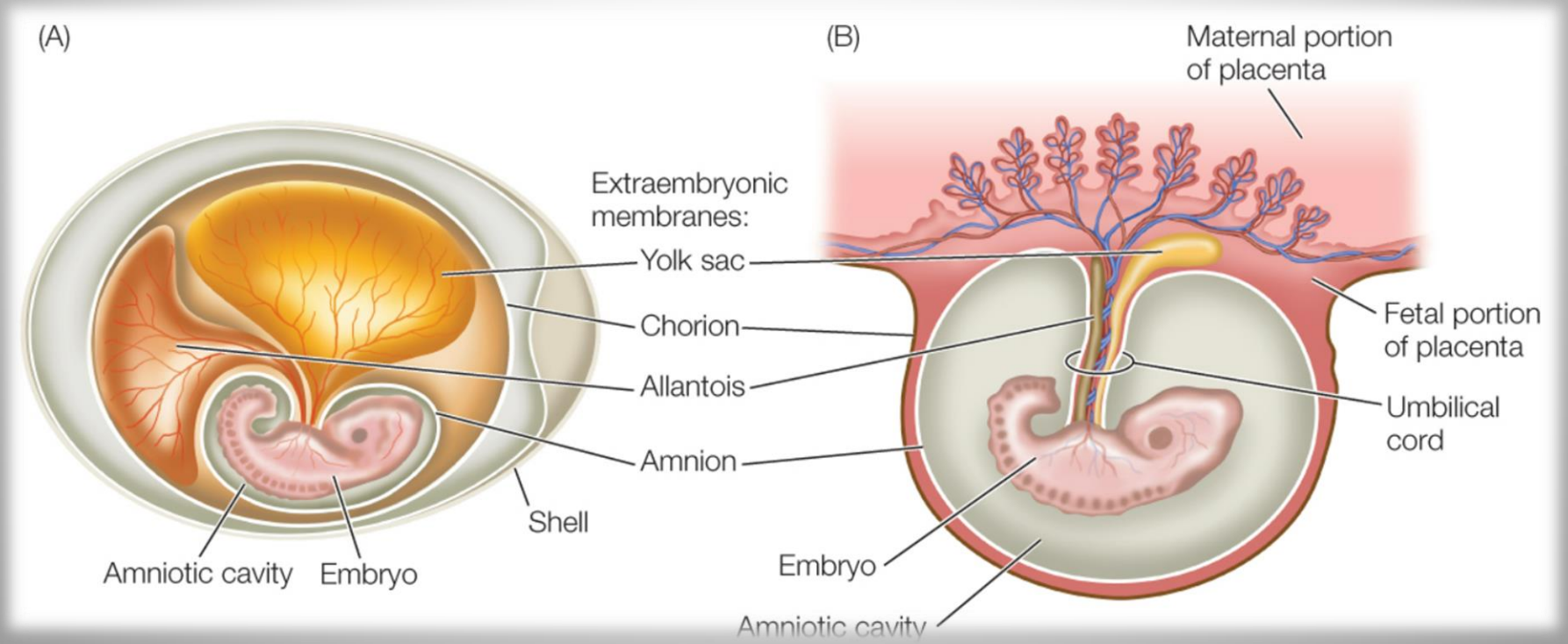
DEVELOPMENTAL BIOLOGY
(22ZOOC22)

AMNIOTES

- PLACENTATION

Amniote Egg and its evolution



(A) Chick egg

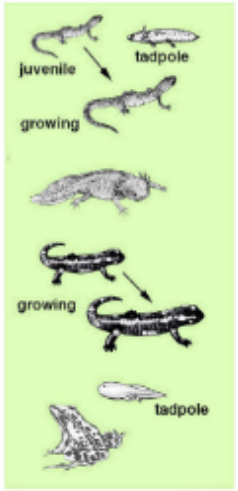
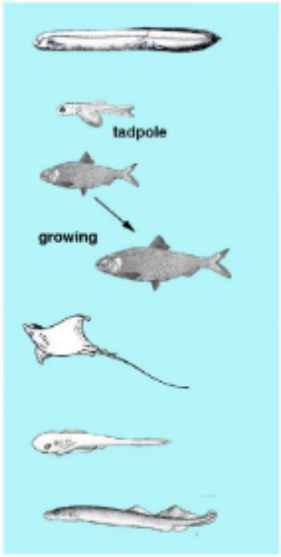
Water-retaining shell, four extraembryonic membranes, and embryo-nourishing yolk, was a major step in adaptation to the terrestrial environment.

