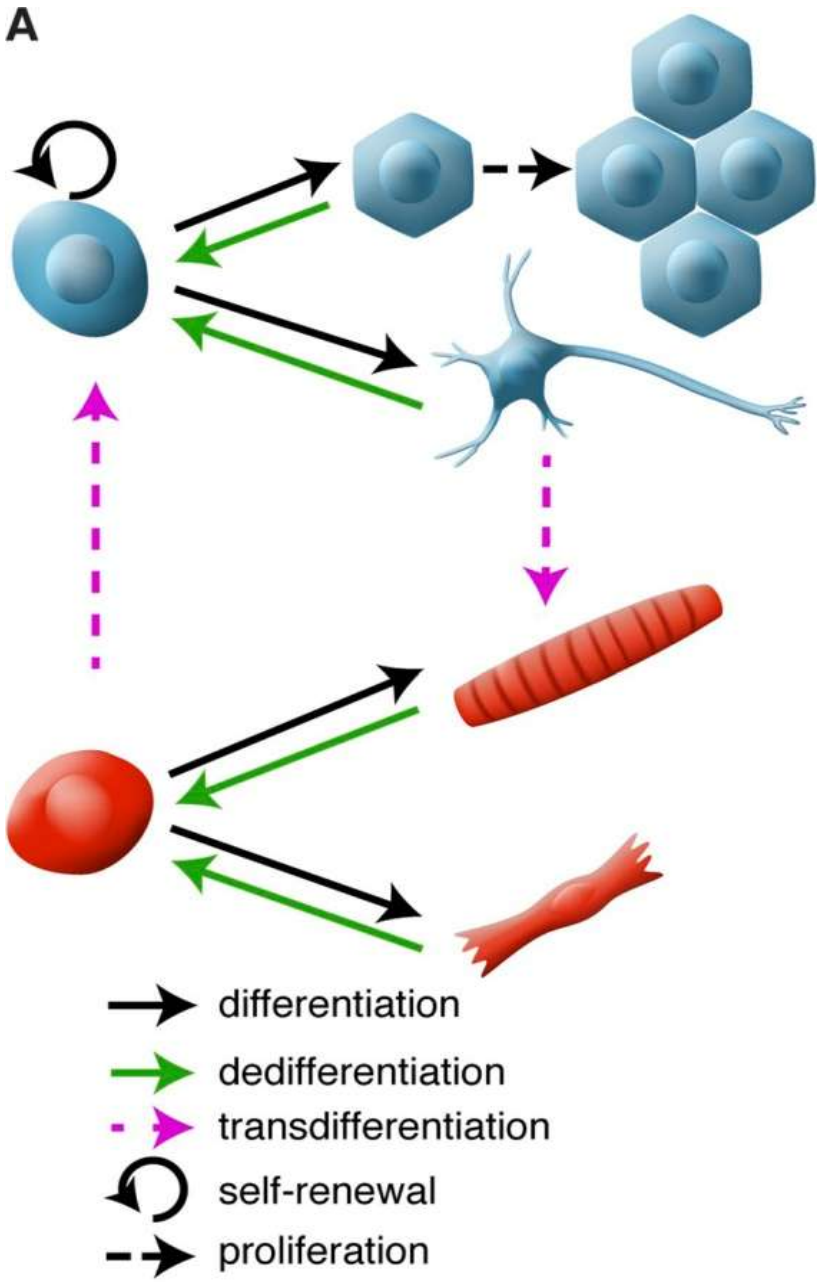
The apparent addition of several regulatory steps (wound healing, blastema formation, dedifferentiation and differentiation) as evolutionary complexity increases.

All regenerative processes need to be

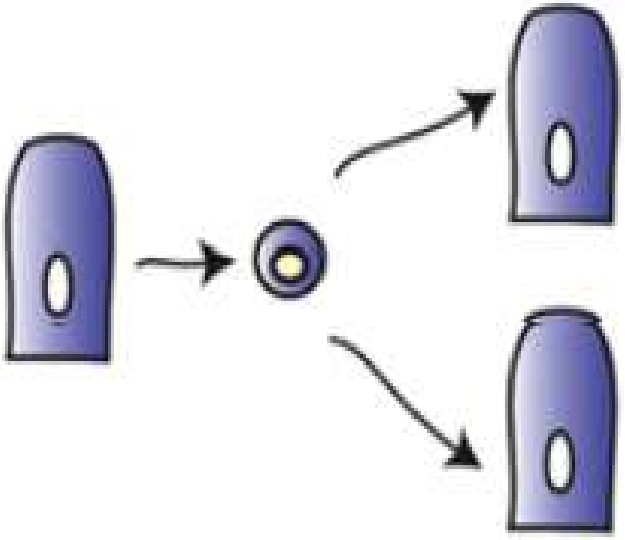
- tightly regulated and
- involve communication between different cell types

Regenerative strategies

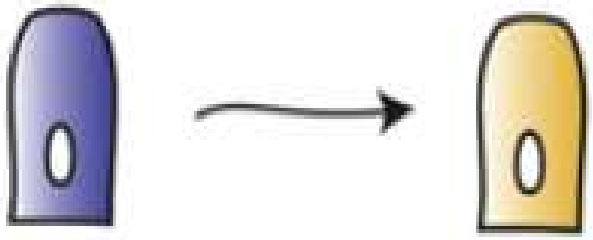
- Rearrangement of pre-existing tissues
- Use of adult somatic stem cells
- De-differentiation
- Re-differentiation
- Transdifferentiation of cells
- Proliferation



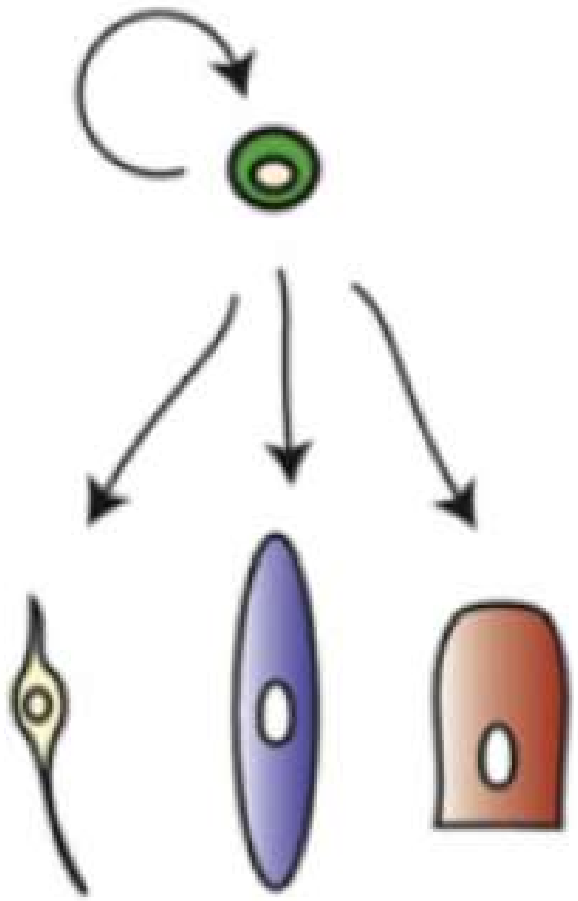
Stem cells self-renew and produce one or more differentiated cells.



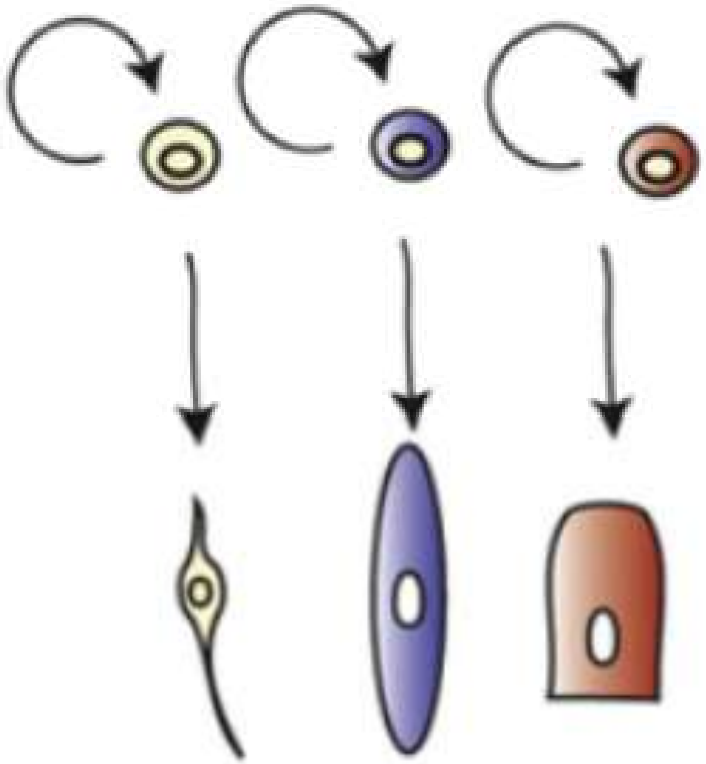
De-differentiation
A cell loses differentiated character to produce a progenitor cell that can divide to produce more differentiated cells.



Transdifferentiation involves the change of one cell type into others. This could occur without division, or following dediffer-entiation of one cell type into a progenitor for additional cell types



A pluripotent progenitor cell (green) produces differentiated progeny cells spanning multiple germ layers. There could exist multiple, and/or self-renewing intermediates along different lineage paths.



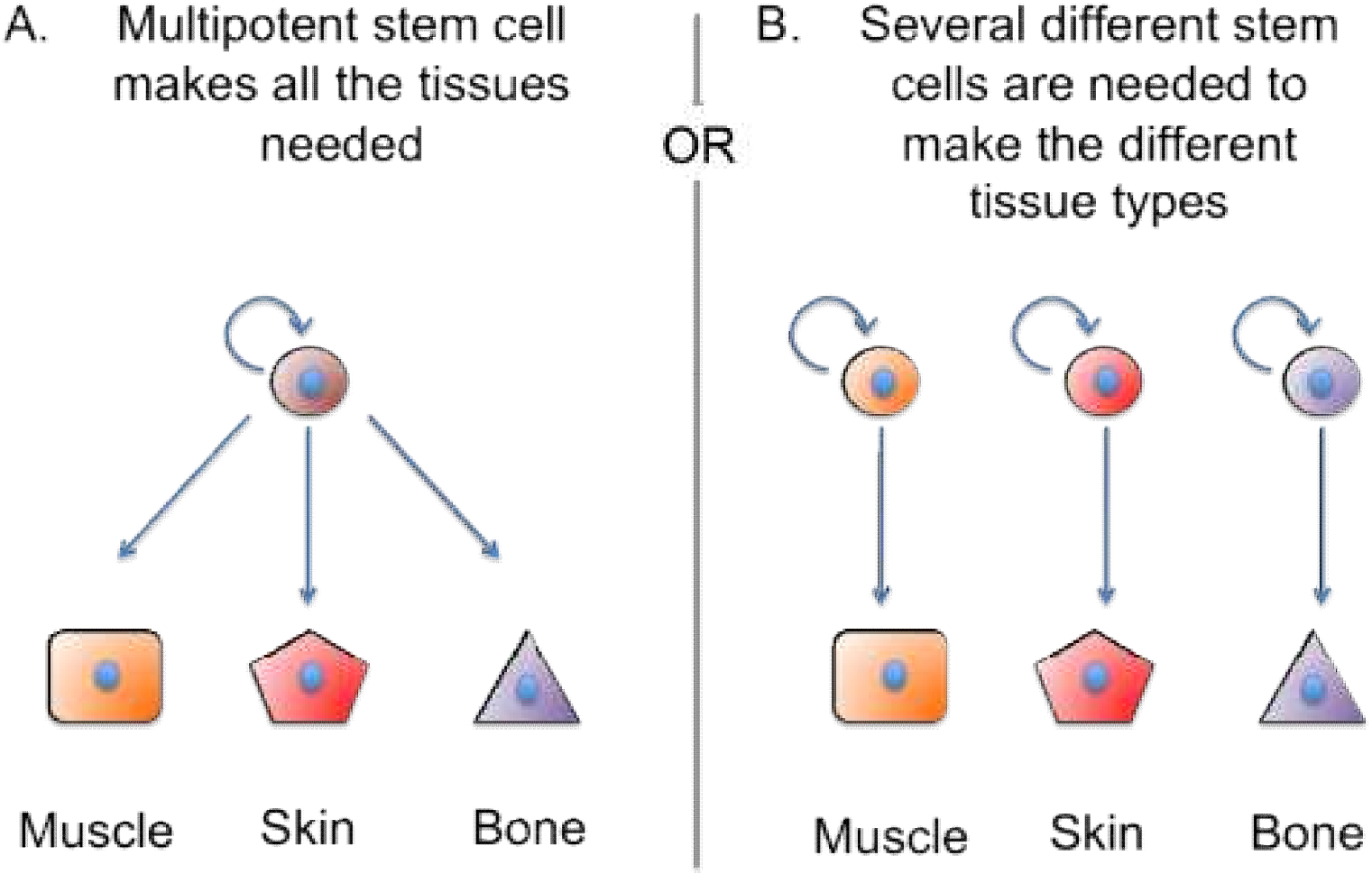
Different lineage-restricted progenitor cells (stem cell types are depicted) each produce different differentiated cells.

Each different tissue separately generates or harbors a restricted stem cell.

These stem cells together can reconstitute the three different tissues, while any individual on its own is not sufficient.

Possibilities for regrowing a limb:

Is one kind of stem cell responsible for making all the different tissues needed (A)? Or is there a separate set of stem cells responsible for making each of the different tissues?

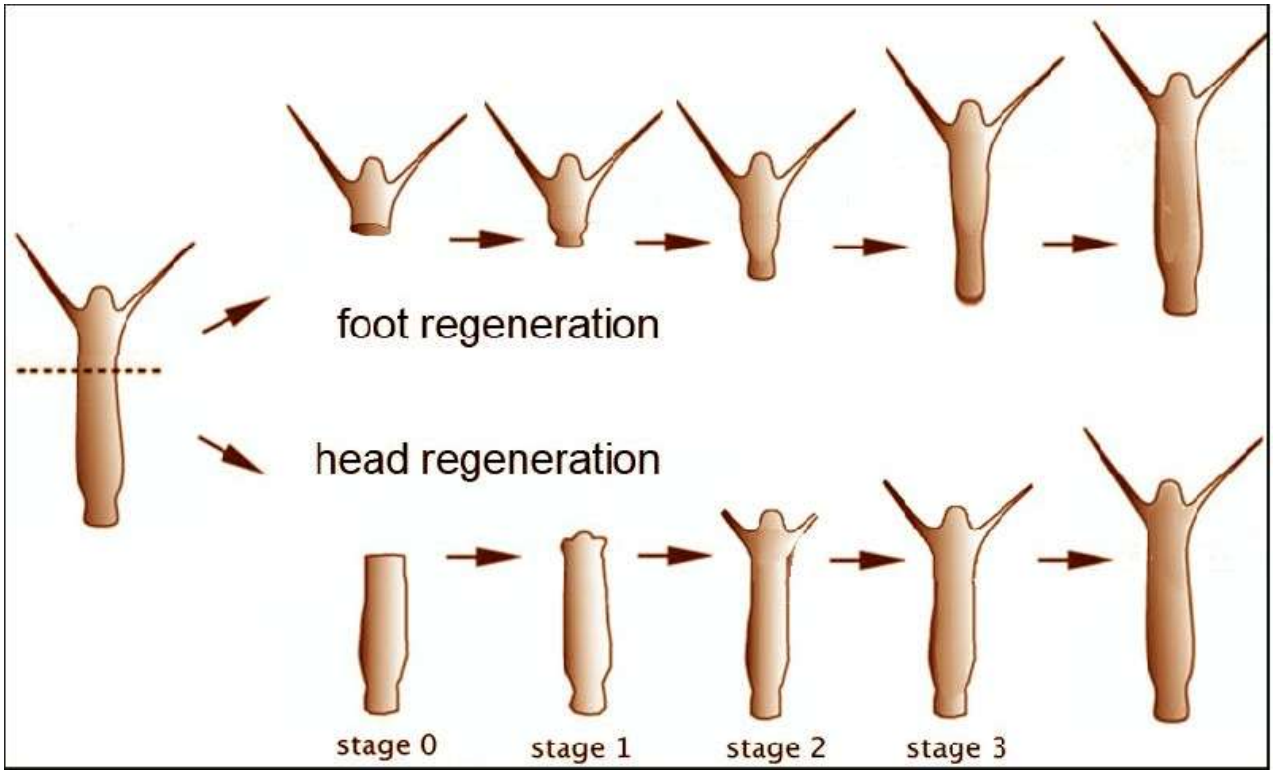


Major ways by which regeneration can occur

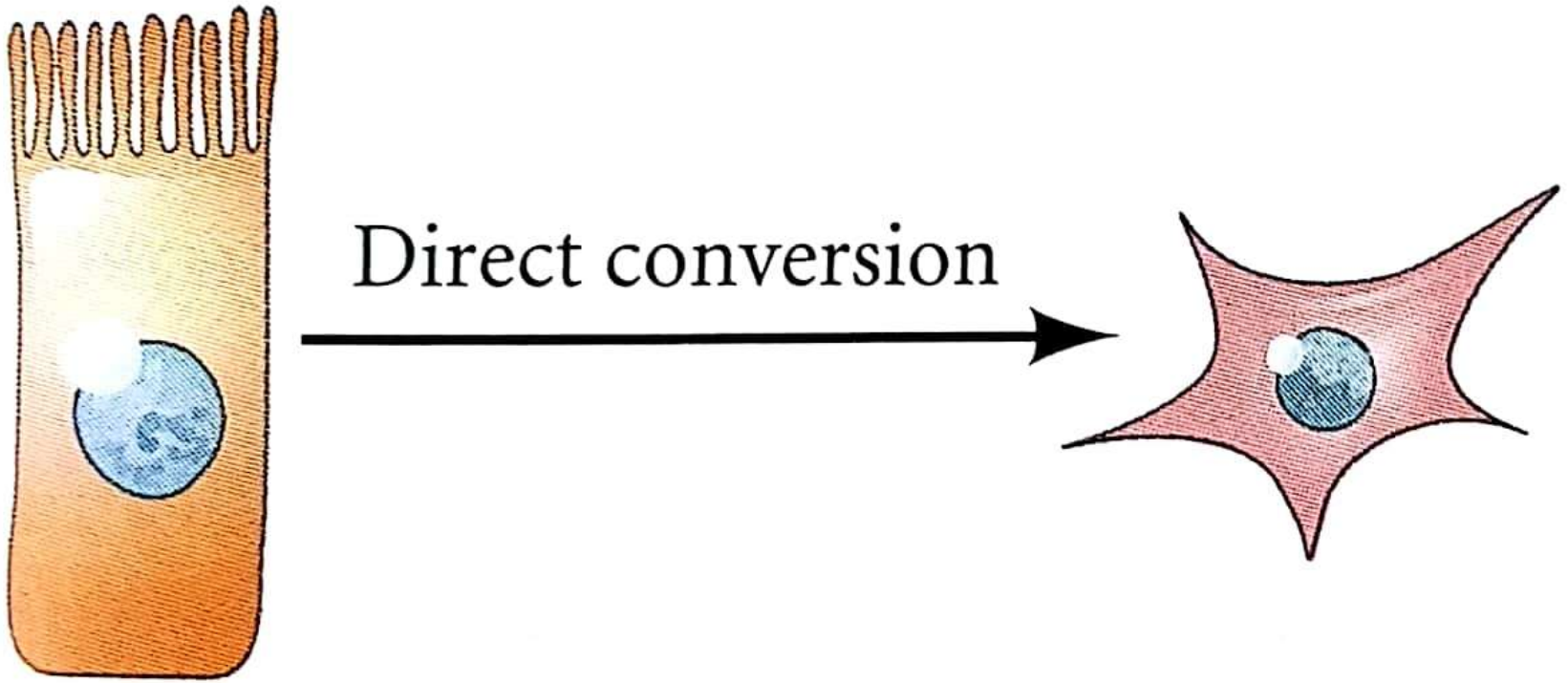
- Morphallaxis
- Epimorphosis
- Compensatory regeneration.
- Tissue regeneration
- Stem cell mediated regeneration

Major ways by which regeneration can occur.

- The first mechanism is called **morphallaxis.** Here, regeneration occurs through the repatterning of existing tissues, and there is little new growth. Such regeneration is seen in hydras.

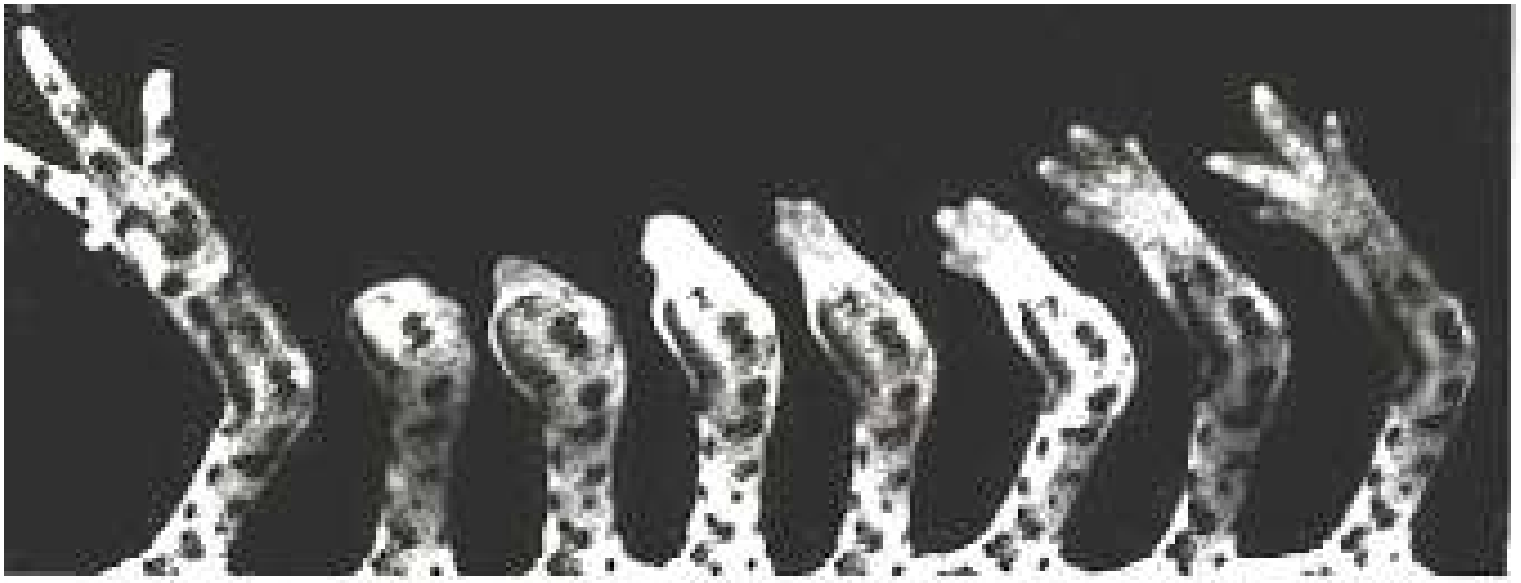


Morphallaxis (transdifferentiation)

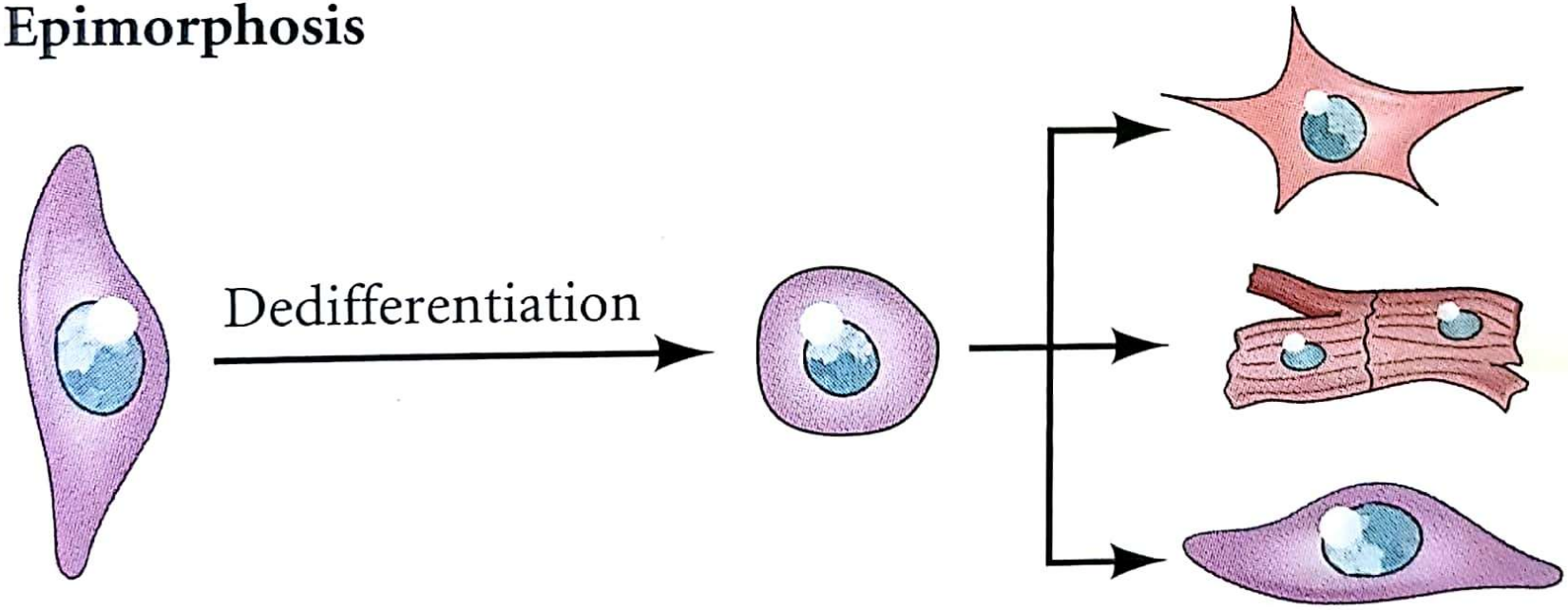


Major ways by which regeneration can occur.

- The second mechanism involves the dedifferentiation of adult structures to form an undifferentiated mass of cells that then become respecified. This type of regeneration is called **epimorphosis** and is characteristic of regenerating limbs. Regeneration via formation of blastema



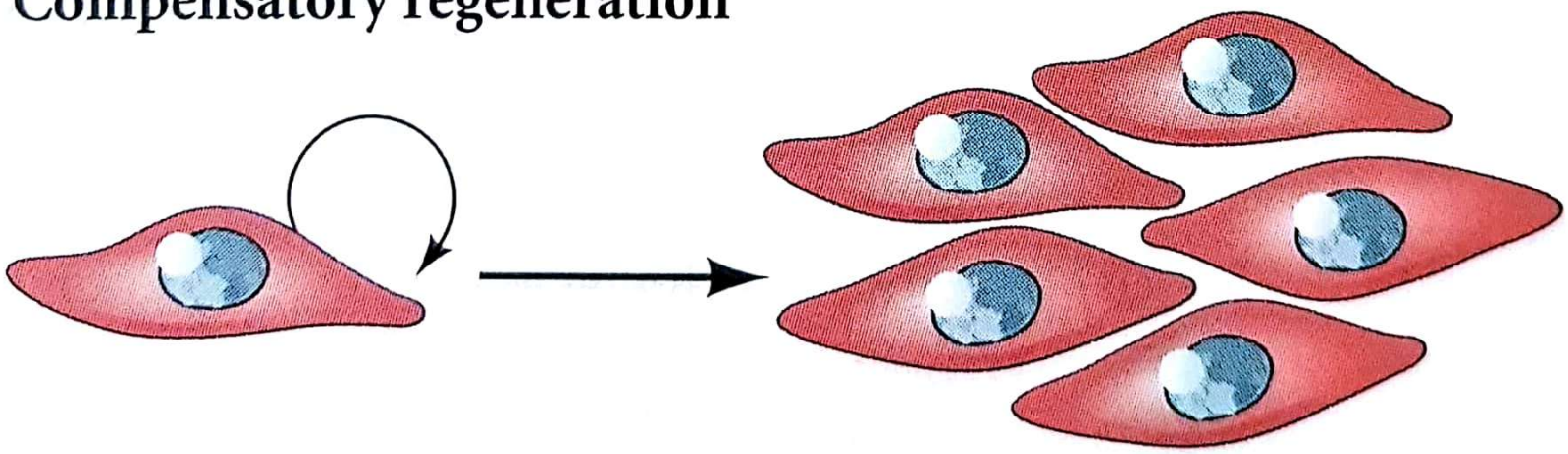
Epimorphosis

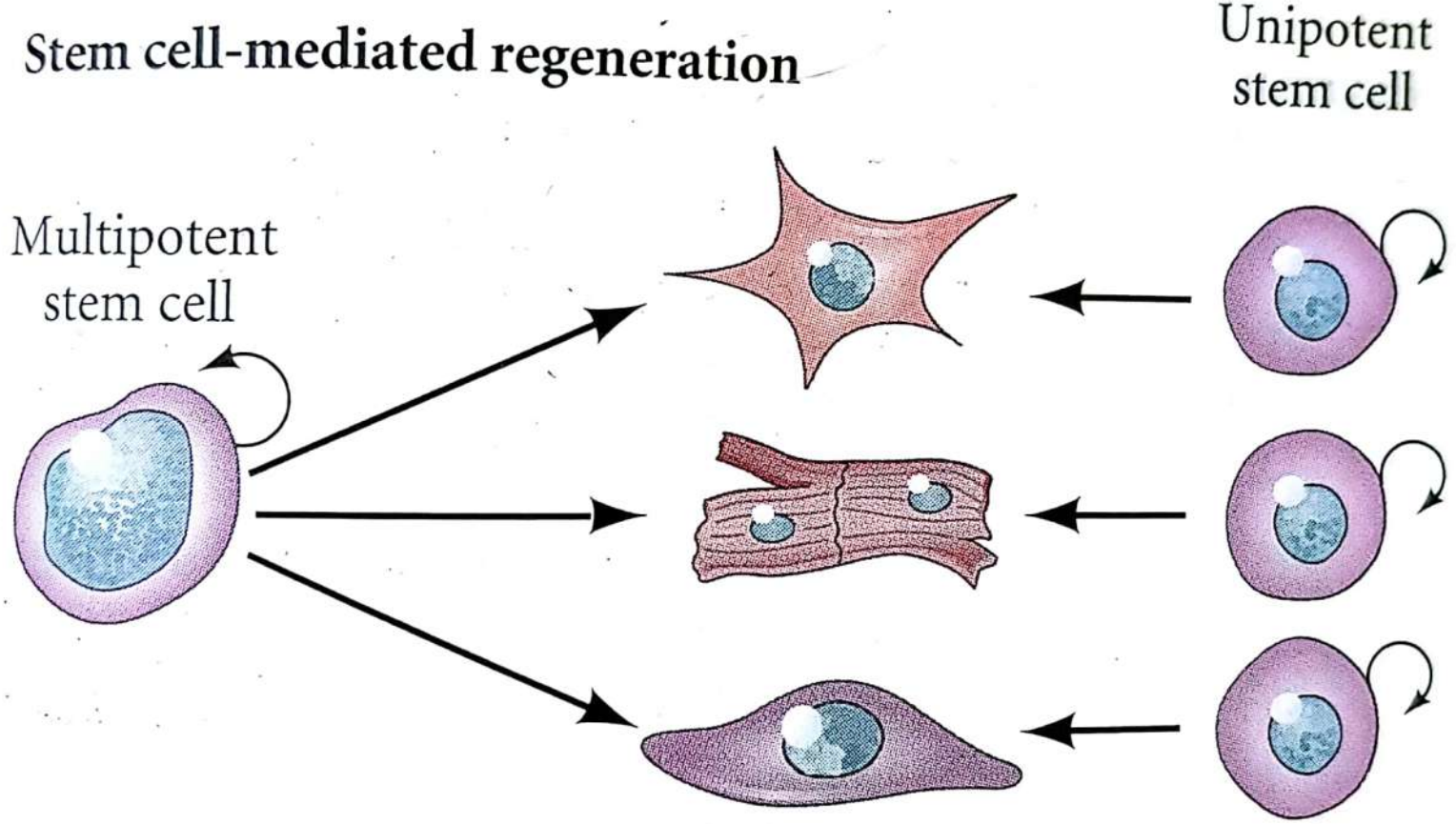


Major ways by which regeneration can occur.

- A third type of regeneration is an intermediate type, and can be thought of as **compensatory regeneration**.
- Here, the cells divide, but maintain their differentiated functions. They produce cells similar to themselves and do not form a mass of undifferentiated tissue.
- This type of regeneration is characteristic of the **mammalian liver.**

Compensatory regeneration

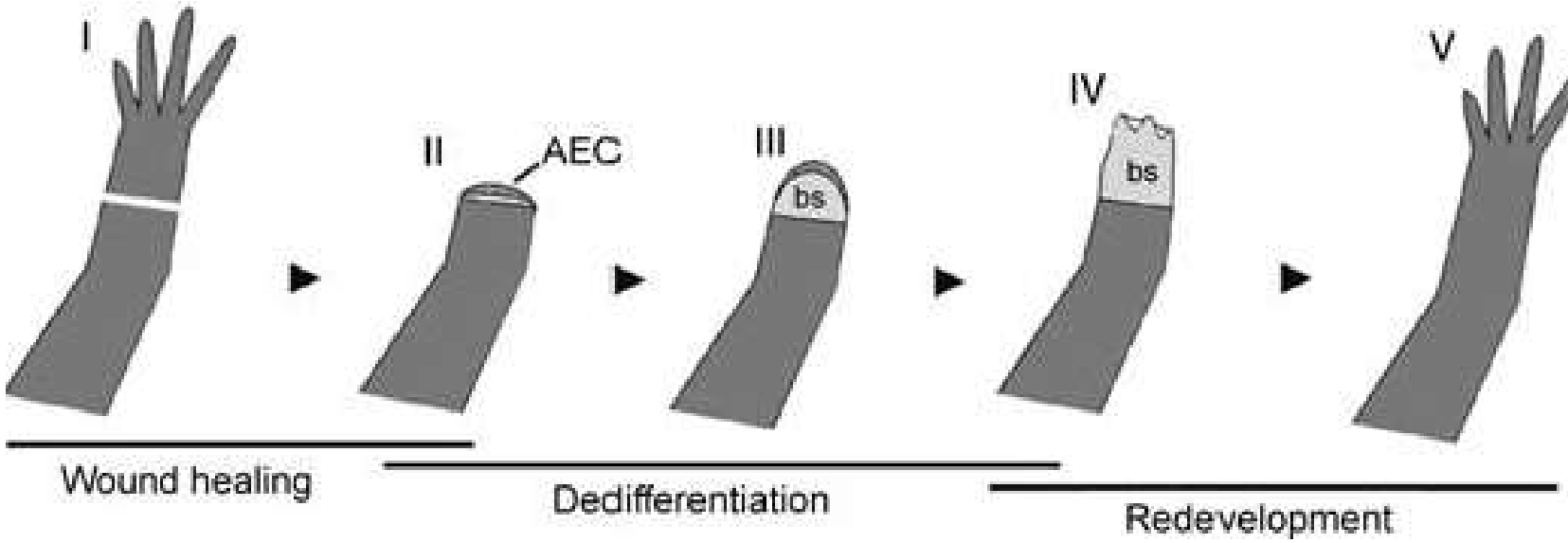



Stem cell-mediated regeneration
Unipotent stem cell
Multipotent stem cell

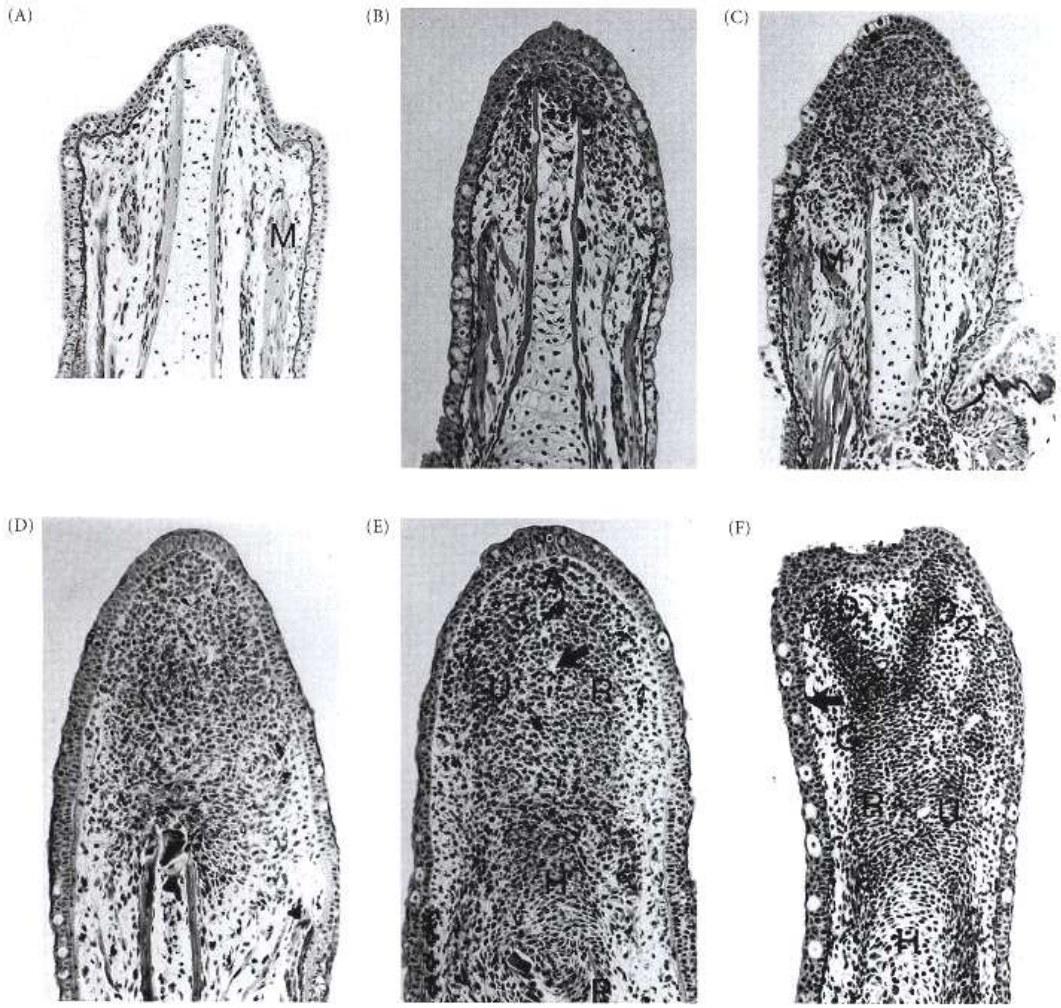
All regenerative processes need to be tightly regulated and involve communication between different cell types

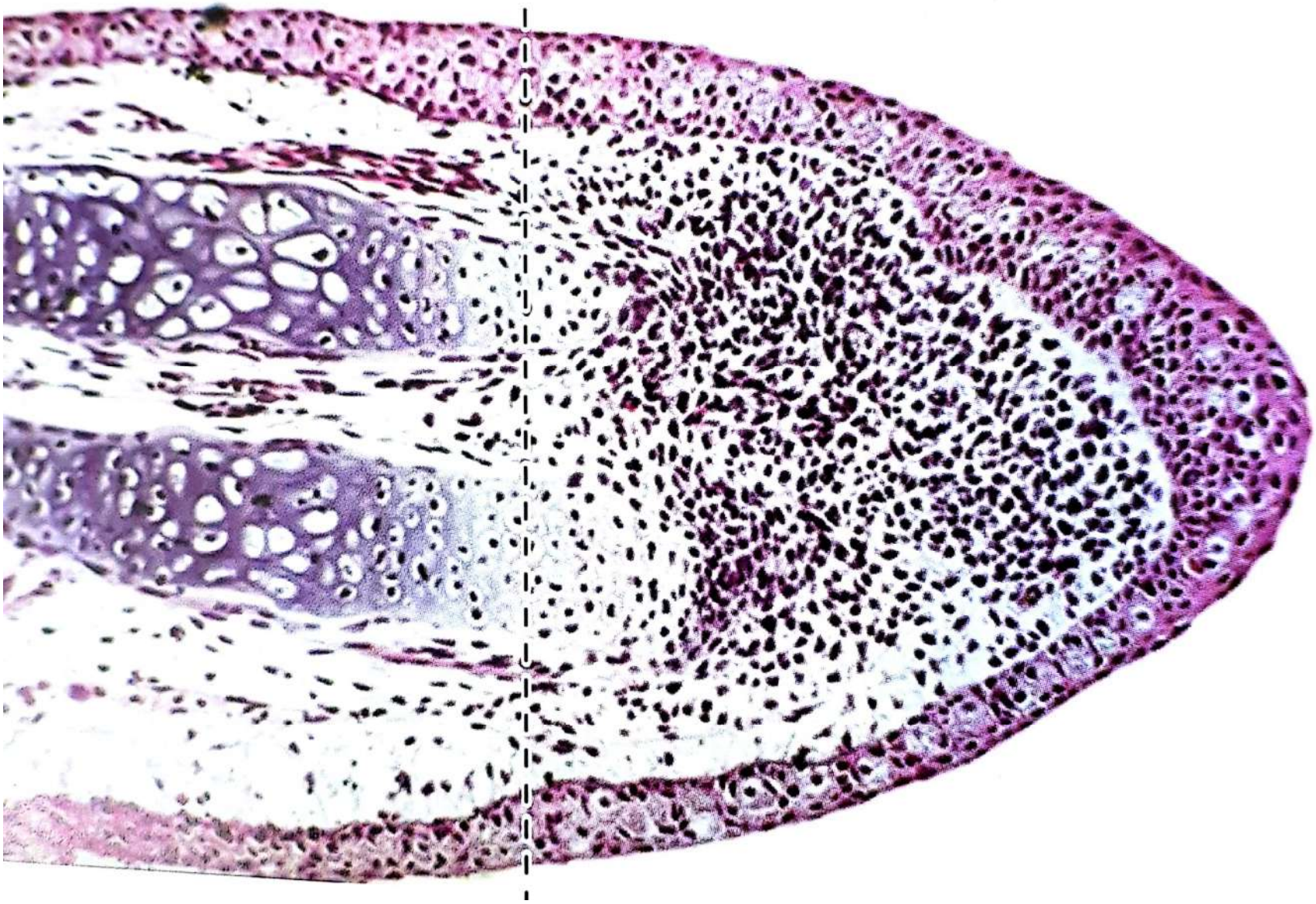
Limb regeneration

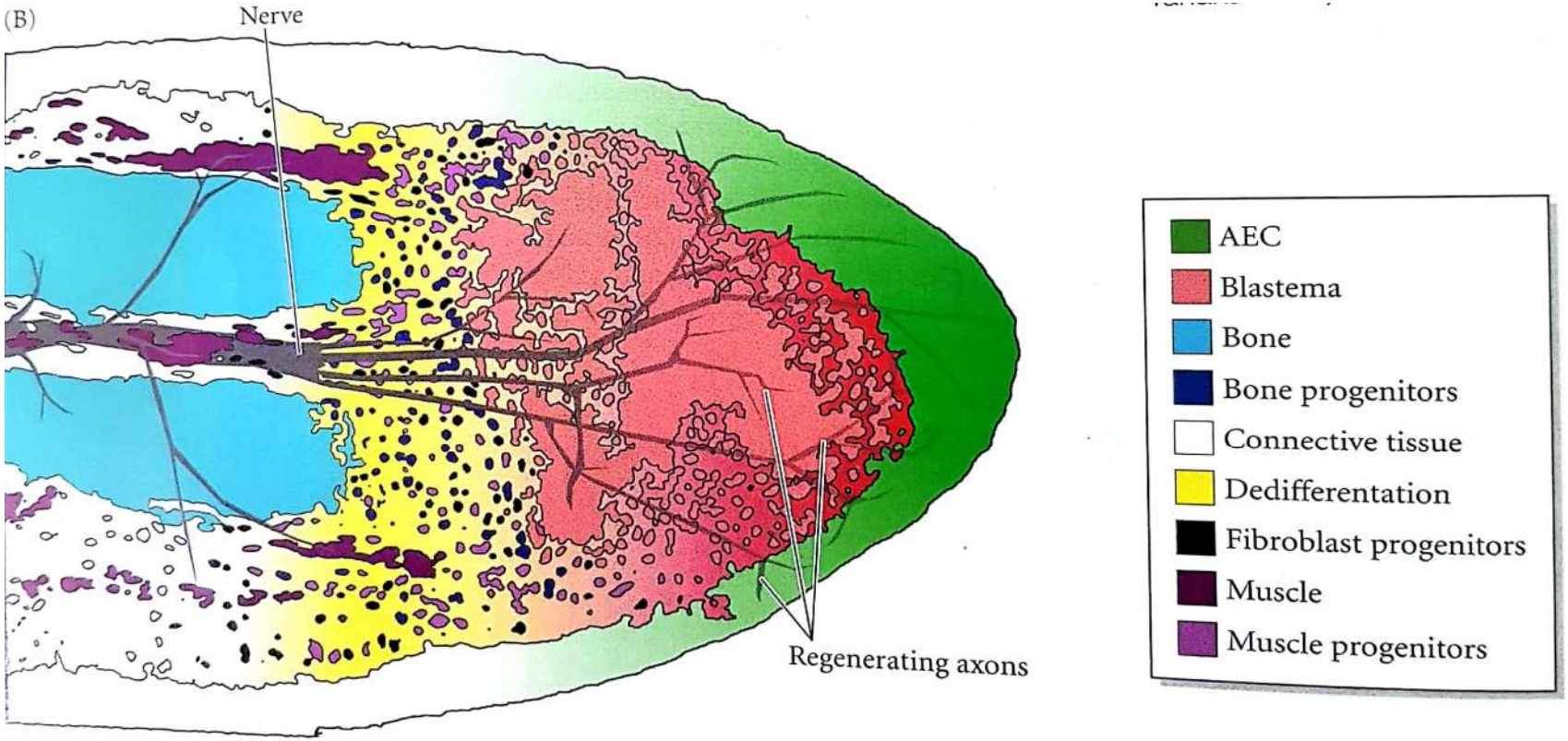
Limb regeneration is one of the best examples of organ/appendage regeneration in vertebrates and has been called ‘epimorphosis’ since it requires blastema formation and proliferation.