(B) Mammalian egg

The developing embryo is retained inside the maternal body, with which it exchanges nutrients and wastes via the placenta.

Note the correspondence between the various membranes in (A) and (B)

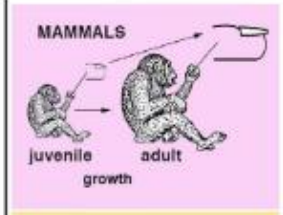
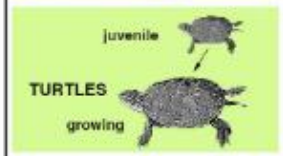
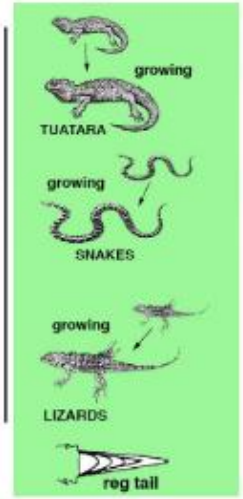
ORGAN REGENERATION IN POST-EMBRYONIC (LARVAL-ADULT) ANAMNIOTES
in submerged-wet-humid environment

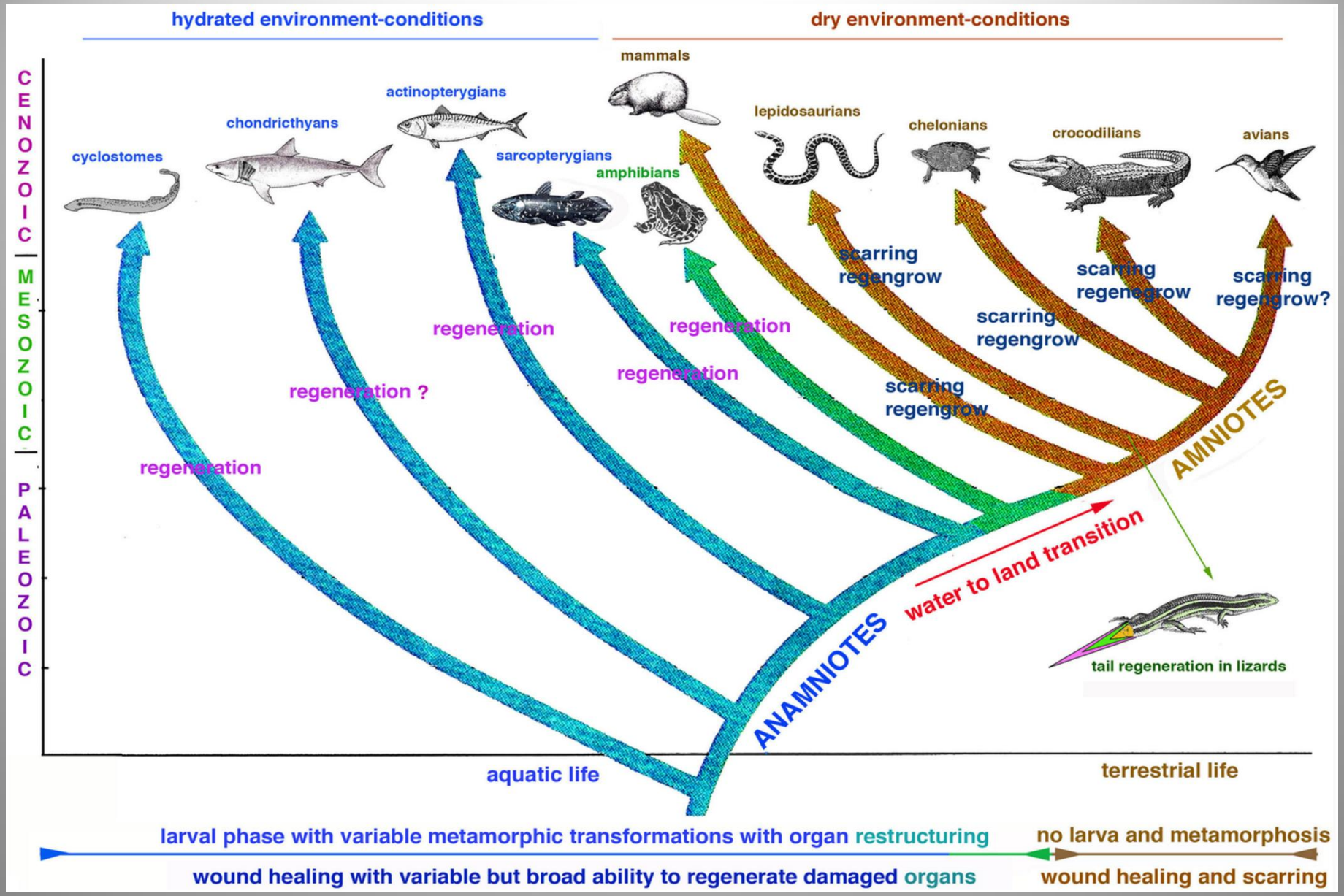
	CLASS	ANAMNIOTE TYPE	ORGAN	PROCESS
INDIRECT		newts	leg, arm, tail digits, jaws spinal cord brain areas eyes, liver heart areas, intestine, gills others	regeneration (blastema or internal cell proliferation)
		tadpoles		
		aquatic salamanders (axolotl)		
		terrestrial salamanders	leg, arm	regengrow heteromorphic regeneration scarring outgrowth
		anurans tadpoles		regeneration
		post-metamorphic frogs		regengrow scarring outgrowth regeneration (heteromorphic)
DEVELOPMENT		lungfish	tail, fins others	regeneration
		teleost larvae	fins, barbels, brain areas spinal cord heart areas eye, scales liver, others	regeneration
		teleosts		
		skate	fin cartilage	regengrow (likely)
		shark (juveniles/adults)	spinal cord fin	regeneration repair
		lamprey (adult and larva)	fin, spinal cord, chord	regeneration