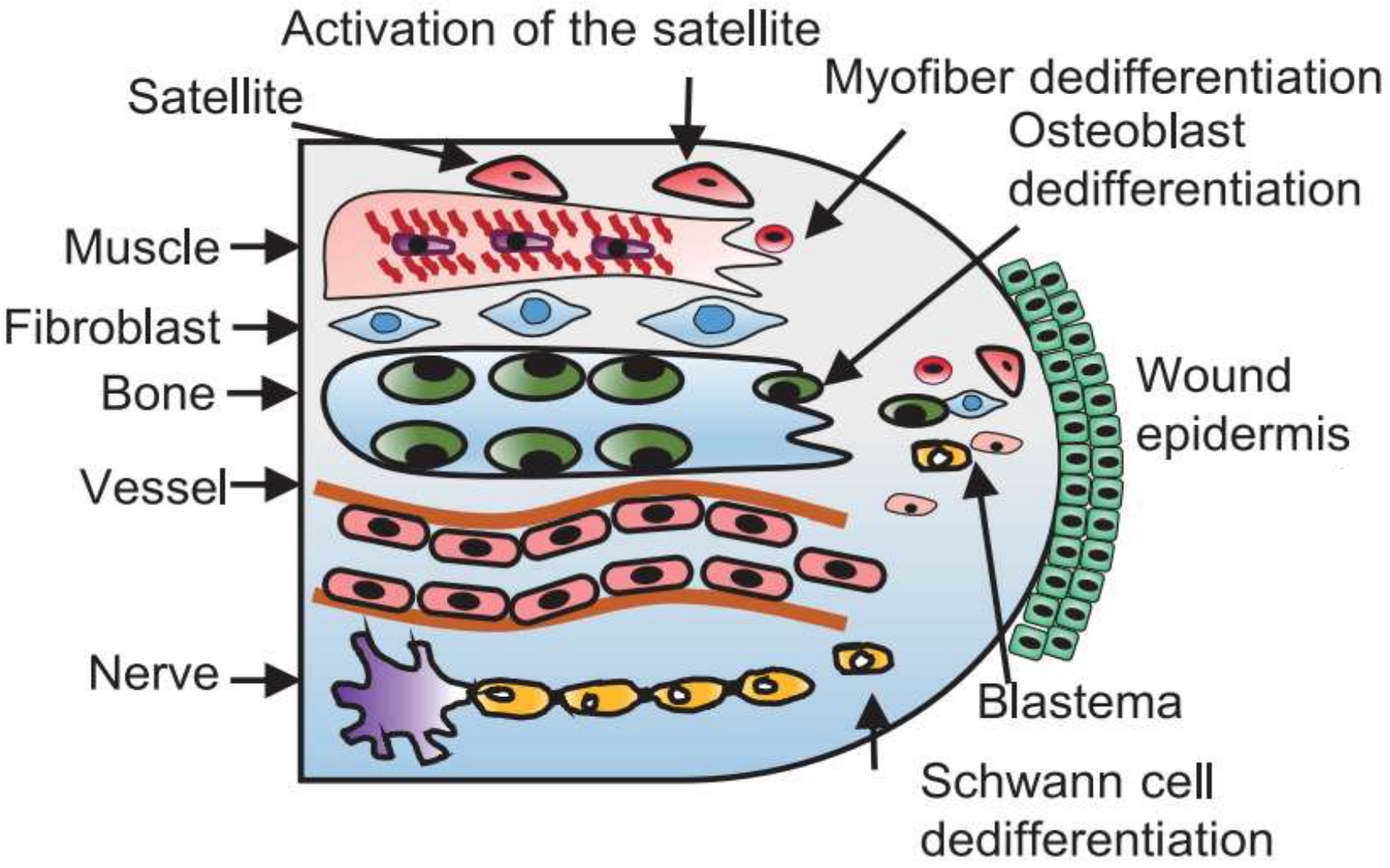

I
II
AEC
III
bs
IV
bs
V
Wound healing
Dedifferentiation
Redevelopment

Regeneration of limb of *Ambystoma maculatum*
A. 2d **B**. 5d thin accumulation of blastemal cells below epidermis **C**. 7d large population of mitotically active blastemal cells **D**. 8d blastema elongates **E**. 9d early redifferentiation **F**.10d precartilaginous condensation (Stocum 1979)

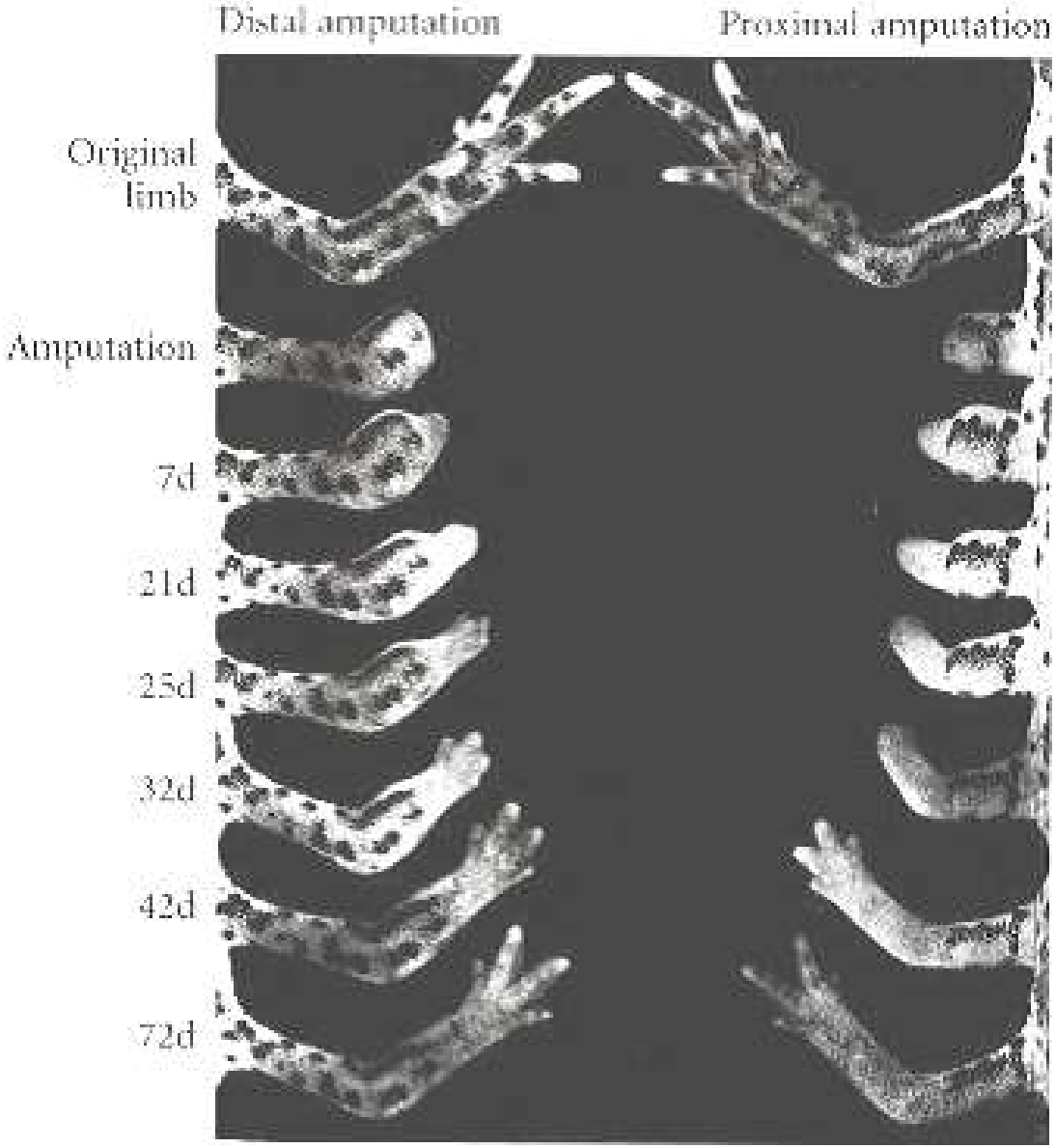





(B)
Nerve
Regenerating axons
AEC
Blastema
Bone
Bone progenitors
Connective tissue
Dedifferentation
Fibroblast progenitors
Muscle
Muscle progenitors



Vertebrate appendages, such as salamander limb regrow from the regeneration blastema



Epimorphic development of salamander forelimb

Wound healing

While urodele amphibians (newts and salamanders) can regenerate limbs as adults, other tetrapods (reptiles, birds and mammals) cannot and just undergo wound healing.

In adult mammals such as mice and humans, the wound heals and a scar is formed after injury, while wound healing is completed without scarring in an embryonic mouse.

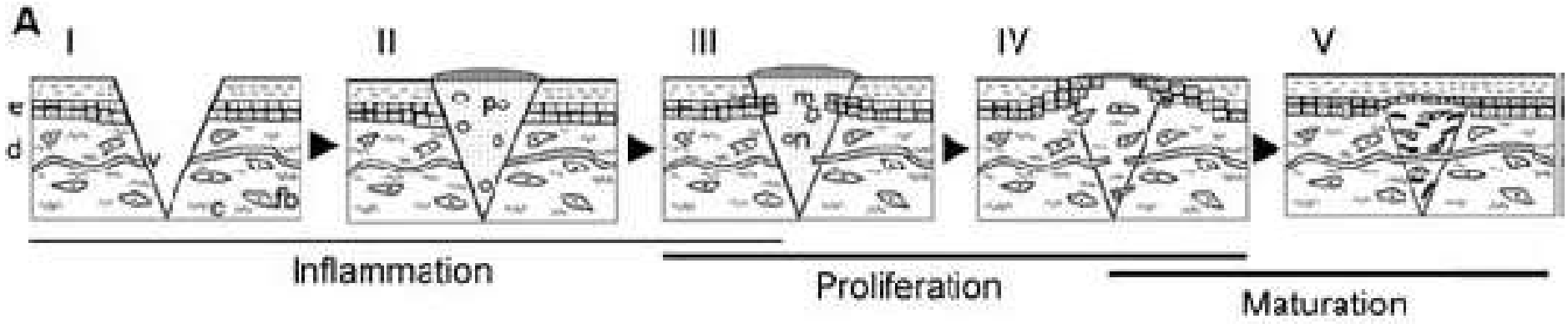
Completion of regeneration and wound healing takes a long time in regenerative and non-regenerative limbs, respectively.

However, it is the early steps that are critical for determining the extent of regenerative response after limb amputation, ranging from: **wound healing with scar formation**, **scar-free wound healing**, **hypomorphic limb regeneration** to **complete limb regeneration**.

Processes occurring after limb amputation: Wound healing versus limb regeneration

When a non-regenerative limb of tetrapods is amputated,wound healing without initiation of the limb regeneration process will occur, although hypertrophy of bones at the stump is observed in neonatal mice.

Damaged skin is healed (healing of other tissues such as bone, muscle and tendon also occurs).



Wound healing process of mammalian adult skin.

I Wounded skin immediately after injury.

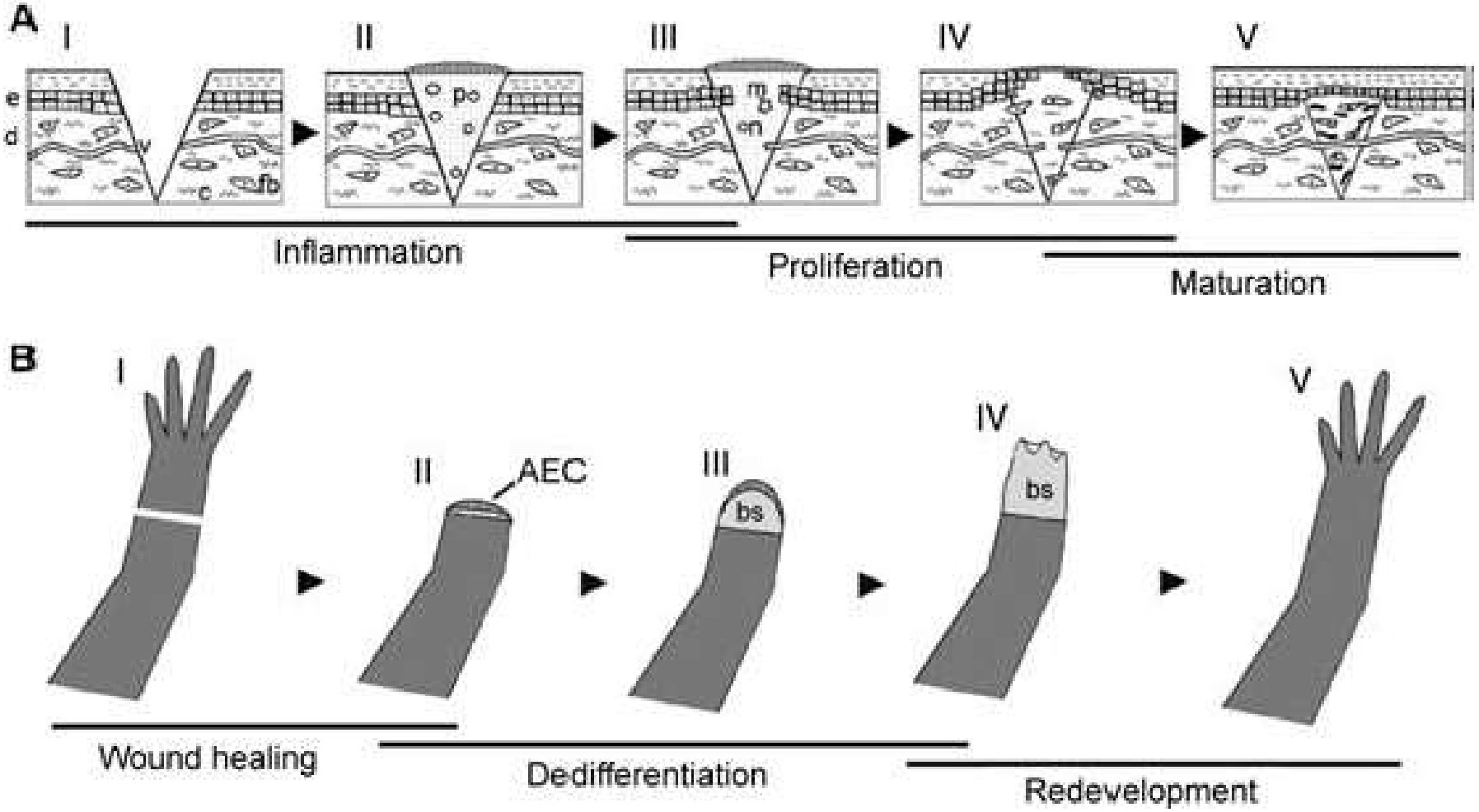
II The clot is formed and platelets are trapped in the clot. The clot has dried at the surface to form a scab (stippled).

III Neuroptophils and then monocytes (which transform into macrophages) enter the wound, re-epithelization begins, and angiogenesis is initiated.

IV Re-epithelization is almost completed and an extensive capillary network develops. Fibroblasts invade the wound, proliferate, and start synthesizing collagen, resulting in the formation of granulation tissue.

V The temporal matrix of collagen is broken down and remodeled into thick bundles of collagen (dark lines).

c,collagen; d,dermis; e,epidermis; fb, fibroblast; m, macrophage; n, neurotrophil; p, platelet; v, blood vessel.


A
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fb
c
Inflammation
Proliferation
Maturation
B
I
II
AEC
III
bs
IV
bs
V
Wound healing
Dedifferentiation
Redevelopment

Signals involved in the initiation of limb regeneration

- *MMP* Matrix metalloproteinases (MMPs) were discovered as proteases capable of digesting collagen in the remodeling tissues of metamorphosing tadpoles

- MMPs are activated in regenerating limbs of newts and salamanders and are also activated during inflammation of wound healing and function to clear inflammatory debris in mammals

- Some MMPs are upregulated very early after amputation and that urodele limb regeneration can be partially inhibited by treatment with a synthetic MMP inhibitor.

- These findings suggest that MMPs are specifically required for limb regeneration, especially during initiation (wound epithelium formation/subsequent blastema formation).

FGF (Fibroblast growth factor)

- Developing limb buds in vertebrates are mainly composed of mesenchyme derived from the **lateral plate mesoderm** (LPM) and epithelium derived from the ectoderm.

- **Epithelial–mesenchymal interactions** are necessary for limb regeneration as well as for outgrowth of a developing limb bud.

- With regards to a developing limb bud, recent studies have revealed that these interactions are mediated by **FGFs** during embryogenesis

- Specifically, ***fgf-10*** **and** ***fgf-8*** are expressed in the **lateral plate mesoderm** of the presumptive limb field and its **overlying epithelium**, respectively, during limb induction.

- FGF-10 in limb bud mesenchyme and FGF-8 in the apical epithelium of the limb bud actually constitute a **positive feedback signaling loop** essential for limb outgrowth in the amniote embryo

- neither *fgf-10* nor *fgf-8* are expressed after amputation of a **non-regenerative limb**

- **reinforcement of this feedback loop** may be a crucial key to enhance the regenerative response of non-regenerative vertebrate limbs.

Wnt/β-catenin

- The Wnt/β-catenin pathway is an evolutionarily conserved signaling pathway that is known to control cell proliferation and cell fate determination by regulating target gene expression

- Wnt/β-catenin has been shown to be involved in the initiation of chick limb development and zebrafish pectoral fin formation, by inducing *fgf-10* expression in the presumptive limb and fin region, respectively

BMP/Msx

- BMPs, a subset of the TGF-β superfamily, have numerous roles during embryogenesis and organogenesis, regulating processes as diverse and fundamental as regional specification and cell proliferation, differentiation, survival and death.
- *Msx* genes are often expressed in regions similar to those in which BMPs are present and mediate BMPs function.

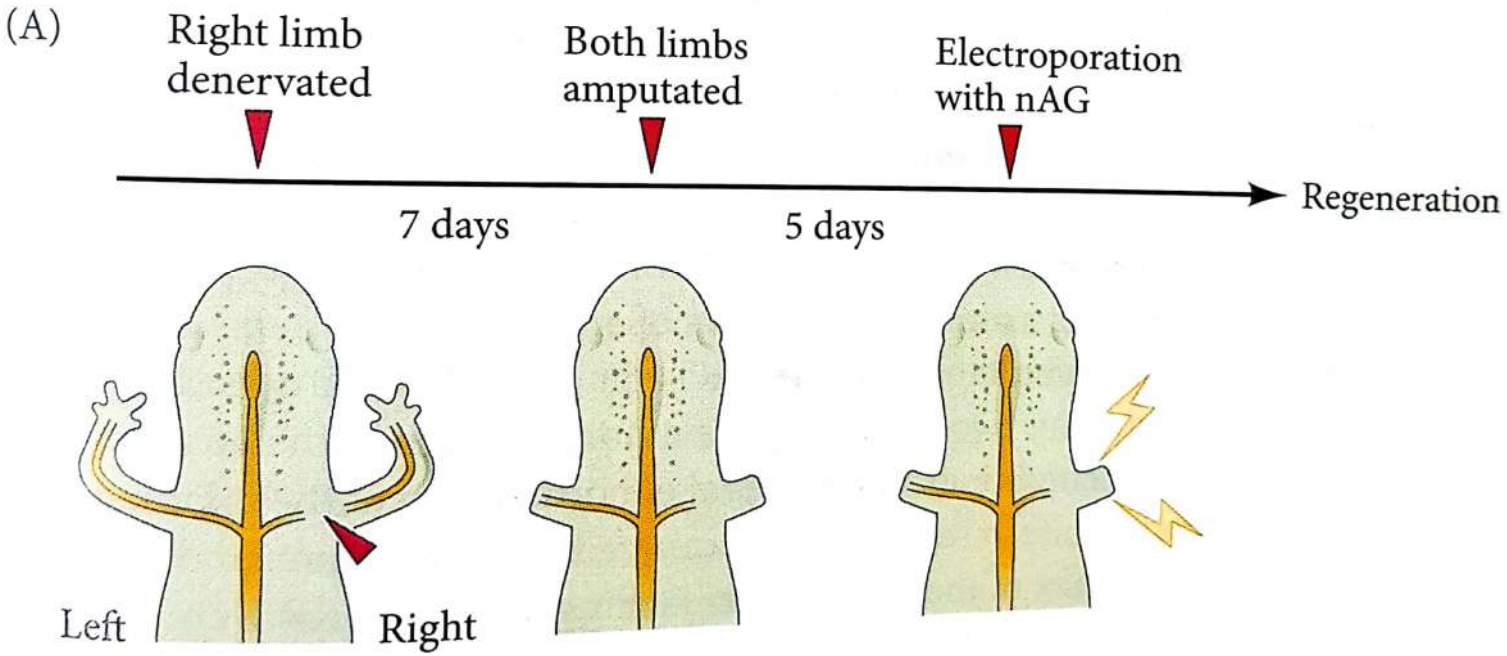
- Both Wnt/β-catenin and BMP signaling have essential roles in the early stages of limb regeneration

- But Wnt/β-catenin signaling is required at an earlier period than is BMP signaling during blastema formation.

Nerve signals and a stepwise model

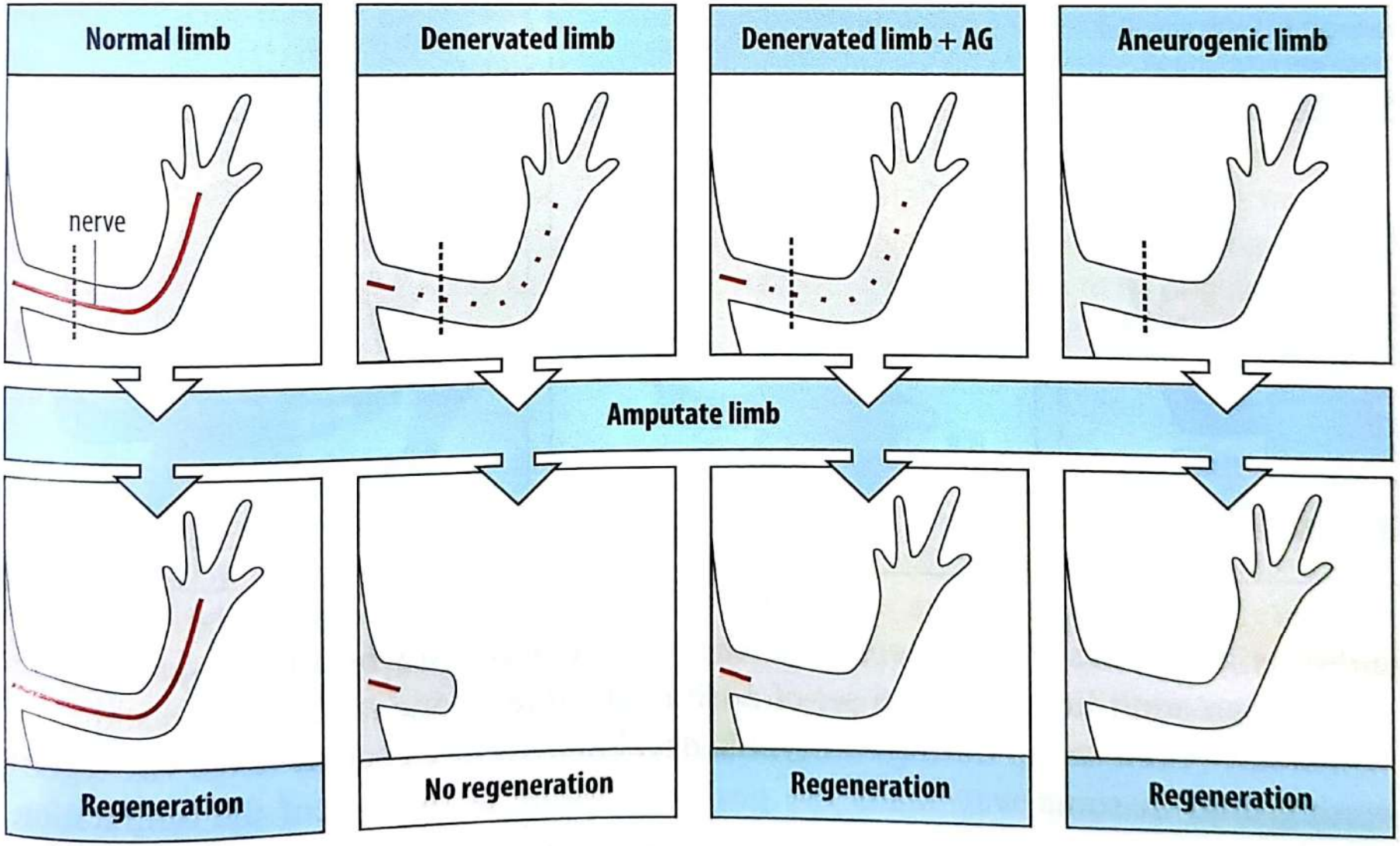
- It is well known that limb regeneration requires neuronal innervation in urodele amphibians
- It has been suggested that axons secrete what have been called ‘neurotrophic factors’ into the amputated limb and that these factors stimulate mitotic activity of blastemal cells and upregulate genes important for the regenerative process. If a limb stump is denervated, it fails to regenerate.
- However, if denervation is done after some stage of blastema formation (medium bud stage), limb regeneration is not blocked .
- In fact, blastemal cells in a denervated limb undergo apoptosis, suggesting that these ‘neurotrophic factors’ are survival factors during limb regeneration.
- There are currently two major candidates for these so-called neurotrophic factors: **GGF and FGF-2**. Glial growth factor (GGF), a member of the neuregulin growth factor family, is present in regenerating blastema and GGF distribution is lost in the limb stump after denervation
- Furthermore, **injection of GGF protein into the denervated blastema** actually increases the rate of regeneration of blastemas

Newt anterior gradient protein (nAG) induces normal limb regeneration



Control

nAG administered


Normal limb
Denervated limb
Denervated limb + AG
Aneurogenic limb
nerve
Amputate limb
Regeneration
No regeneration
Regeneration
Regeneration

Steps during limb regeneration

Wound healing

- Non regenerating: Large wound, takes days to close, accumulation of collagen bundle that form scar tissue

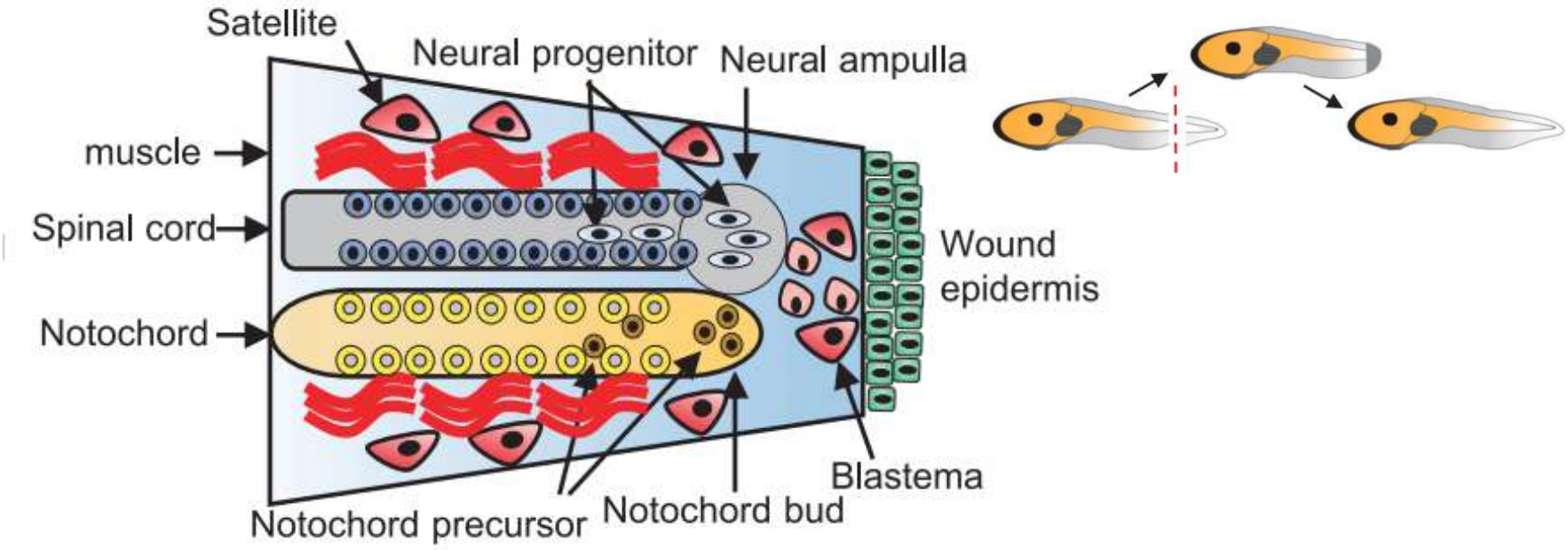
- Regenerating: Surface of wound is covered rapidly with epidermal cells forming “Wound epidermis” (WE)

- Matrix metalloprotease (MMPs) up-regulated help matrix degradation and formation of wound epidermis.

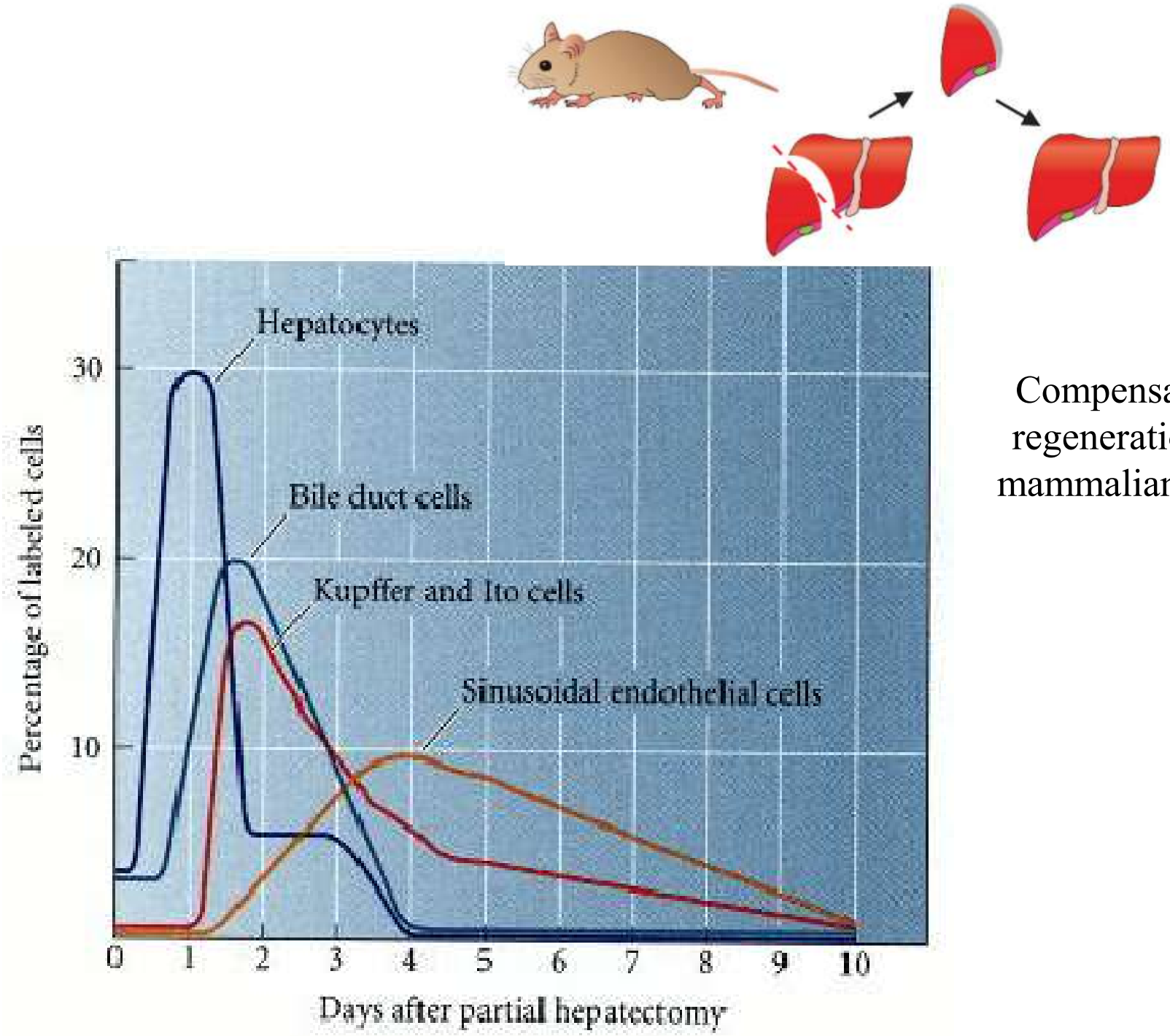
- WE becomes a specialized structure : Apica epithelial cap (AEC) differs from normal epithelium and thought to be similar to apical epidermal ridge (AER) which is present in developing limb bud in amniots.

Blastema formation

- Signal from WE induce formation of regeneration blastema
- How blastema is formed?
- 1. dedifferentiation of differentiated cells
- 2. activation of resident stem cells
- Why blastema is formed?
- 1. Blood cloting proteinase thrombin
- 2. dermis also contribute in blastema formation. Cells from dermis can give rise to multiple cell types
- During blastema formation the WE express fgf8 (urodels and anurans)
- Nerve derived signals i.e. neurotrophic factors up-regulate genes important for the regenerative process (fgf8,fgf10, max1, tbx5, prx1found in the late blastema

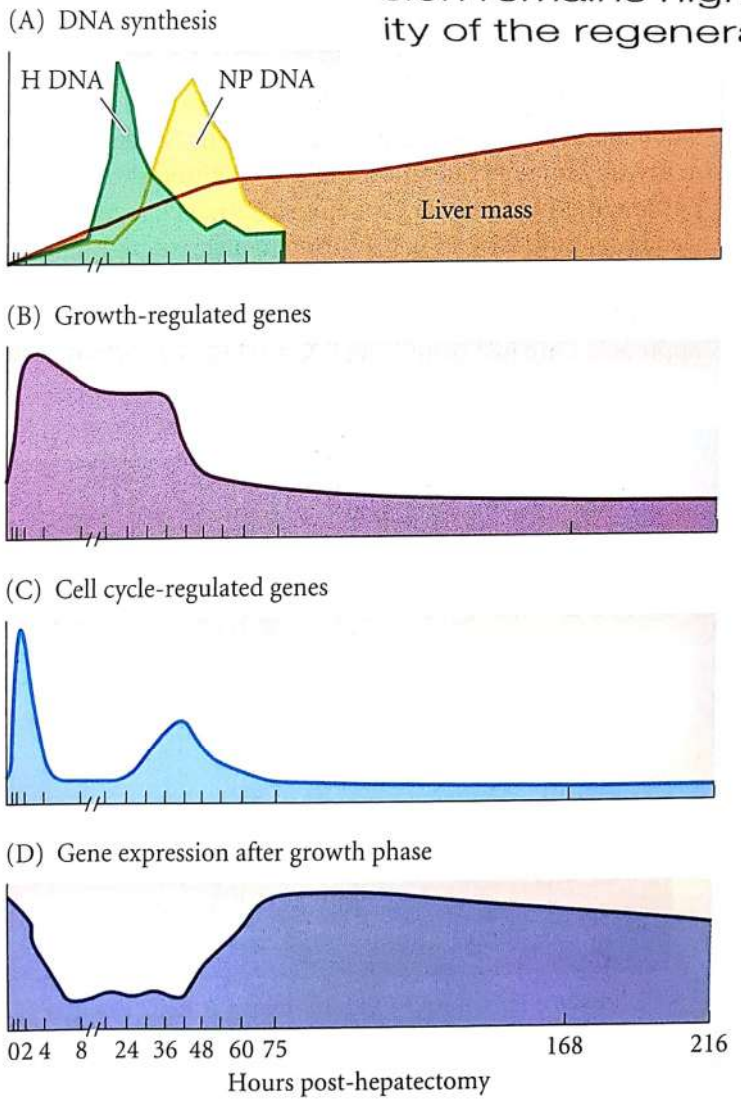


Frog tadpole tail regrows from the regeneration bud containing neural ampulla, notochord bud, and blastema. Lineage-restricted progenitor cells localized in the spinal cord, notochord, and muscle are activated and then migrate to form the three components of the regeneration bud, respectively.



Compensatory regeneration in mammalian liver

(A) Initial peaks in DNA synthesis are seen in both hepatocytes (H DNA; green) and thereafter in nonparenchymal cells (NP DNA; yellow). This burst in DNA synthesis corresponds with the upregulation of growth-regulated (B) and cell cycle-regulated (C) gene expression, both of which taper off as the liver mass (brown shading in panel A) reaches its normal volume. (D) Overall gene expression remains high after the growth phase, reflecting the functionality of the regenerated liver tissue. (After Taub 2004.)



When hepatocytes fail to regenerate, "the Oval cells" divide to form new hepatocytes. These are reserve cells.

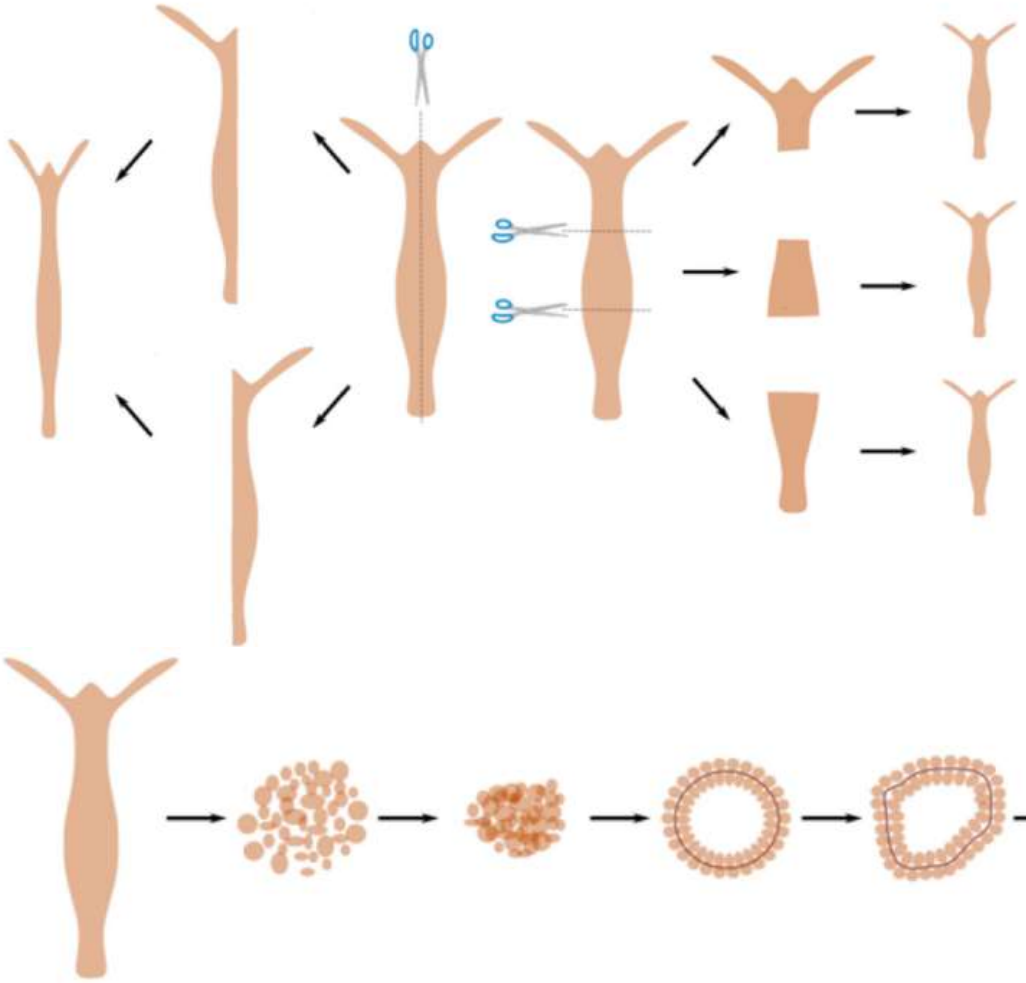
Morphallactic
regeneration in Hydra
Budding in hydra

Hydra is a genus of freshwater cnidarians. Most hydras are about 0.5 cm long. A hydra has a tubular body, with a "head" at its distal end and a "foot" at its proximal end. The "foot," or basal disc, enables the hydra to stick to rocks.

The "head" consists of a conical hypostome region (containing the mouth) surrounded by a ring of tentacles (which catch its food).

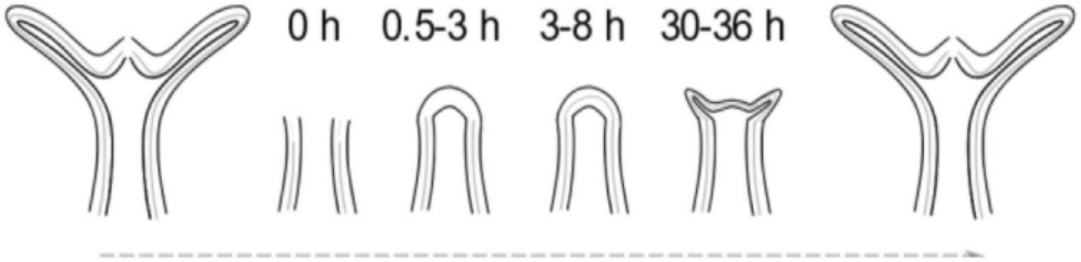
Hydras have only two epithelial cell layers, lacking a mesoderm. They can reproduce sexually, but do so only under adverse conditions, such as severe crowding.

They usually multiply by budding off a new individual
The buds form about two-thirds of the way down the body axis.

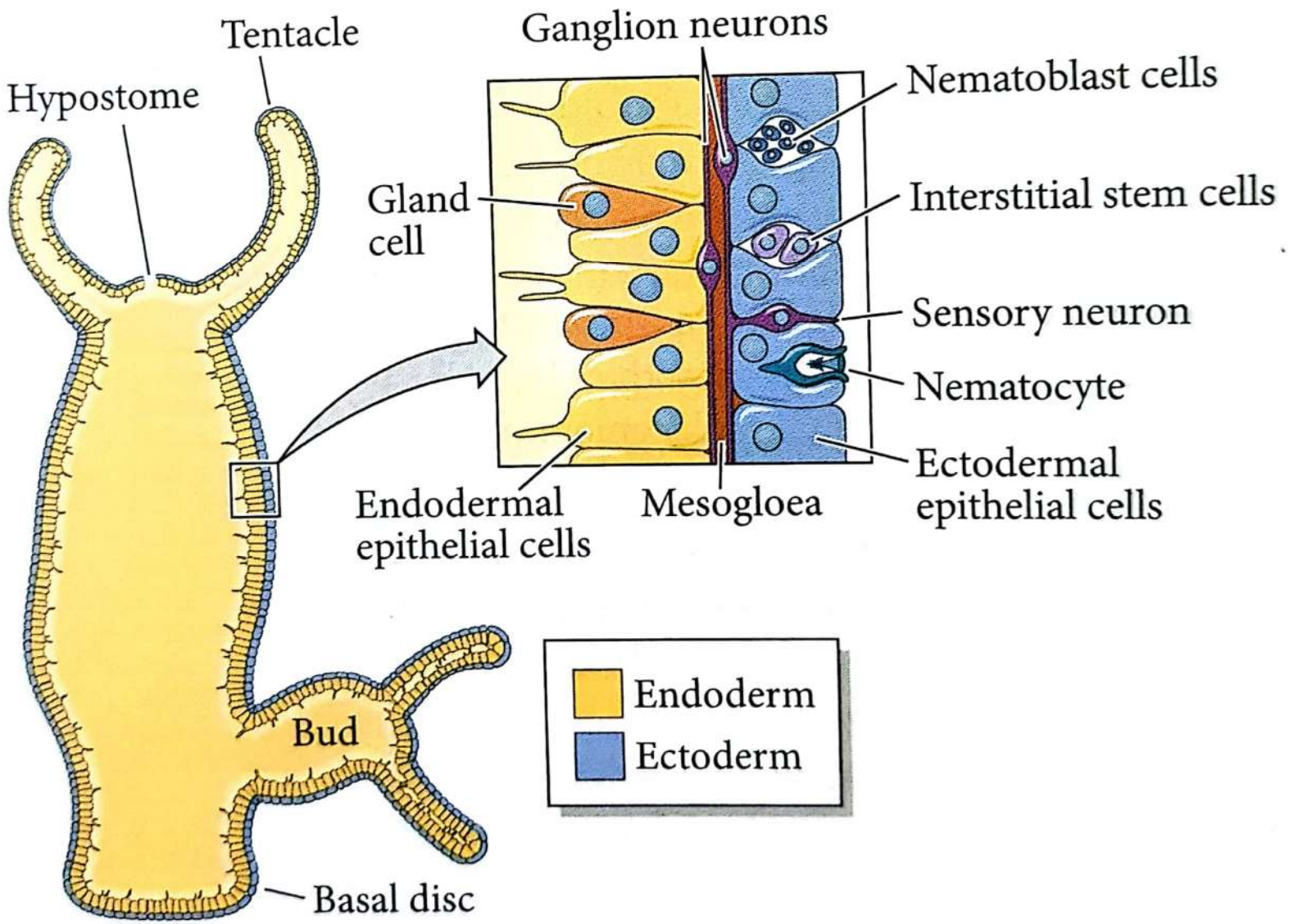


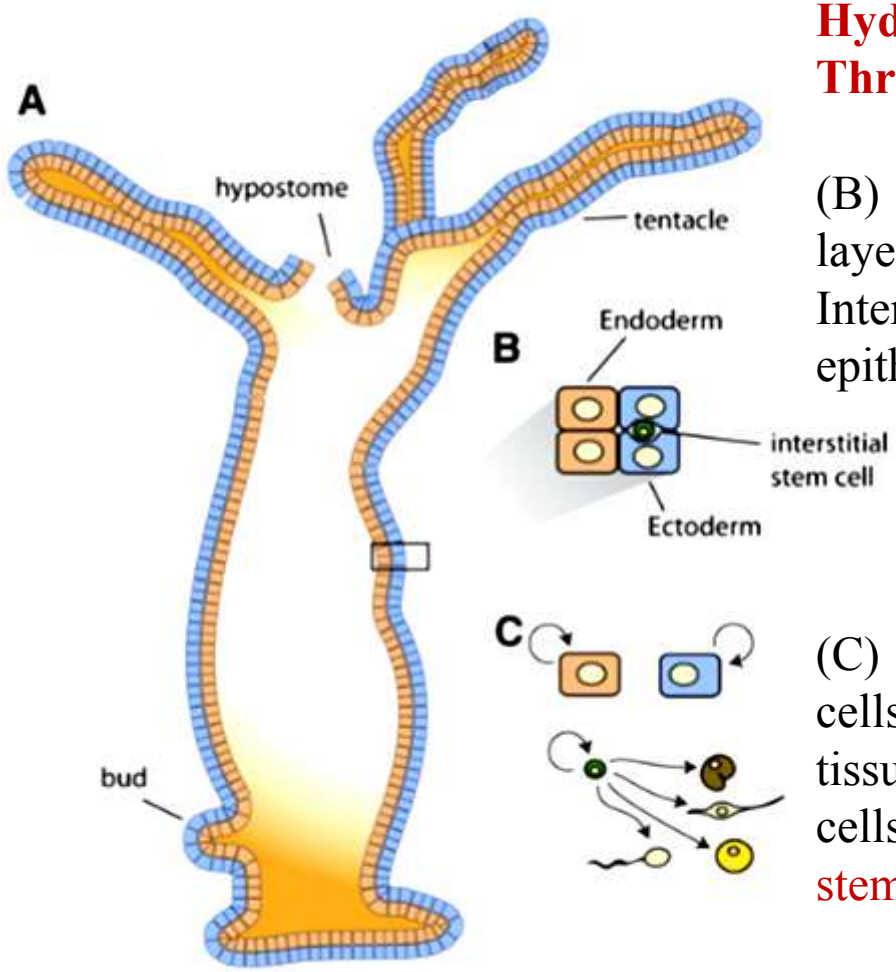
Hydra can regenerate missing parts upon both transverse and longitudinal dissection.

Regeneration from dissociated and reaggregated cells.



Kinetics of gross morphological changes during Hydra head regeneration


Tentacle
Hypostome
Ganglion neurons
Nematoblast cells
Gland cell
Interstitial stem cells
Sensory neuron
Nematocyte
Ectodermal epithelial cells
Endodermal epithelial cells
Mesogloea
Bud
Basal disc
Endoderm
Ectoderm

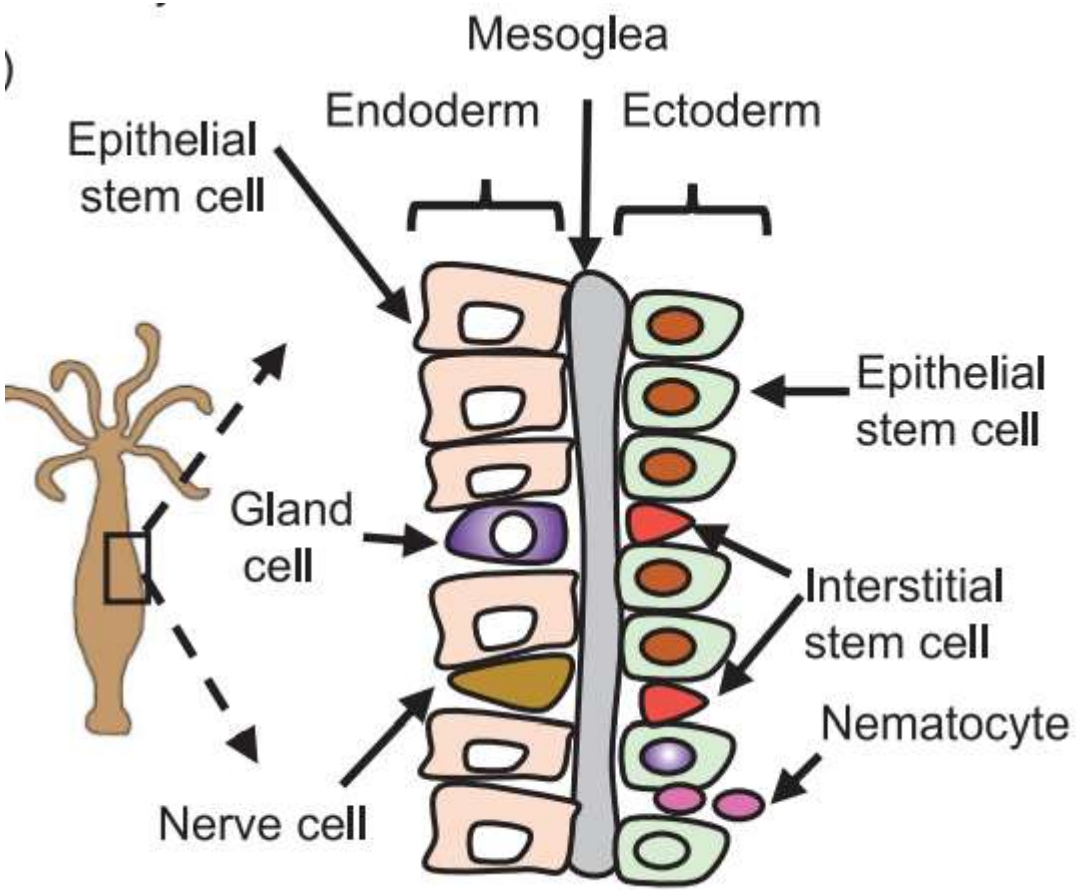


Hydra Regeneration is Accomplished with Three Different Stem Cells

(B) The body wall contains two epithelial cell layers, ectodermal and endo-dermal epithelial cells. Interstitial stem cells exist within the ectodermal epithelial cell layer.