ORGAN REGENERATION IN POST-EMBRYONIC (JUVENILE-ADULT) AMNIOTES

in terrestrial environment (ecthotherms and endotherms)

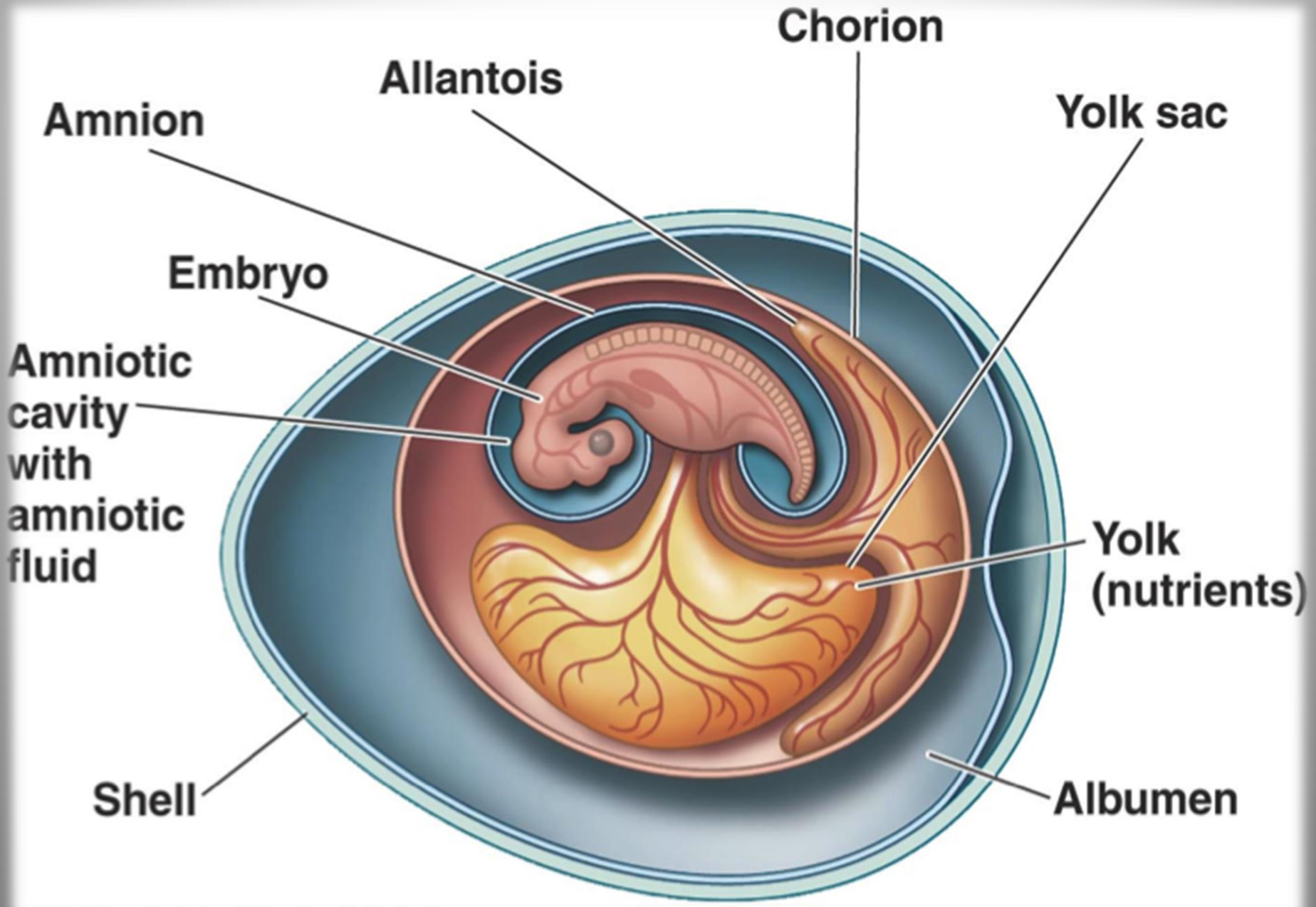
DIRECT DEVELOPMENT

CLASS	AMNIOTE TYPE	ORGAN	PROCESS
	juvenile primates (children)	fingertips	regengrow (nail bed)
	juvenile rodents	fingertips	
	spiny/mutant mice juvenile rabbit	ear holes	regeneration (regengrow) ?
	juvenile bats	wing holes	
		liver	inner proliferation regeneration (osteogenic blastema)
	deers	antlers	
	chicken fowls	liver	inner proliferation
		feathers (pluching)	regeneration
	turtles tortoises	areas of the carapace	regengrow
		tail repair spinal cord liver ?	regeneration (heteromorphic)
	crocodile alligator	maxilla areas tail repair (juveniles) liver ?	regengrow
LEPIDOSAURIANS 	tuatara	tail liver ?	regengrow
	snakes	scales skull bones liver ?	regengrow
	lizards	scales	regeneration
		articular cartilage (knee epiphyses)	regeneration
		areas of maxilla and mandible	regengrow
		lens, optic nerve	regeneration (heteromorphic)
		part of cerebral cortex optic tectum	
		bones (vertebrae and skull)	cartilaginous (osteogenic)
		liver, par of kidney	inner proliferation
		spinal cord,	regeneration (heteromorphic)
		limb(occasionally)	regengrow (scarring outgrowth)
		tail (extensively)	regeneration