(C) The **ectodermal** and **endodermal** epithelial cells proliferate continuously to maintain these tissue layers, producing differentiated epithelial cells, and are therefore considered to be distinct stem cells.

A third stem cell type, the multipotent, **interstitial stem cell** can self-renew and produce neurons, nematocytes, secretory cells, and gametes.

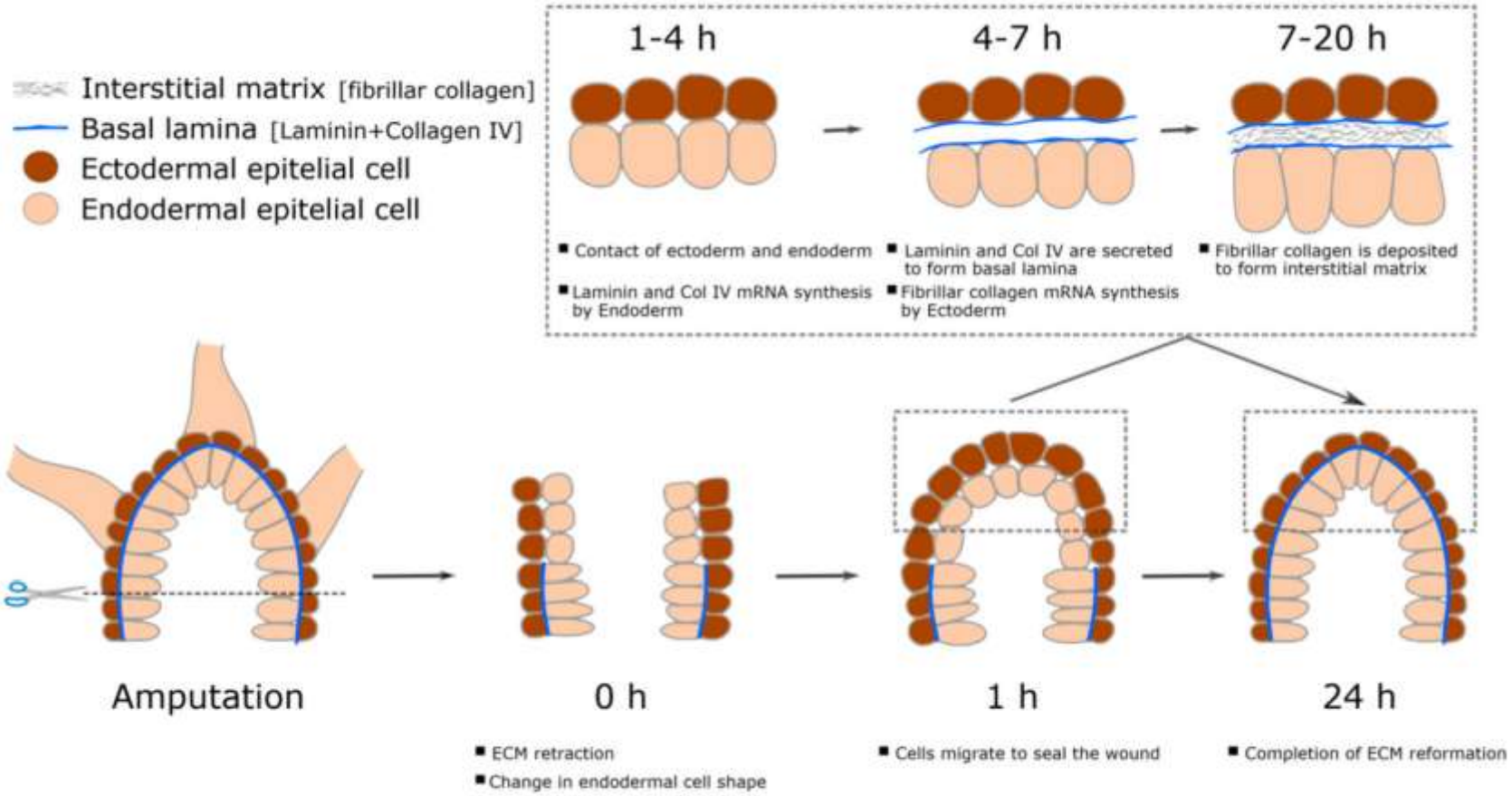


Hydra regeneration involves **three stem cells**

1.endoderm

2. ectoderm epithelial cells

3. interstitial stem cells

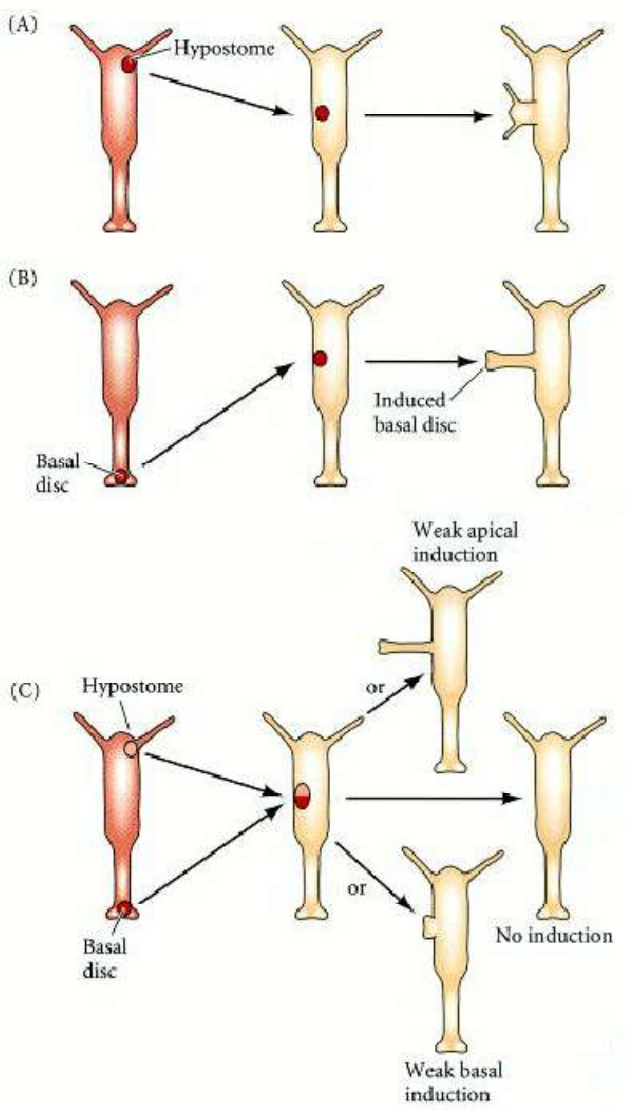


Cellular and structural changes during head regeneration in *Hydra*

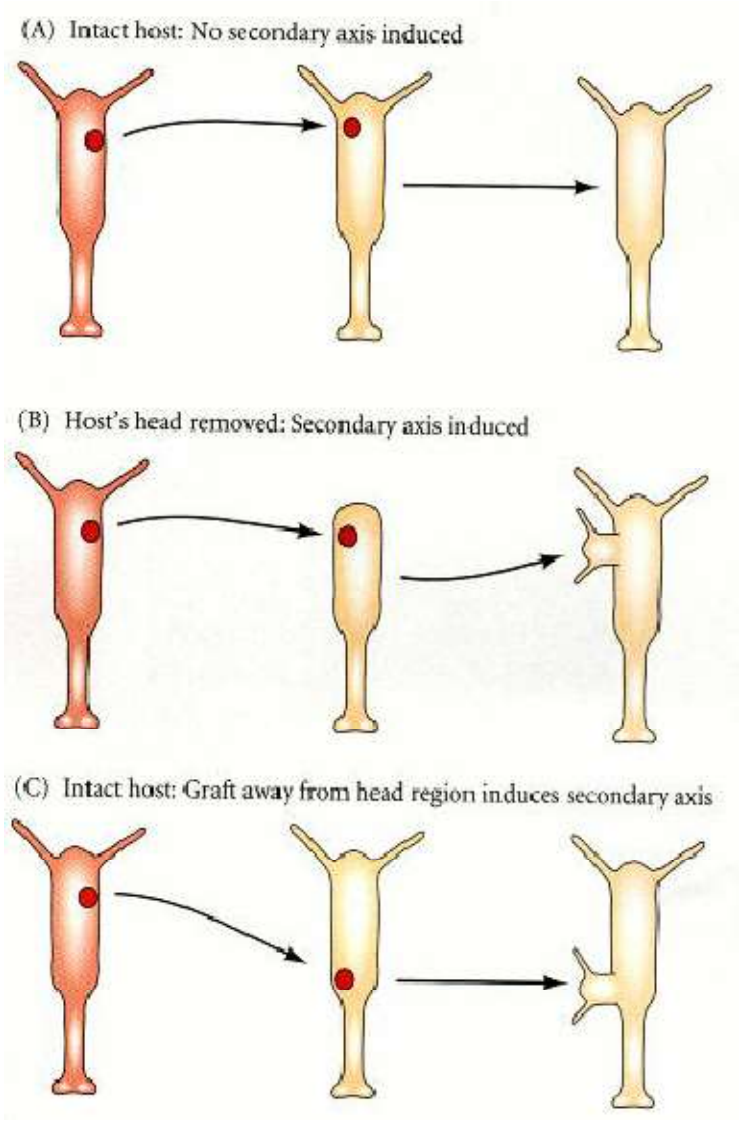
ECM is retracted immediately upon decapitation followed by change in endodermal cell shape.

This is succeeded by wound closure and secretion of ECM components completing the early stages of head regeneration.

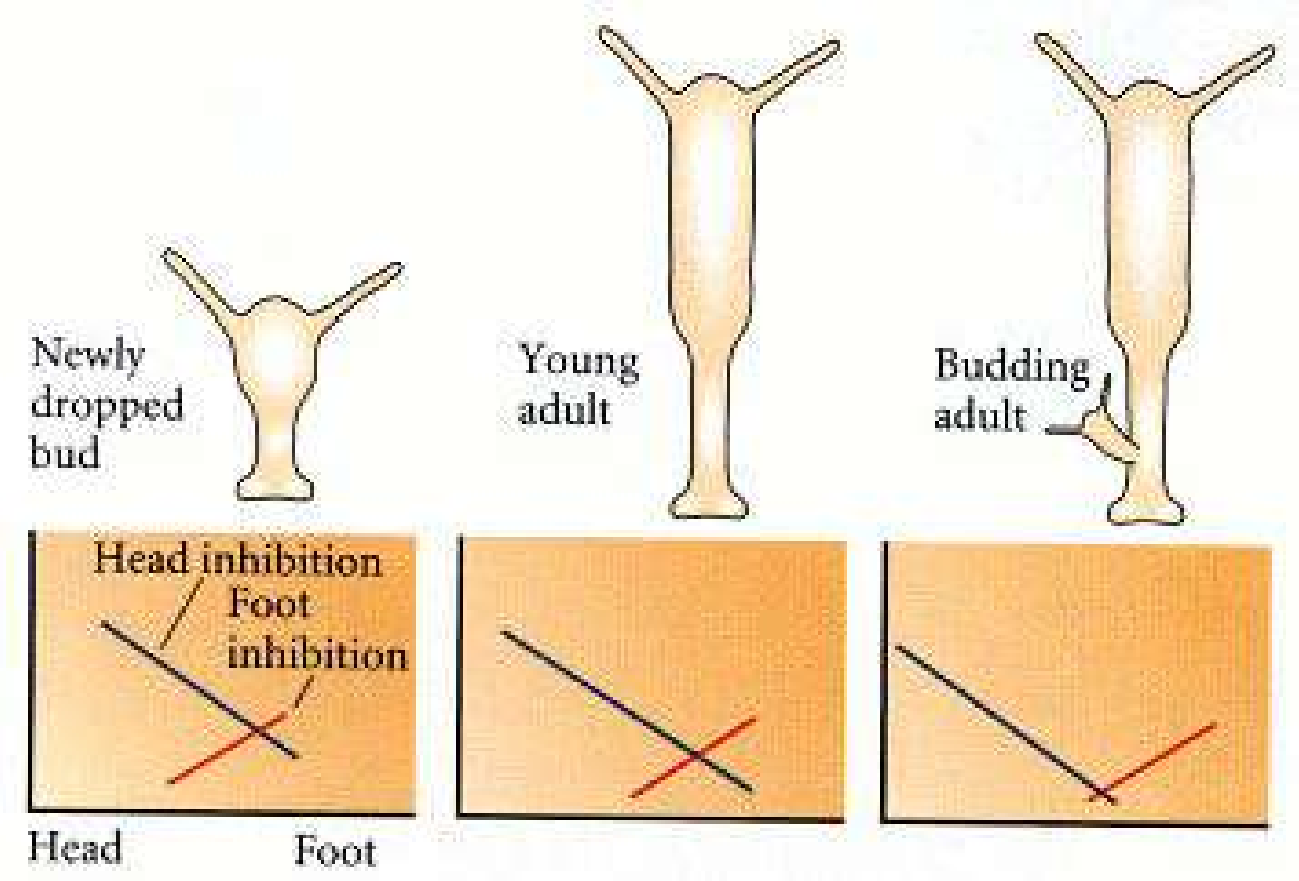
Morphogenetic capacity in different regions of hydra apical-basal axias



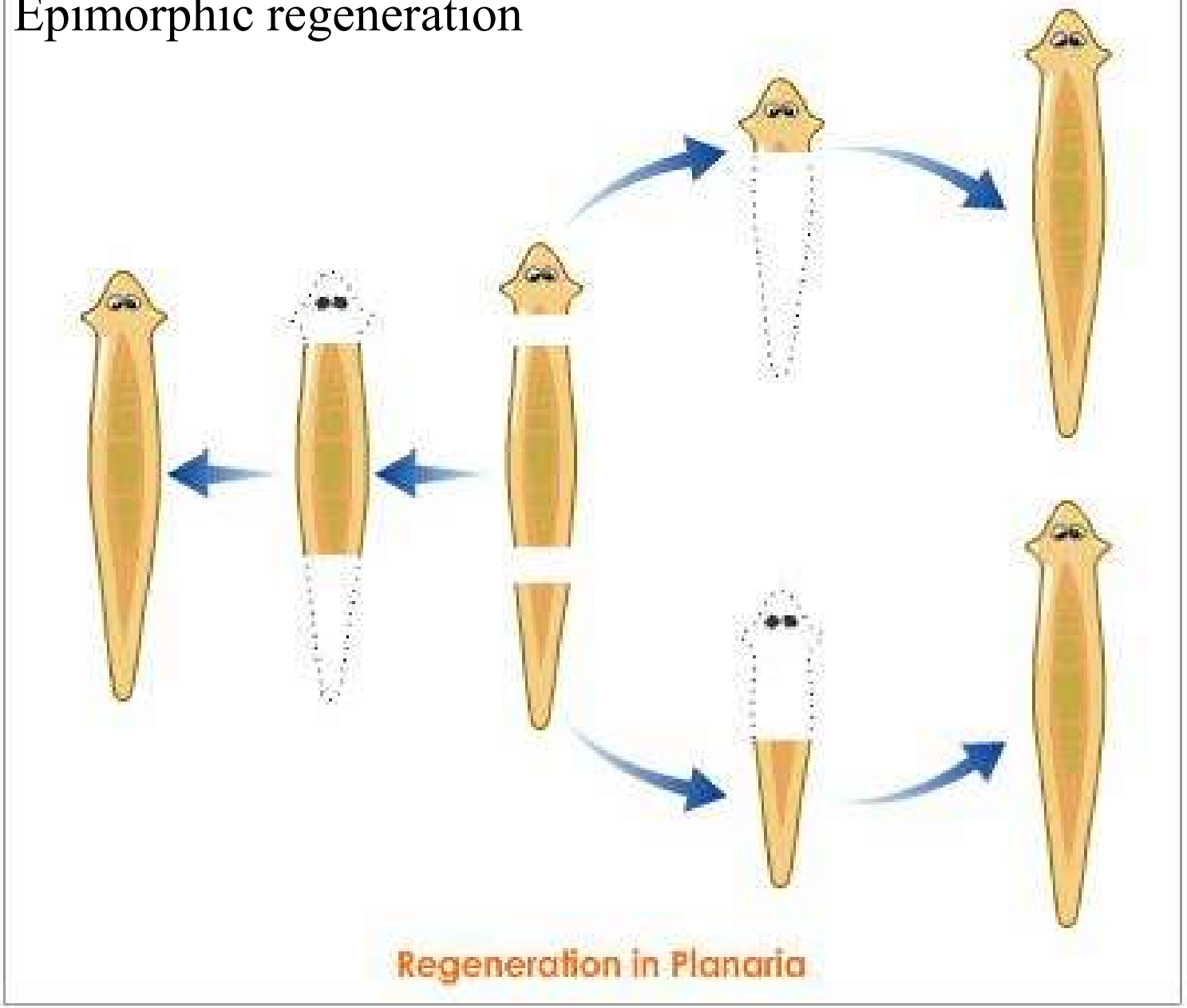
Grafting experiment showing head inhibition gradient

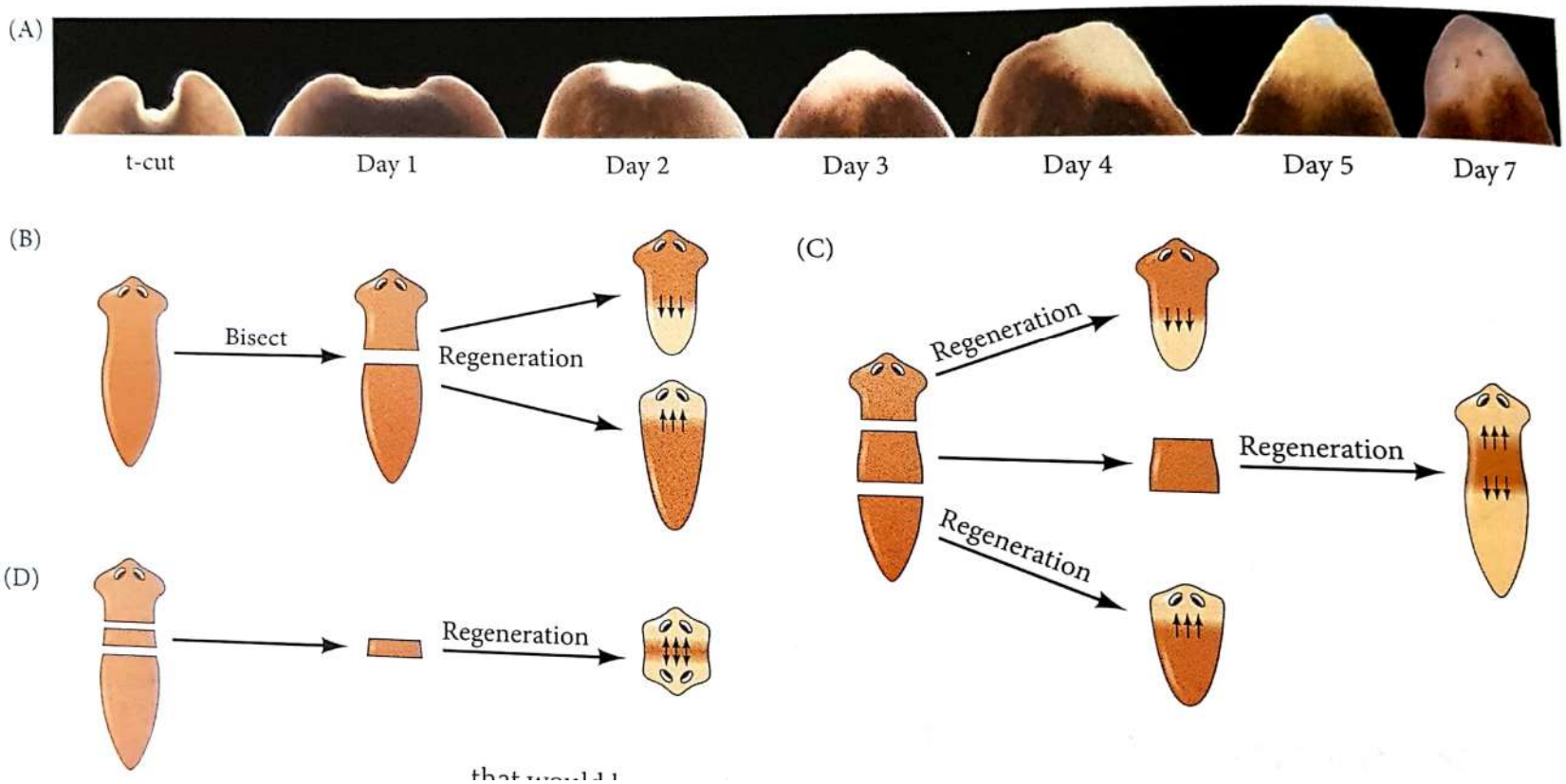


Bud location as a function of head and foot inhibition gradients



Epimorphic regeneration

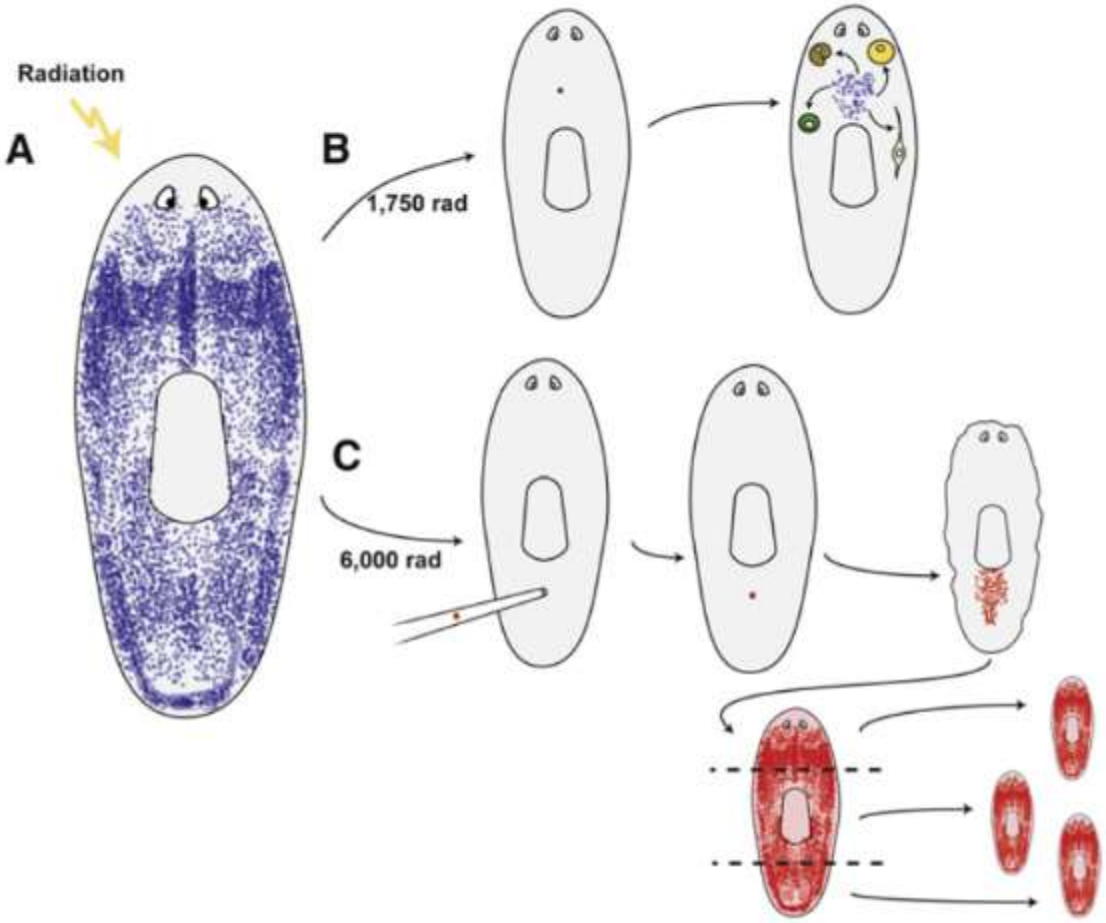




Stem cell mediated regeneration in Planaria

Planarian Regeneration Requires a Proliferative Cell Population

A population of adult dividing cells, called "**neoblasts,**" has long been prominent in planarian regeneration research. In the late1800s, dividing cells with simple morphology were described to exist in the bodies of flatworms.



(A) Neoblasts (blue) are the somatic dividing cells of planarians and are depicted in blue.

Dividing cells are scattered throughout the body, but restricted to behind the eyes and absent from the pharynx (centrally located).

Planarian Regeneration Requires a Proliferative Cell Population

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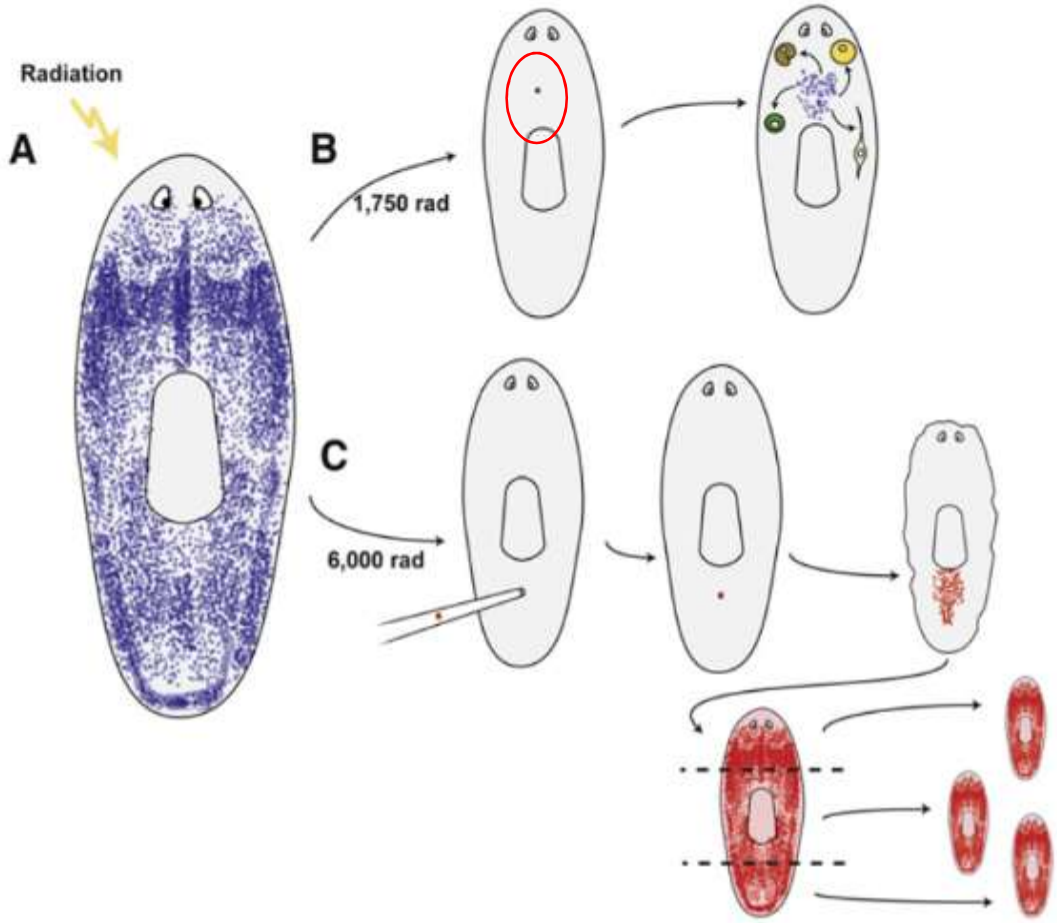
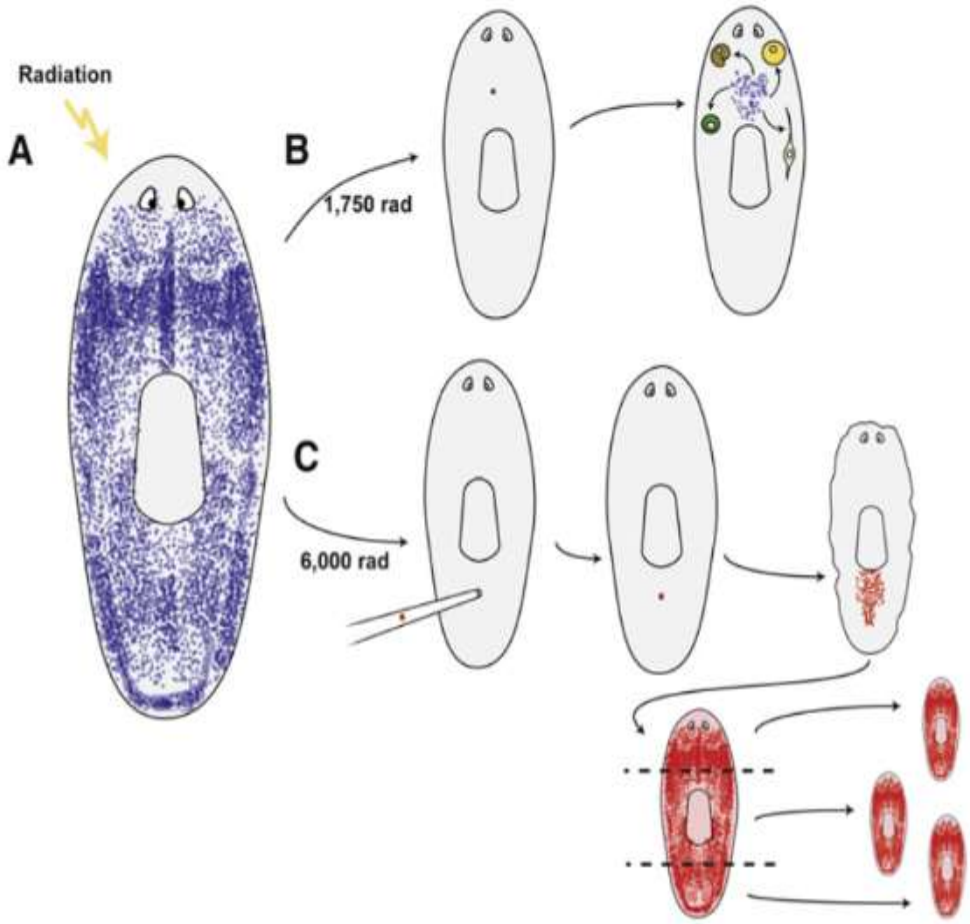


B. Irradiation with 1750 rad can result in animals with a single surviving dividing cell. This single cell, a clonogenic neoblast (cNeoblast), can divide and produce a colony of dividing cells, ultimately producing differentiated cells spanning germ layers For example, individual cNeoblasts can generate both neurons and intestine cells, as well as defined dividing cell progeny populations.

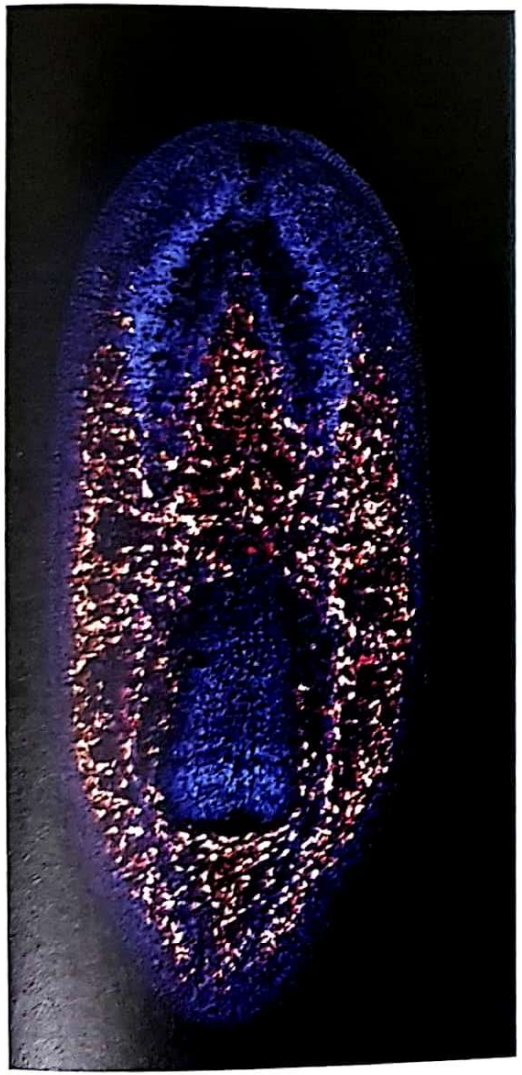
Planarian Regeneration Requires a Proliferative Cell Population

A population of adult dividing cells, called "**neoblasts,**" has long been prominent in planarian regeneration research. In the late1800s, dividing cells with simple morphology were described to exist in the bodies of flatworms.

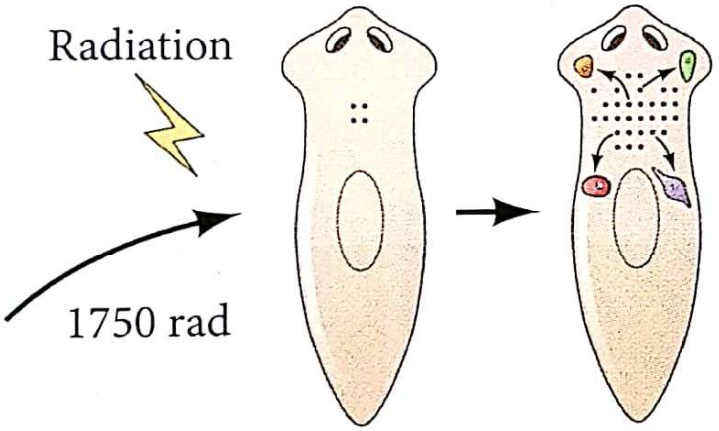


Irradiation with 6000 rad eliminates all dividing cells. Transplant of a single cNeoblast from a donor strain (red) results in clonogenic growth and, ultimately, the restored capacity for regeneration.

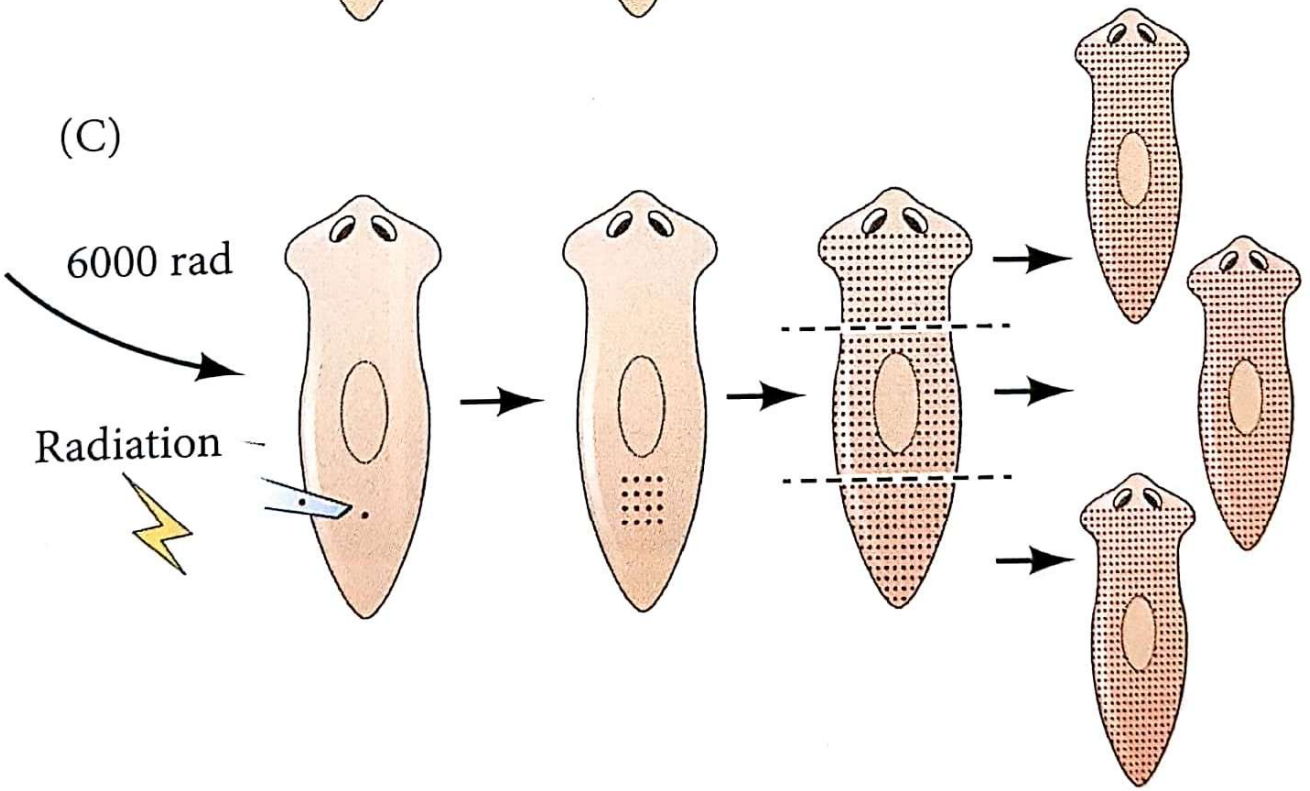
(A)

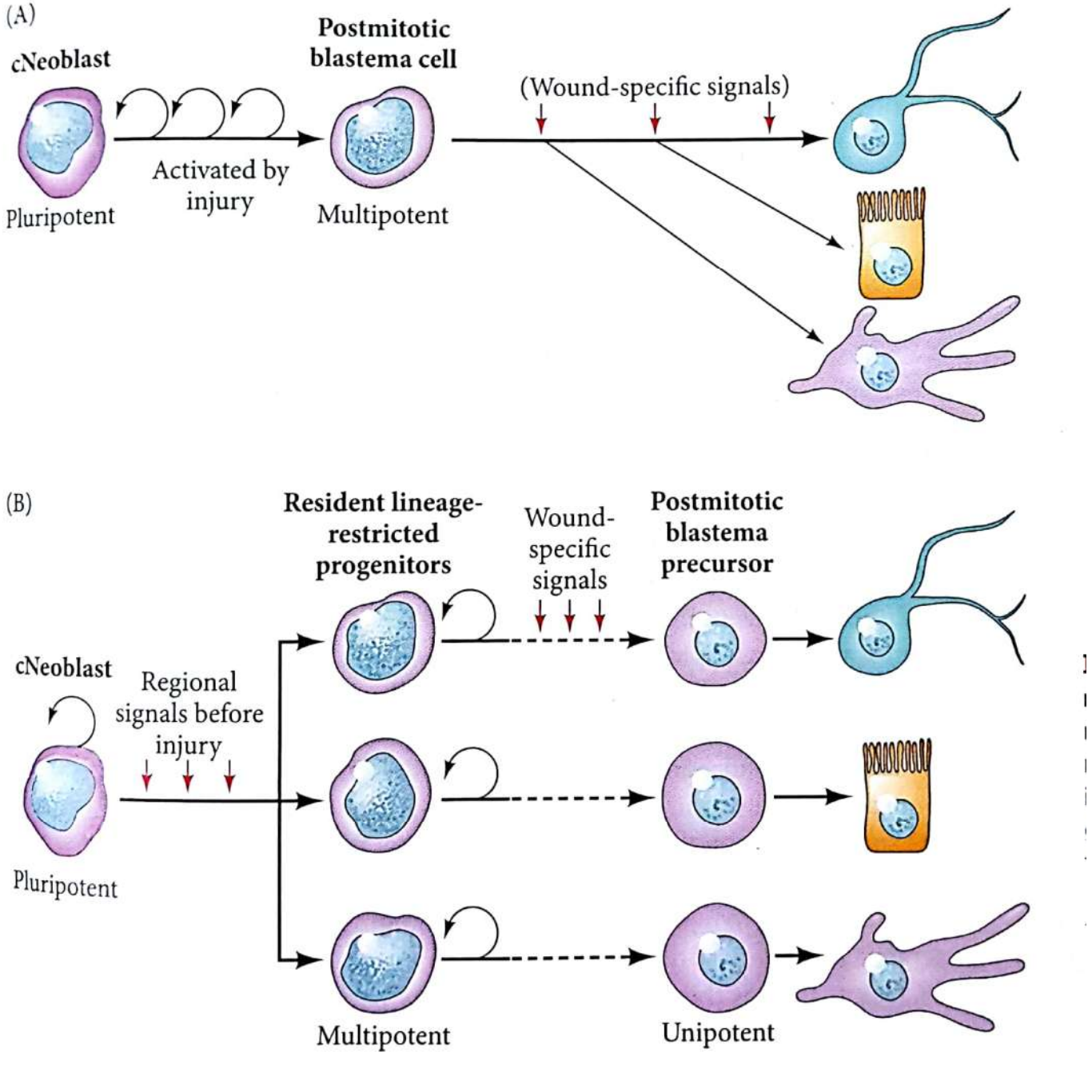


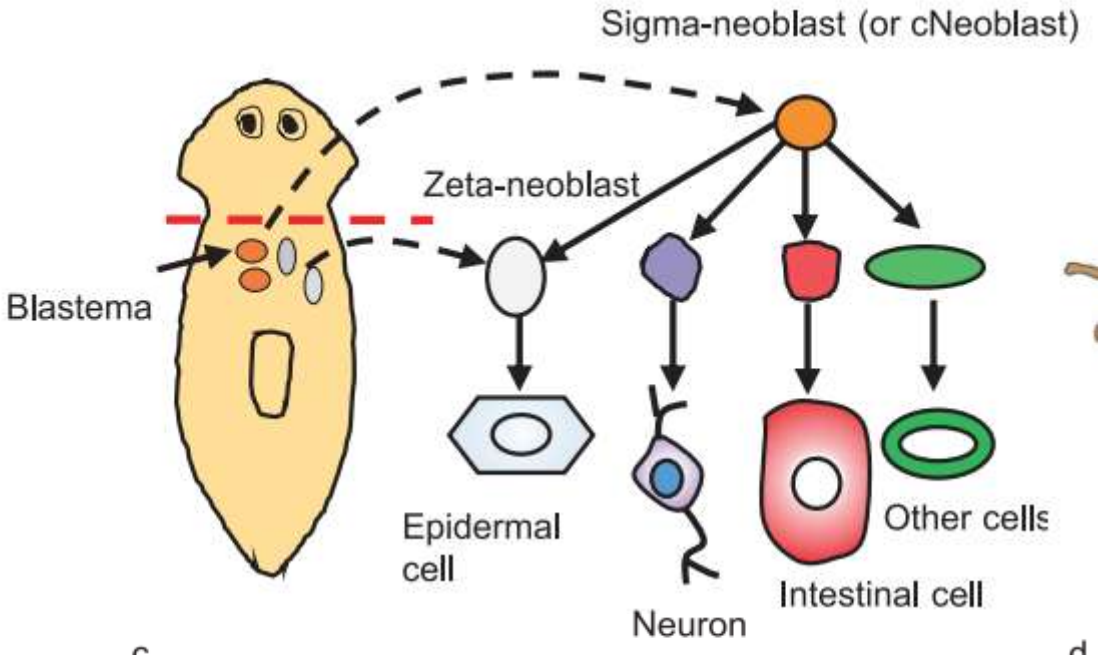
(B)



(C)

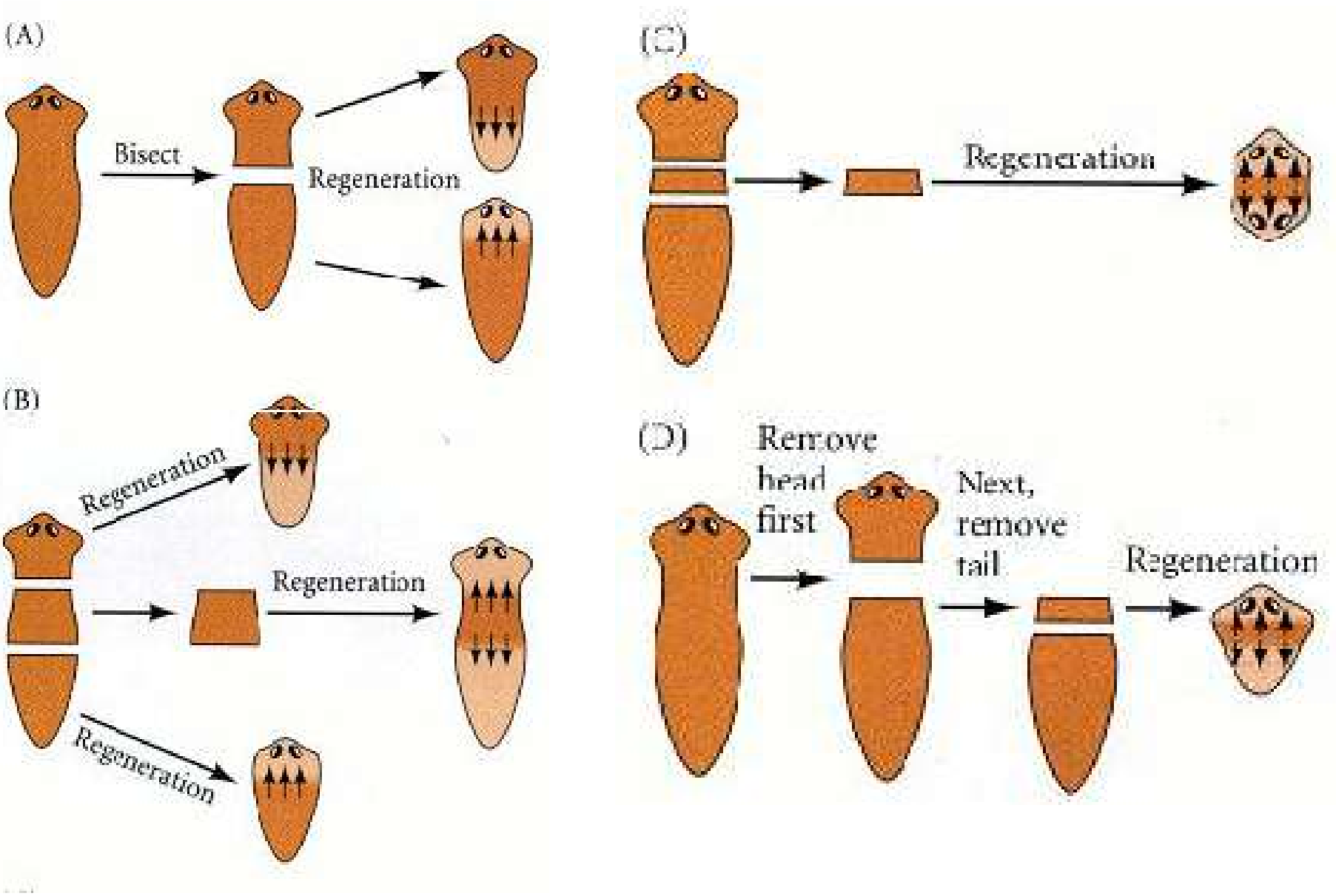



(A)
cNeoblast
Postmitotic blastema cell
(Wound-specific signals)
Activated by injury
Pluripotent
Multipotent
(B)
Resident lineage-restricted progenitors
Wound-specific signals
Postmitotic blastema precursor
cNeoblast
Regional signals before injury
Pluripotent
Multipotent
Unipotent

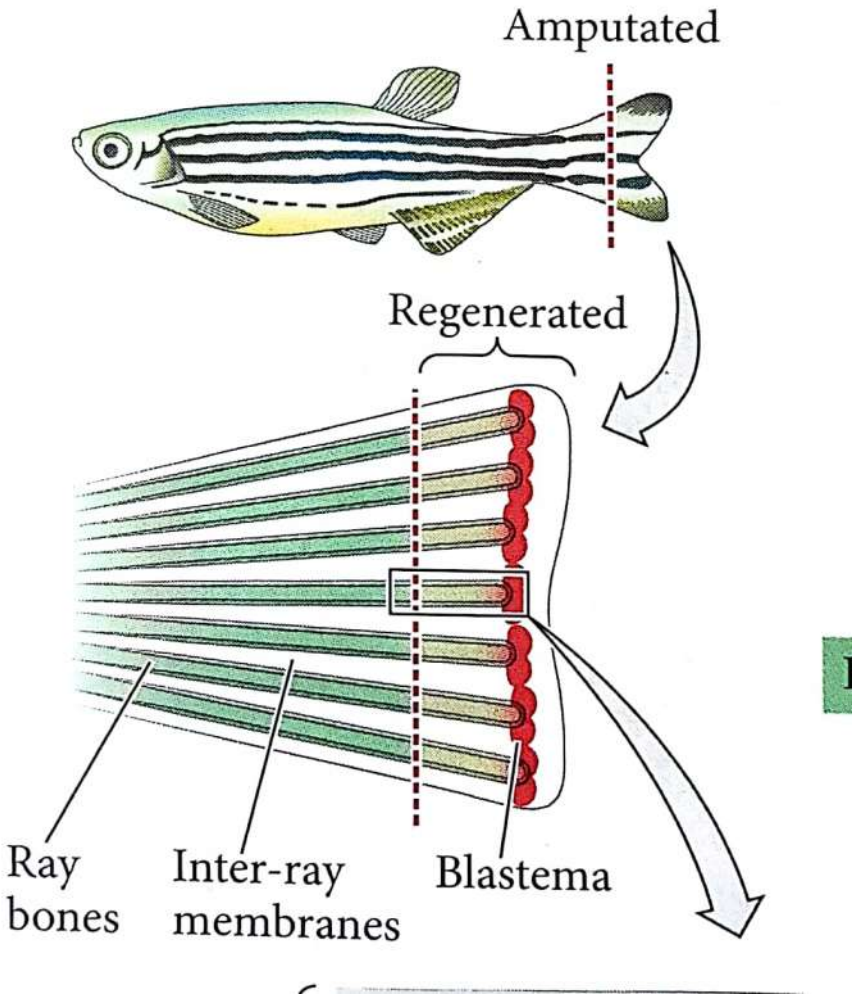


Planarians' neoblasts consist of the
1. pluripotent class (sigma-neoblast or cNeoblast) and
2. the lineage-restricted progenitor class (zeta-neoblast).