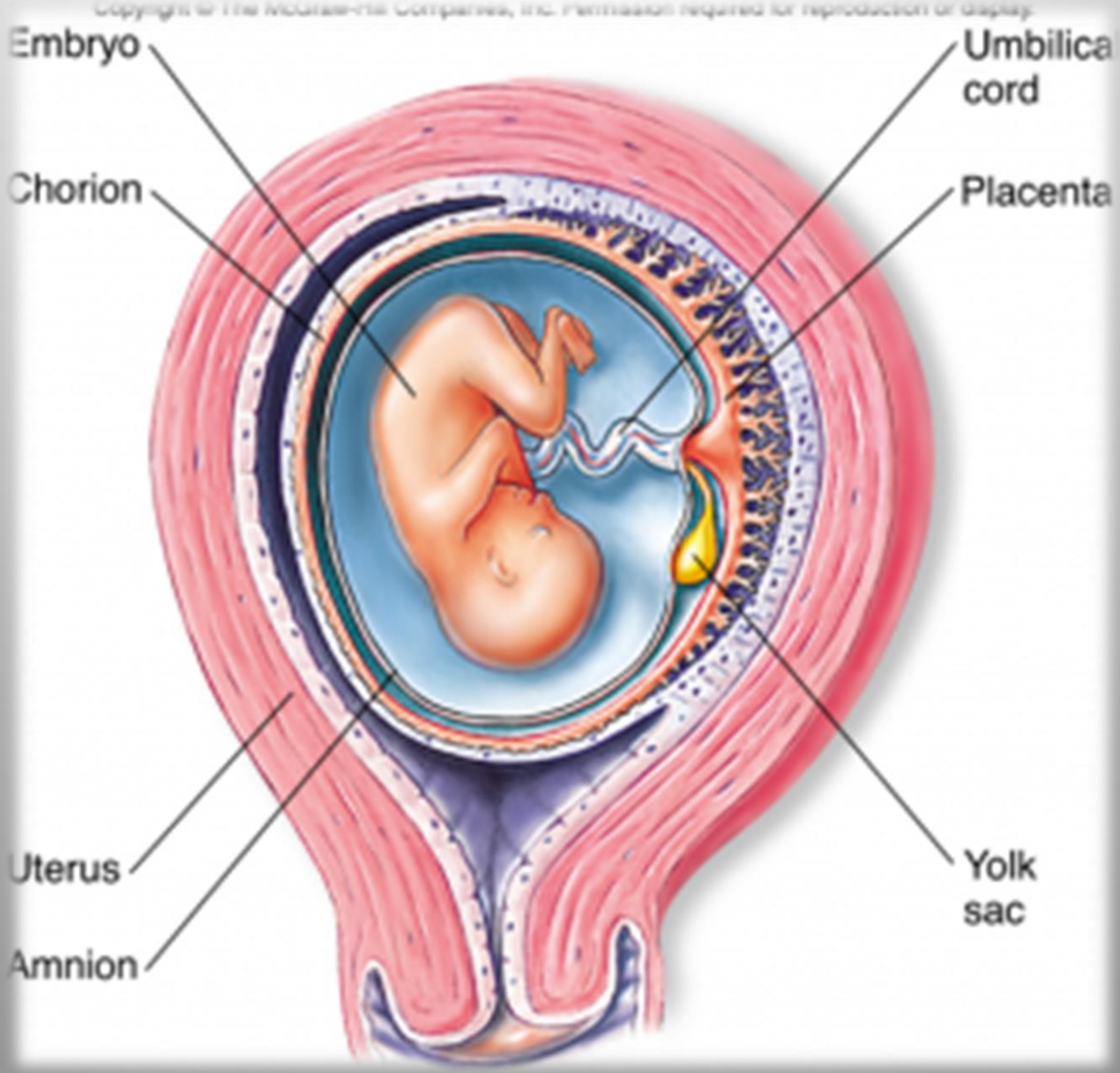


https://anatomypubs.onlinelibrary.wiley.com/doi/epdf/10.1002/dvdy.341

Amniotic egg



Amniotic egg



Placenta:

The **placenta** is **an organ** that develops in maternal uterus