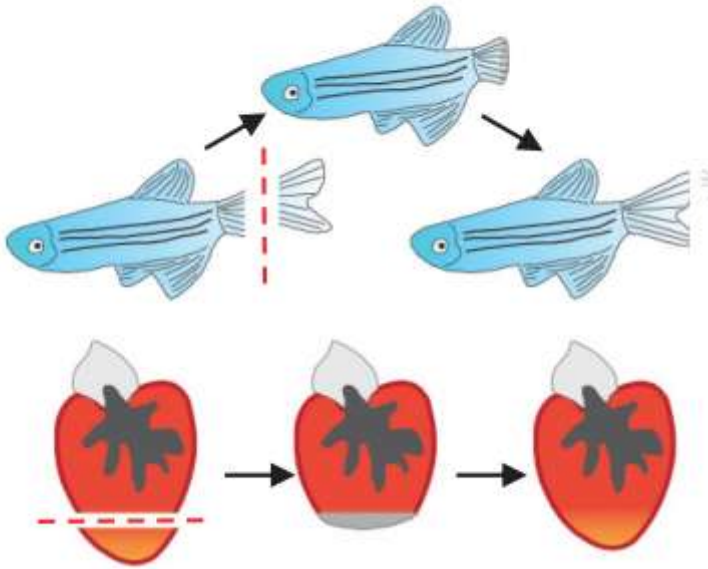
Flat worm regeneration: Gradient of Head and Tail is essential

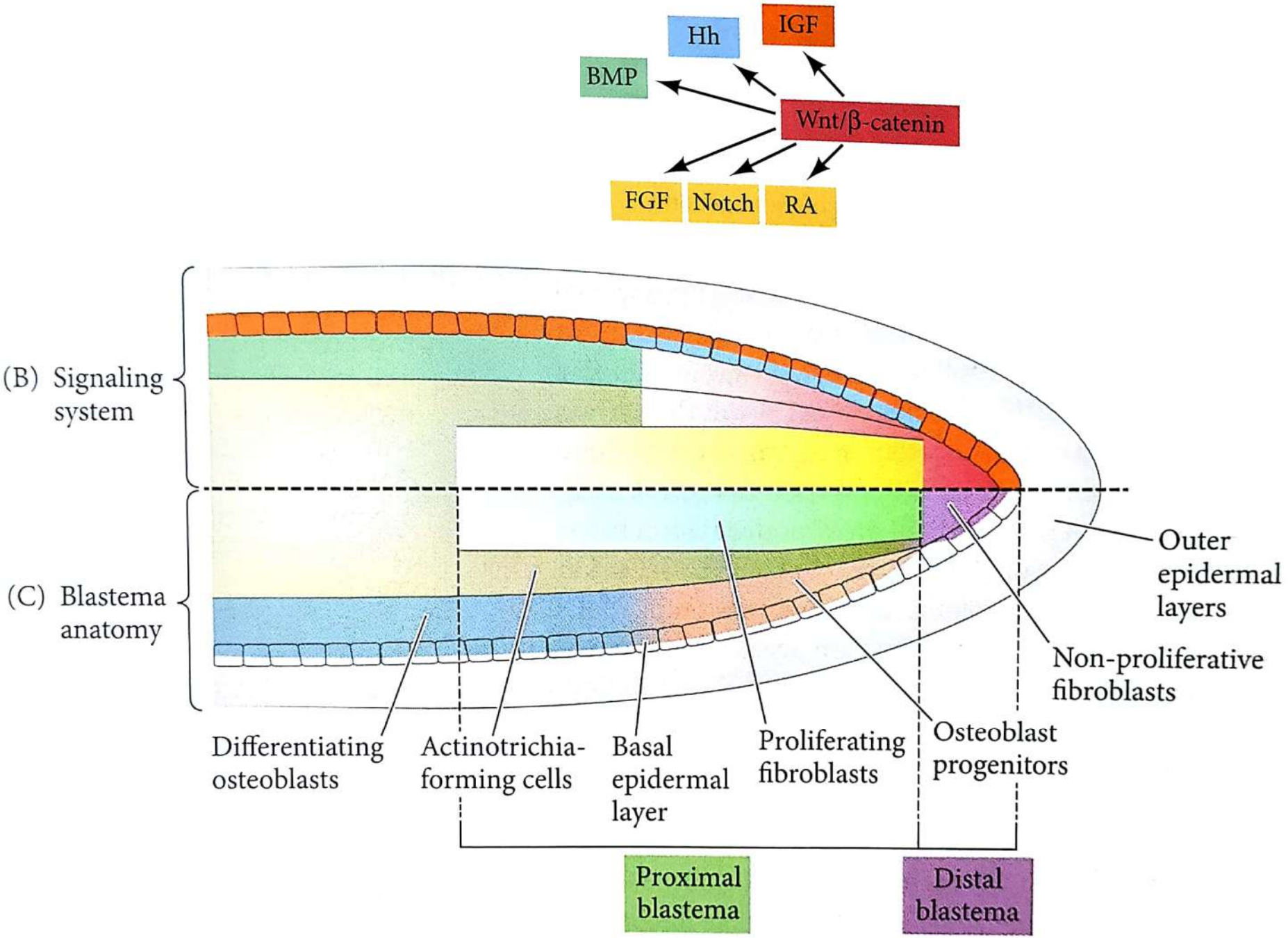


(A)



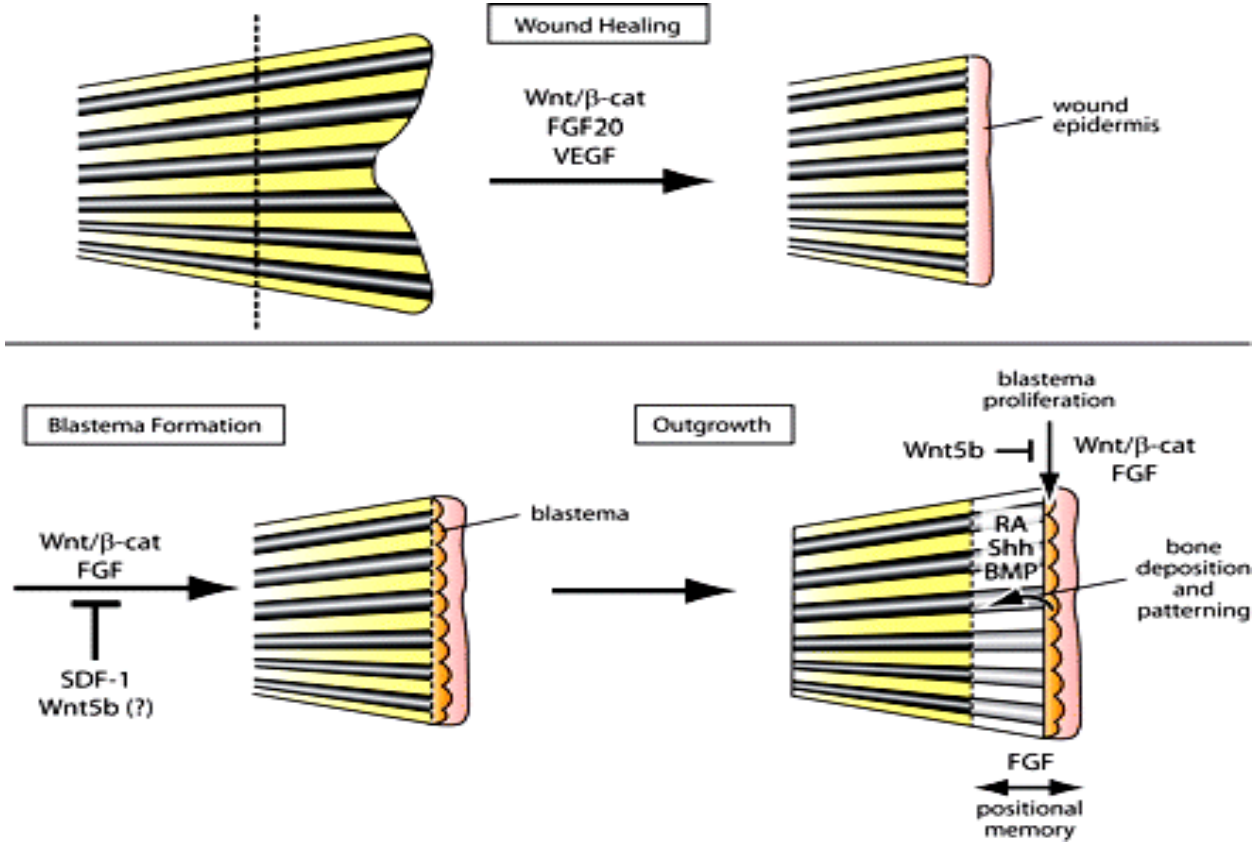
Zebrafish heart and fin regeneration

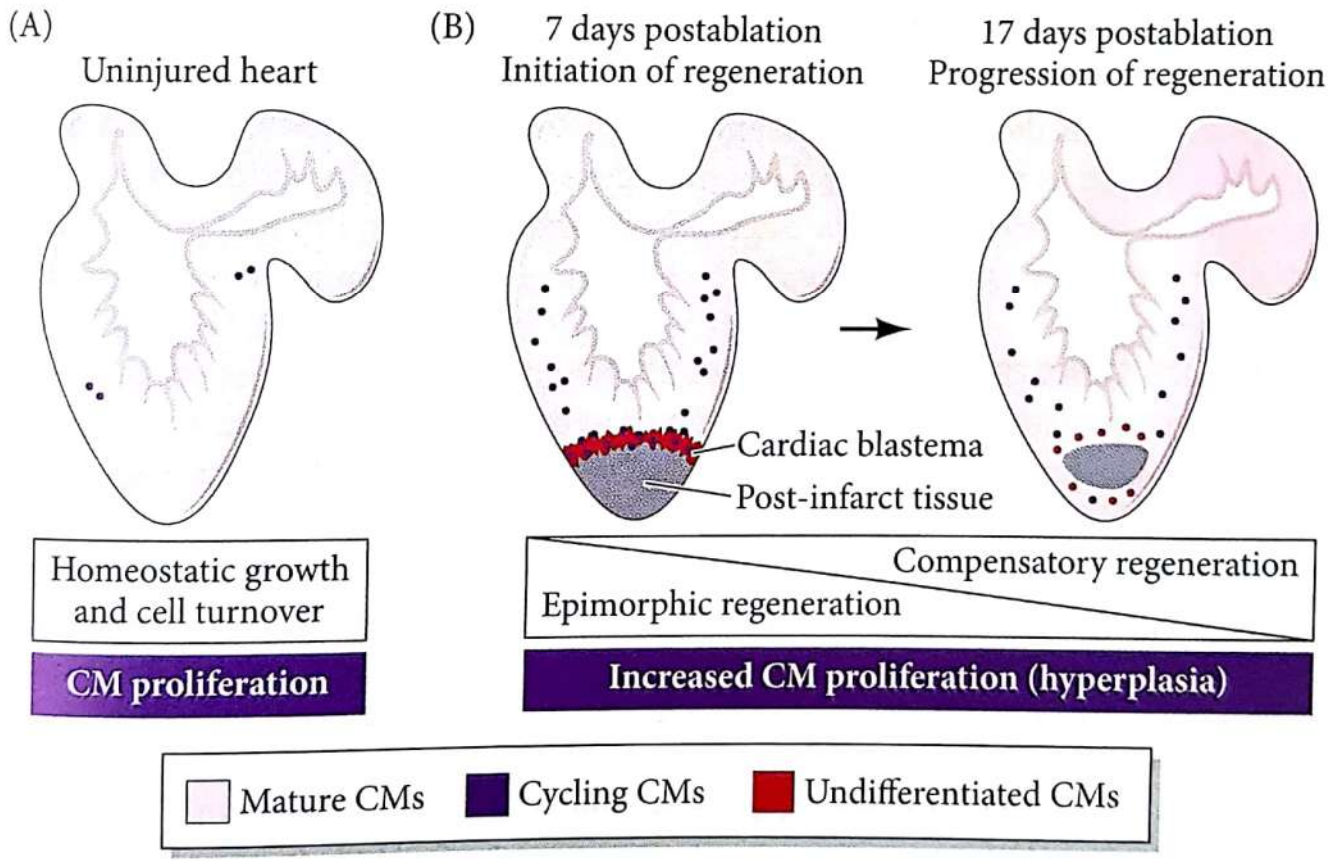




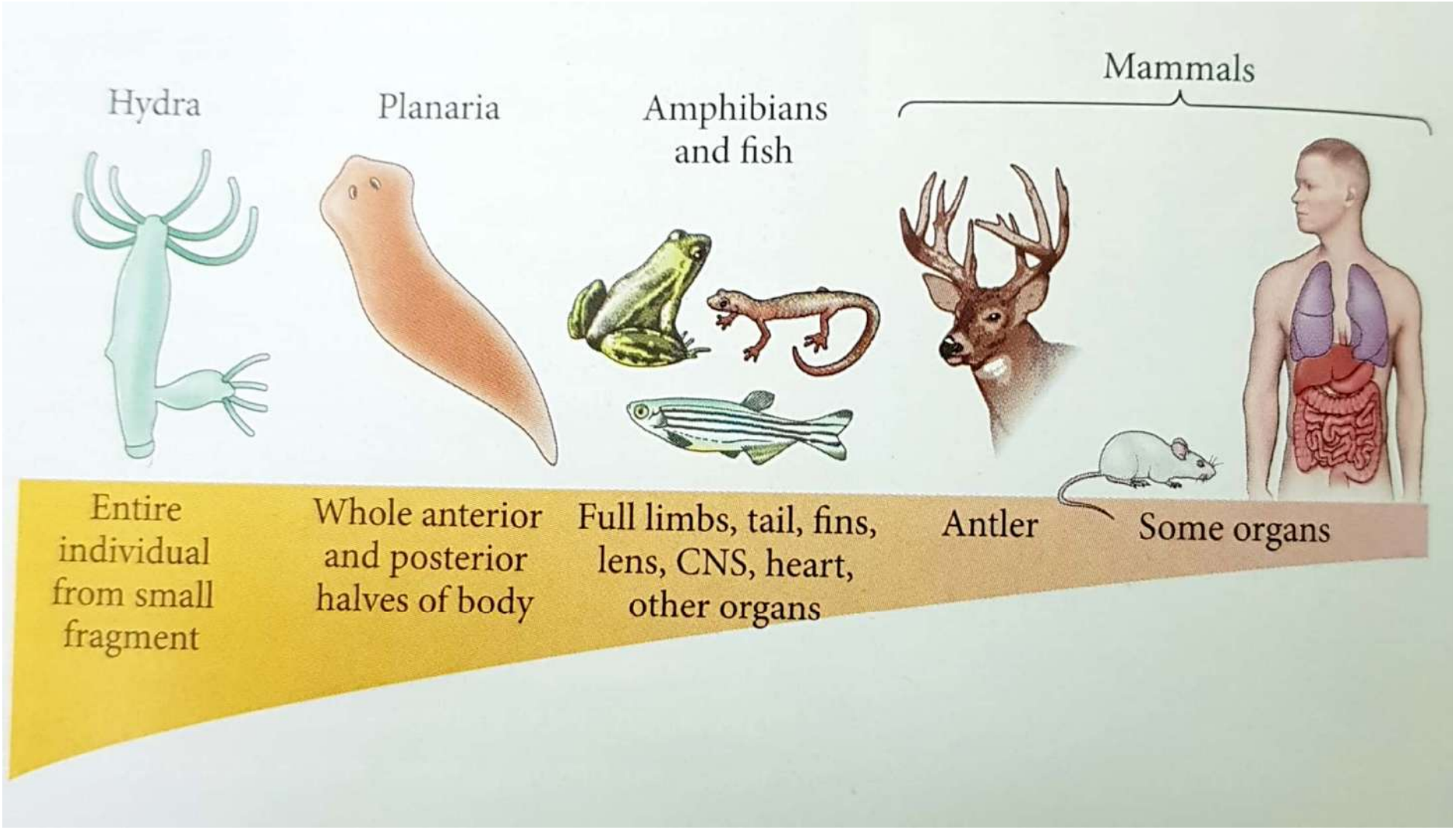
IGF-insulin like growth factor, Hh-Hedgehog

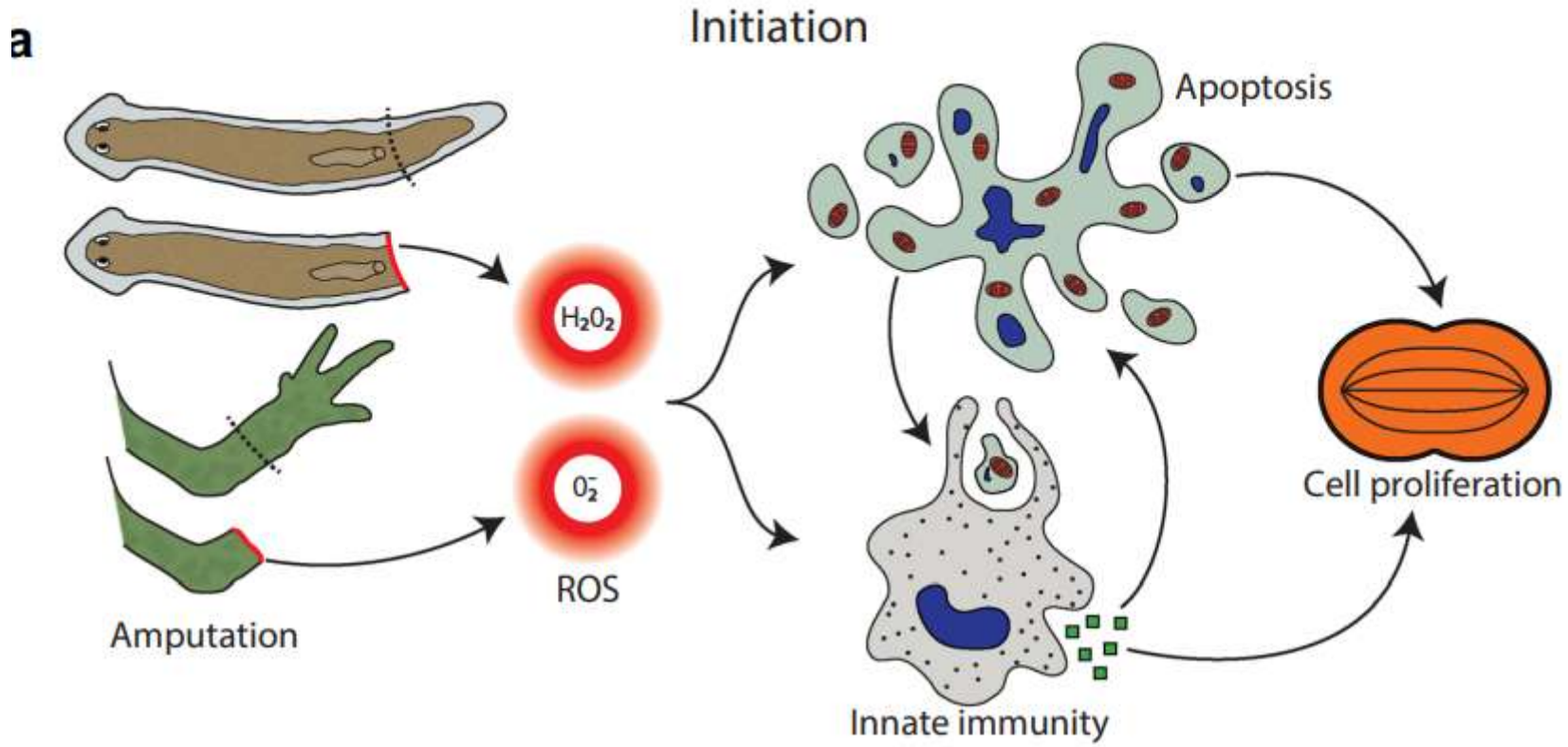
Major signaling events regulating zebrafish fin regeneration. The cartoon combines signals that have been found to be required in vivo or are implicated to be involved by overexpression data. Regeneration occurs in three steps. (1) Wound healing and formation of a specialized WE requires Wnt/ -catenin and FGF signaling. Regenerative angiogenesis begins at this stage and requires VEGF signaling.
(2) Wnt/ -catenin and FGF signaling pathways are also required for blastema formation. SDF-1 and Wnt5b might act as negative regulators of blastema formation.
(3) Wnt/ -catenin and FGF signaling are required for regenerative outgrowth. FGF signaling is also required for positional memory. Shh, BMP, and RA all participate in patterning the regenerating bones. Wnt5b acts as negative regulator of fin outgrowth.



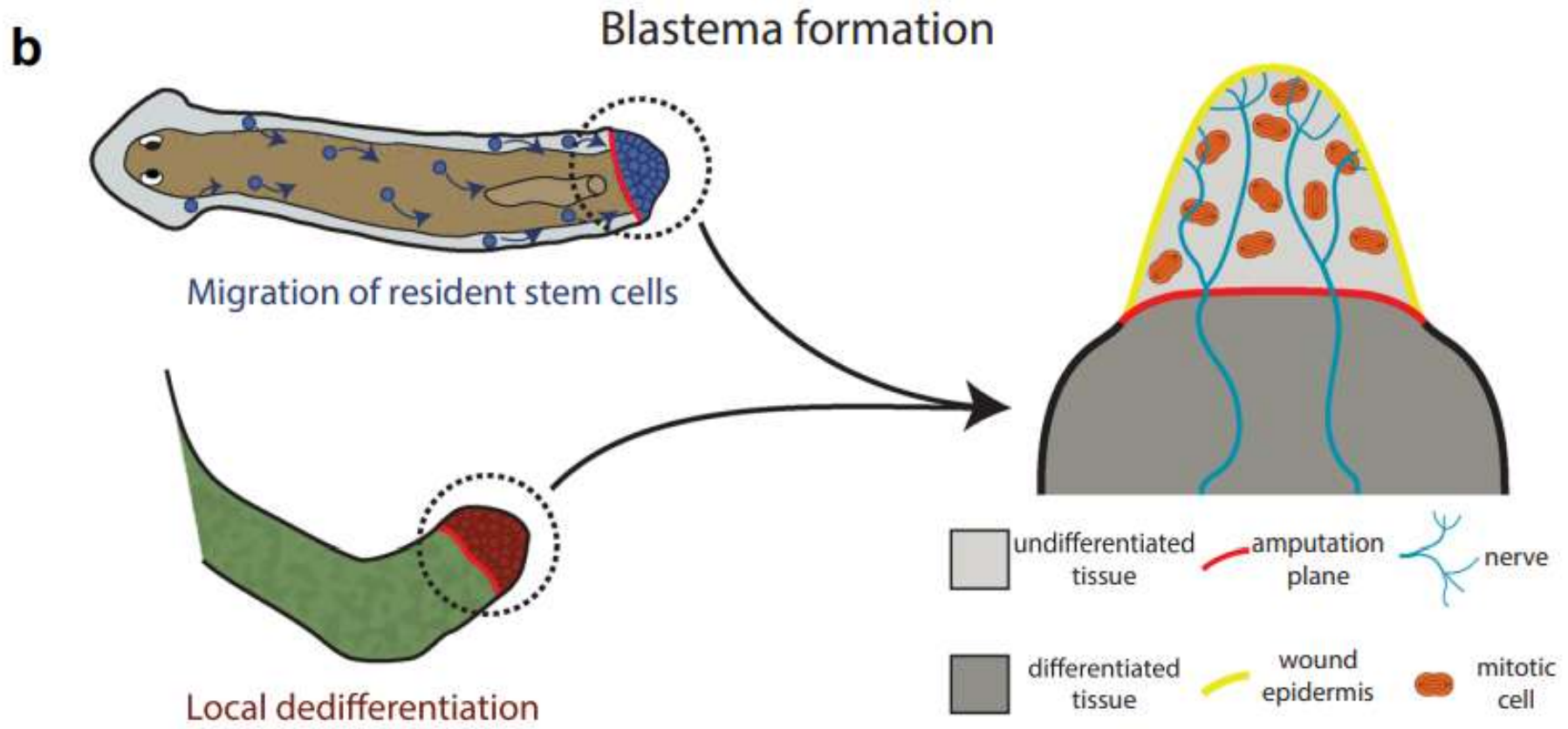


Epimorphosis and compensation in adult Zebra fish heart regeneration.
Regenerating heart produces a focal blastema at the wound surface, fueled by epimorphic processes during which cardiomyocytes (CMs) dedifferentiate, proliferate and redifferentiate in new ventricular cardiomyocytes

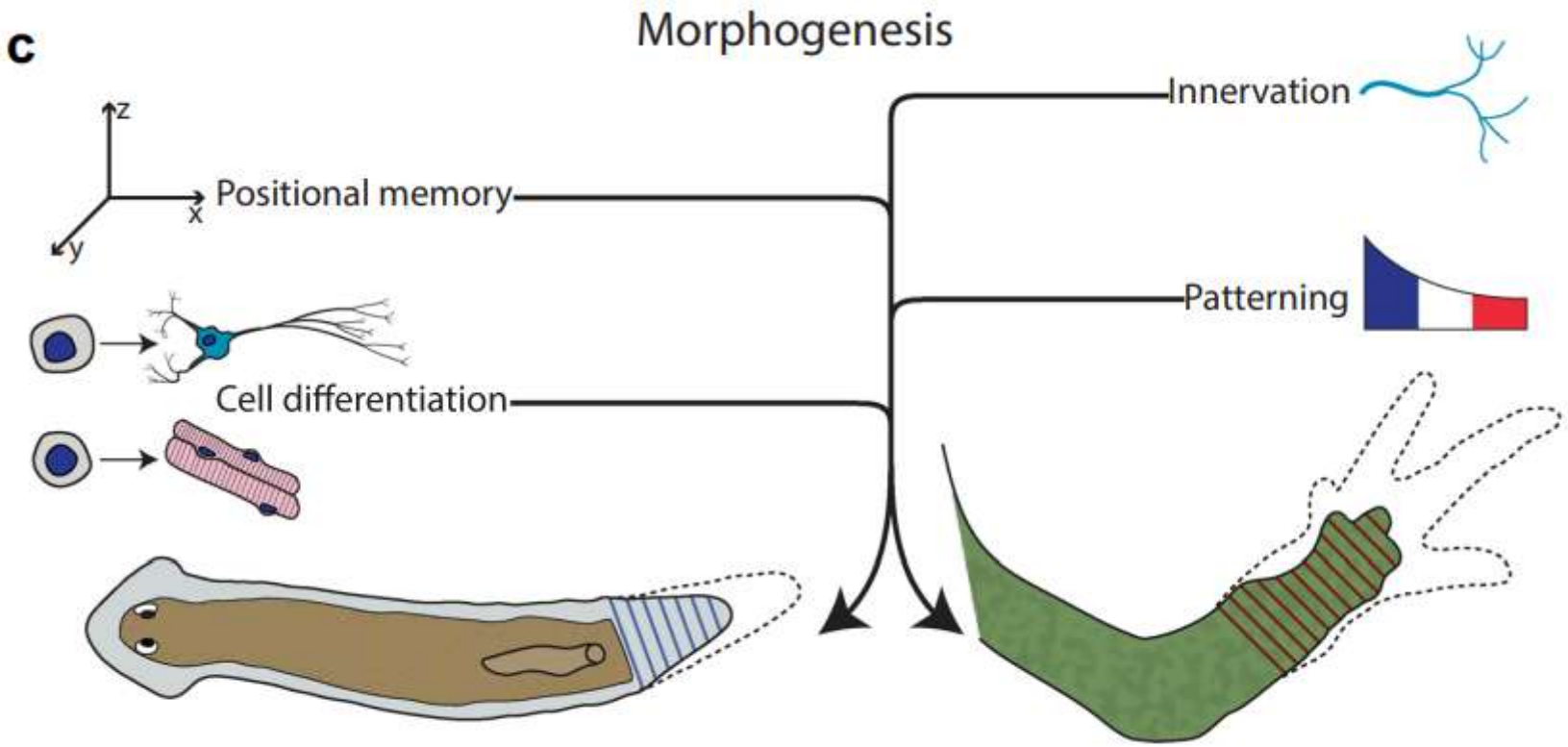

Hydra
Planaria
Amphibians and fish
Mammals
Entire individual from small fragment
Whole anterior and posterior halves of body
Full limbs, tail, fins, lens, CNS, heart, other organs
Antler
Some organs



Initiation of regeneration: Within minutes after amputation, reactive oxygen species (ROS) are produced at the wound site. Their accumulation promotes apoptosis, and activates innate immunity. Innate immunity is also activated by apoptotic cells and produces **pro-infammatory cytokines (green squares)** that sustain apoptosis. Surrounding cells enter mitosis through apoptosis-induced proliferation (AiP) and the difusion of mitogenic pro-infammatory cytokines.

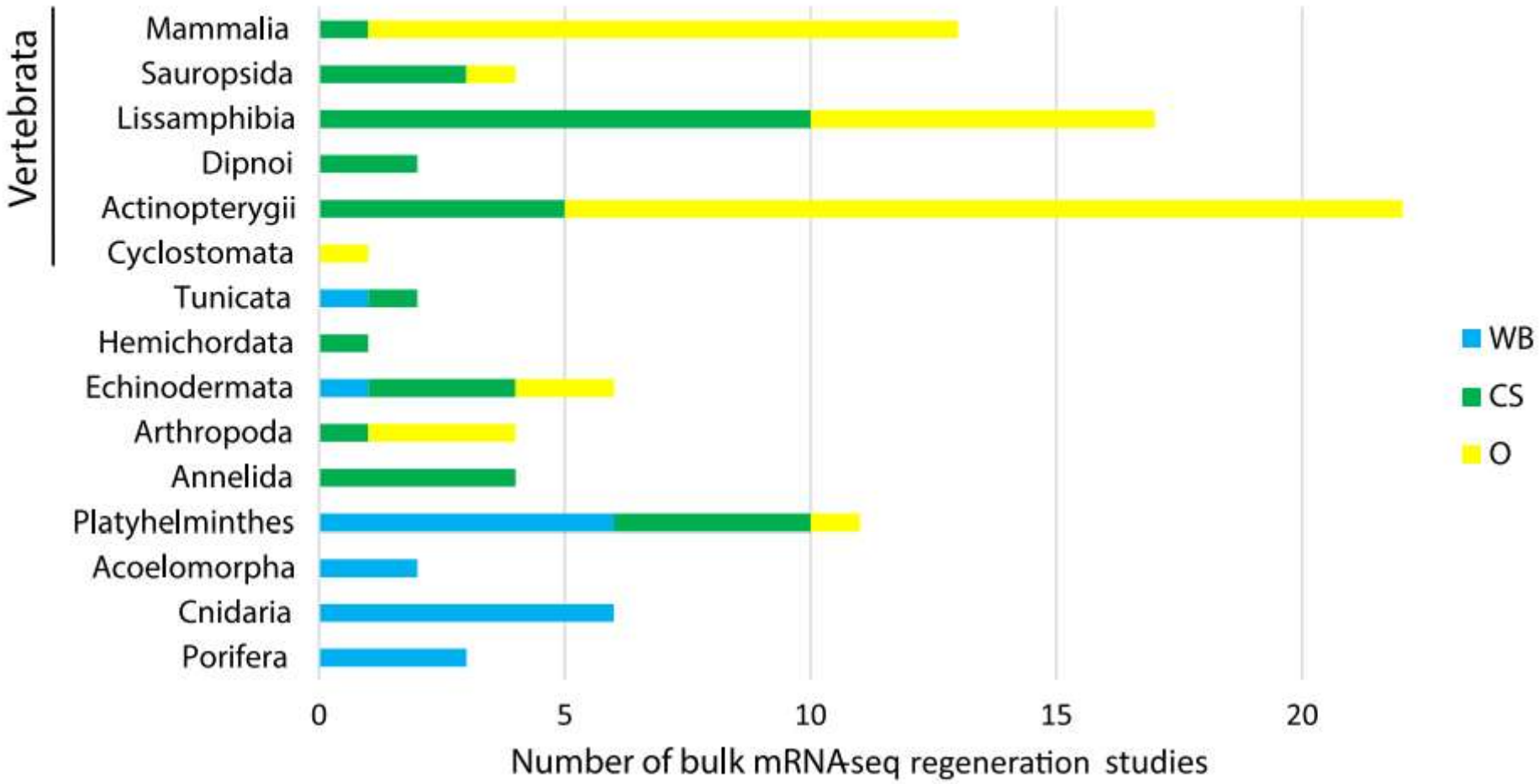


Blastema formation: A blastema, whatever is its origin (migration of resident stem cells or local dediferentiation) is always composed of undiferentiated mesenchymal cells covered by a wound epidermis. Cell proliferation within the blastema as well as its innervation are crucial for a successful regeneration.

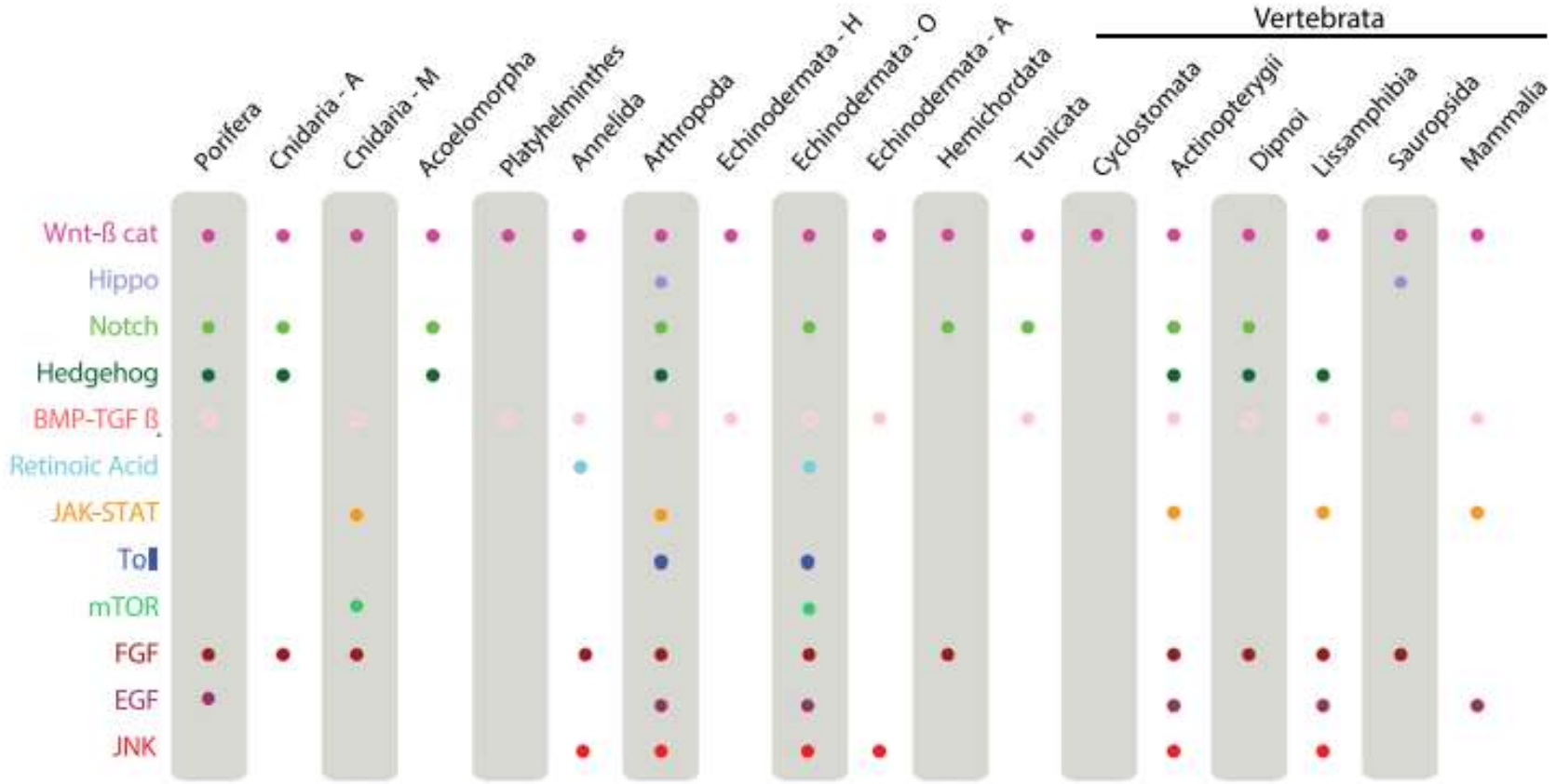


Morphogenesis: Four important aspects of this complex step are depicted:

1. role of innervation,
2. positional memory of the remaining tissue,
3. patterning of the regenerated region in particular in term of axes,
4. cell diferentiation

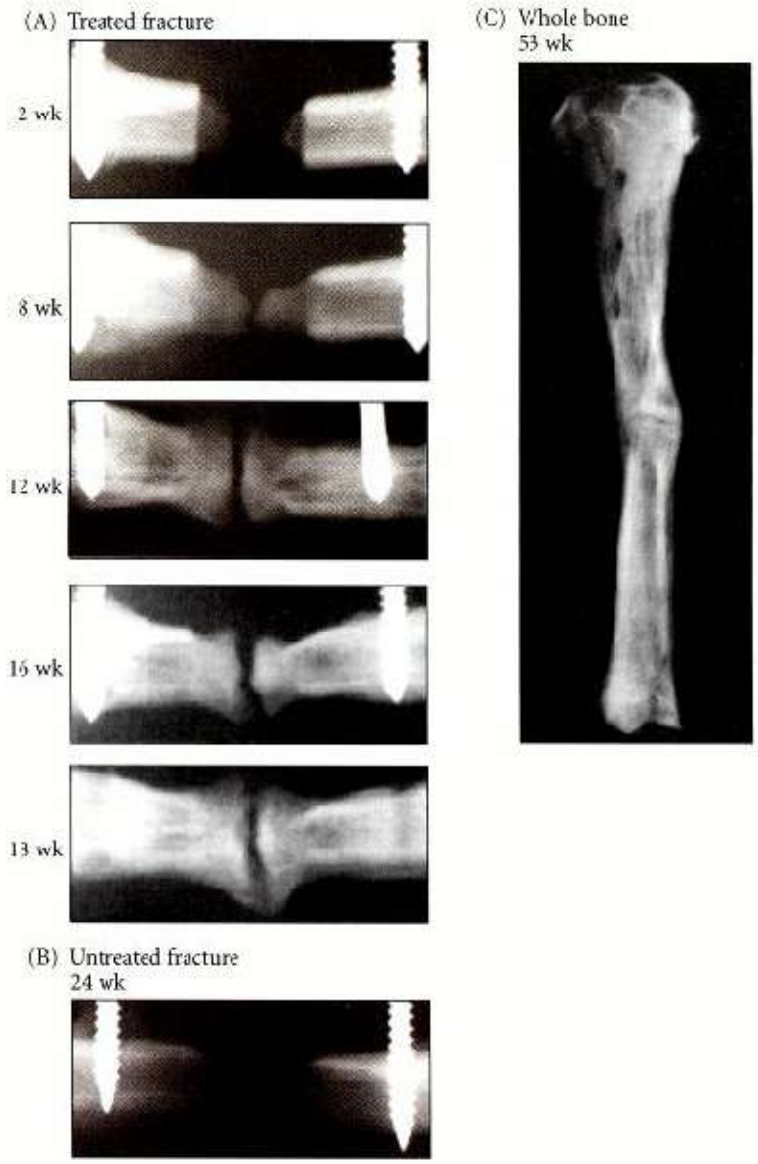


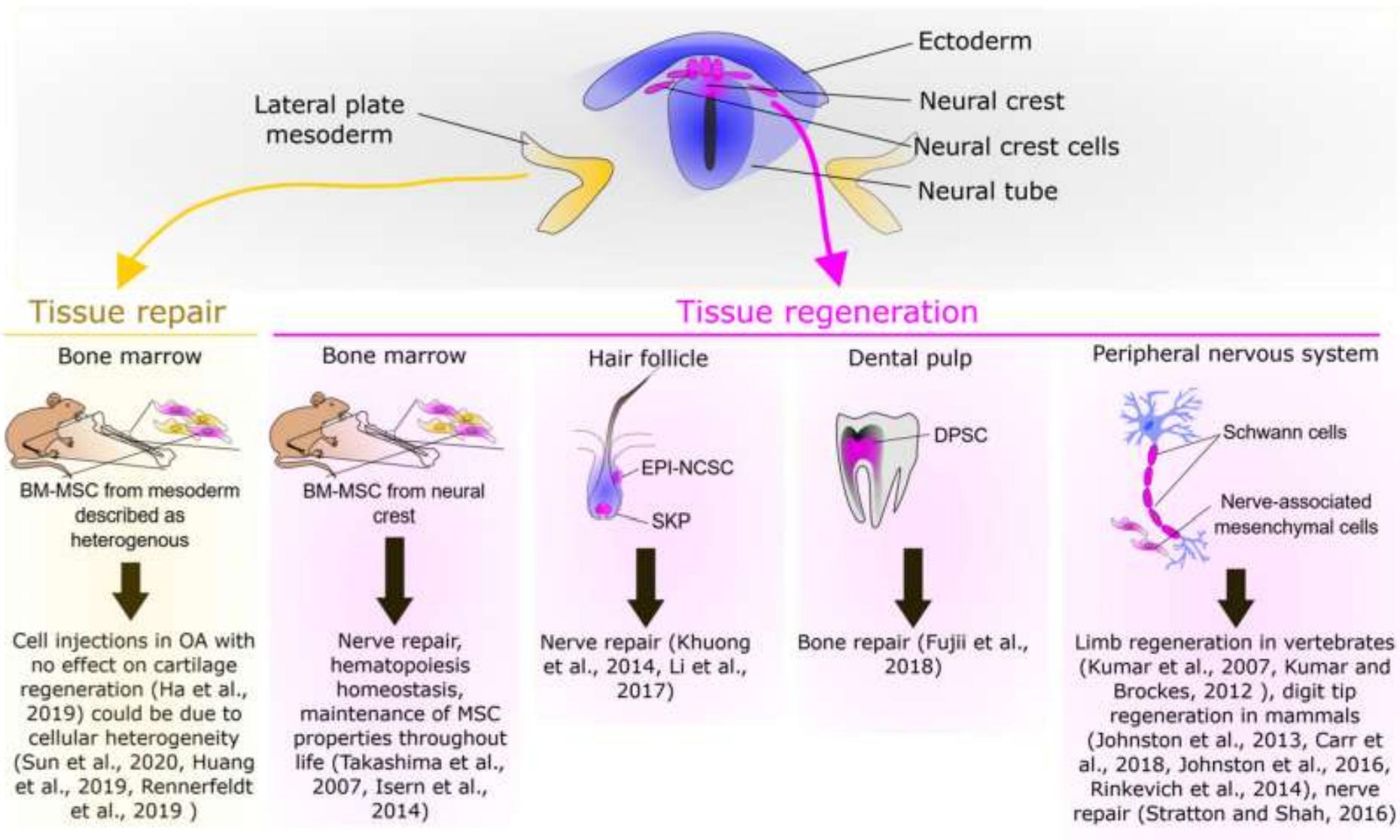
Diversity and number of bulk mRNA-seq regeneration studies. Major groups of metazoans and number of regeneration studies dealing with whole-body regeneration (WB), complex structure regeneration (CS) and organ regeneration (O) in each group are mentioned



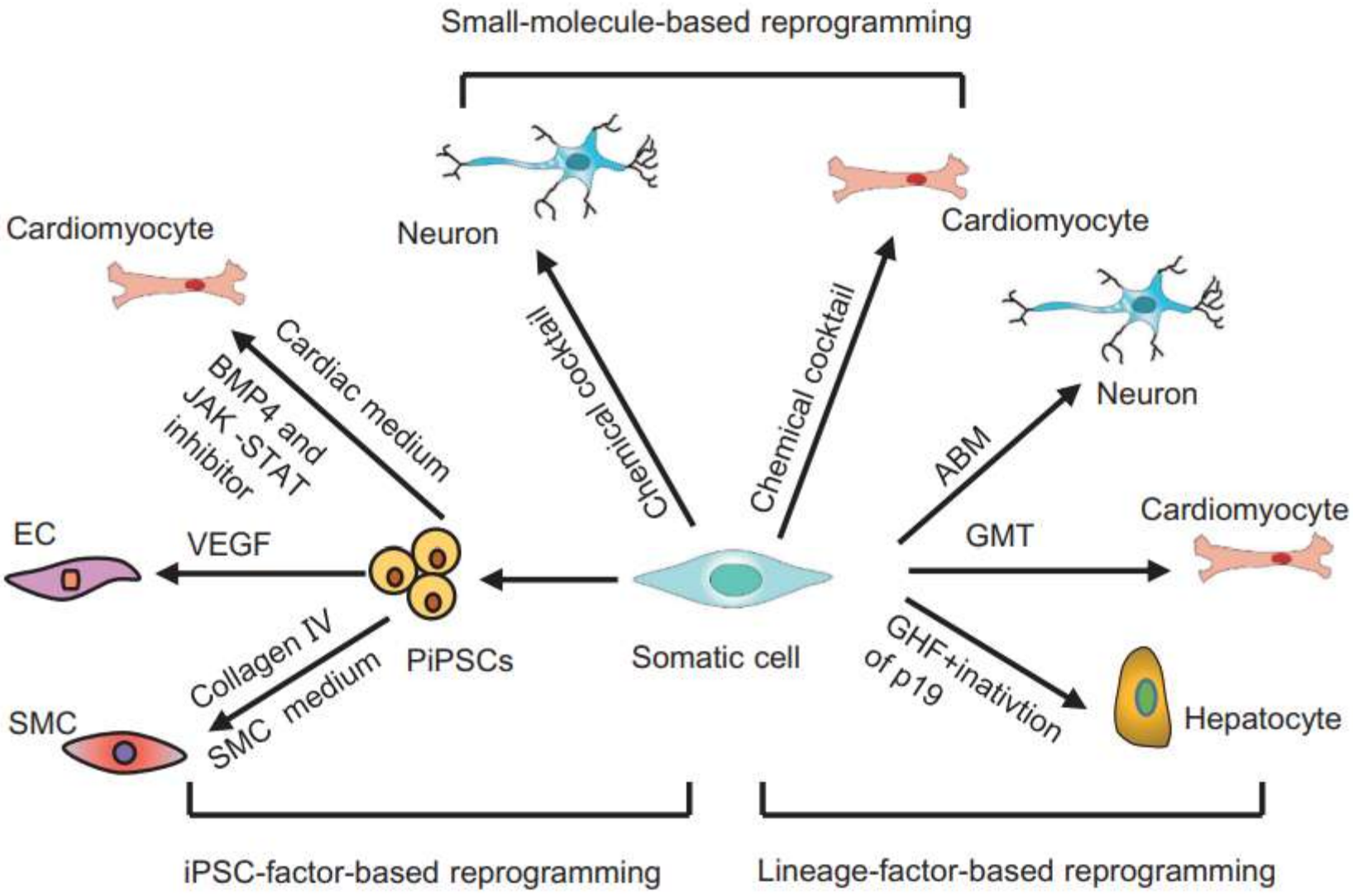
Majors signaling pathways' components are dynamically expressed in various metazoan regeneration contexts. Transcriptomic data highlight the potential importance of 13 major signaling pathways during regeneration of 15 main metazoan lineages. Circles indicate that at least one transcriptomic study reports diferential expression of those pathway components. Cnidaria—A Anthozoa; Cnidaria—M Medusozoa; Echinodermata—H Holothuroidea; Echinodermata—O Ophiuroidea; Echinodermata—A Asteroidea

Bone formation from collagen matrix containing plasmids bearing human parathyroid hormone (collagen gel)
Uses: large bone fractures and osteoporosis





The neural crest cells (NCC) are a transient embryonic cell population emerging during neurulation in vertebrates and give rise to many derivatives and their migratory potential allows their presence in virtually all the tissues.



Somatic cells can be directly reprogrammed to another one by several reprogramming approaches

frontiers in Cell and Developmental Biology

REVIEW
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From the Basis of Epimorphic Regeneration to Enhanced Regenerative Therapies

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Developmental Cell
Review

The Cellular Basis for Animal Regeneration

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Cellular and Molecular Life Sciences

REVIEW

Animal regeneration in the era of transcriptomics

Loïc Bideau[1] · Pierre Kerner[1] · Jerome Hui[2] · Michel Vervoort[1] · Eve Gazave[1]