during pregnancy. Word “placenta” was termed by **Mossman**.

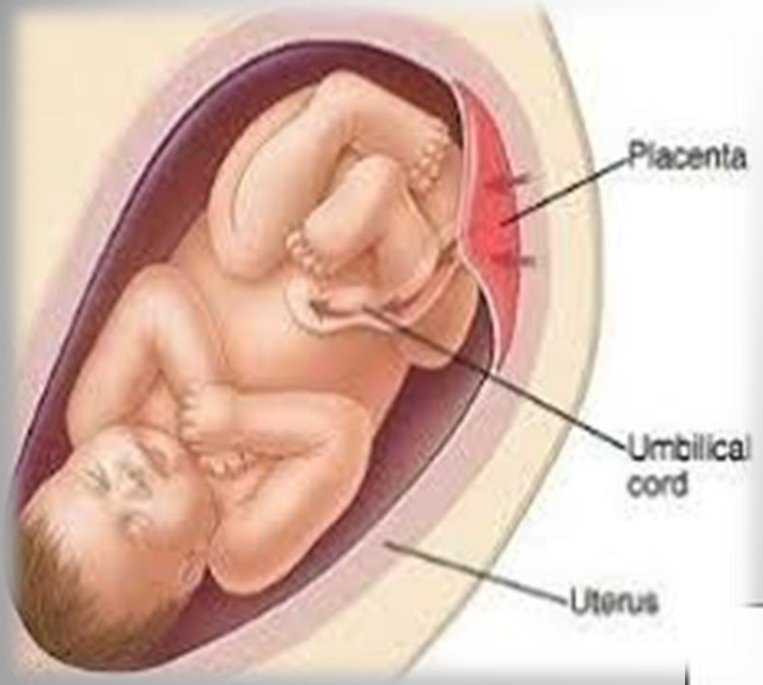
This structure provides **oxygen and nutrients to the foetus** and **removes waste products from foetus’ blood**.

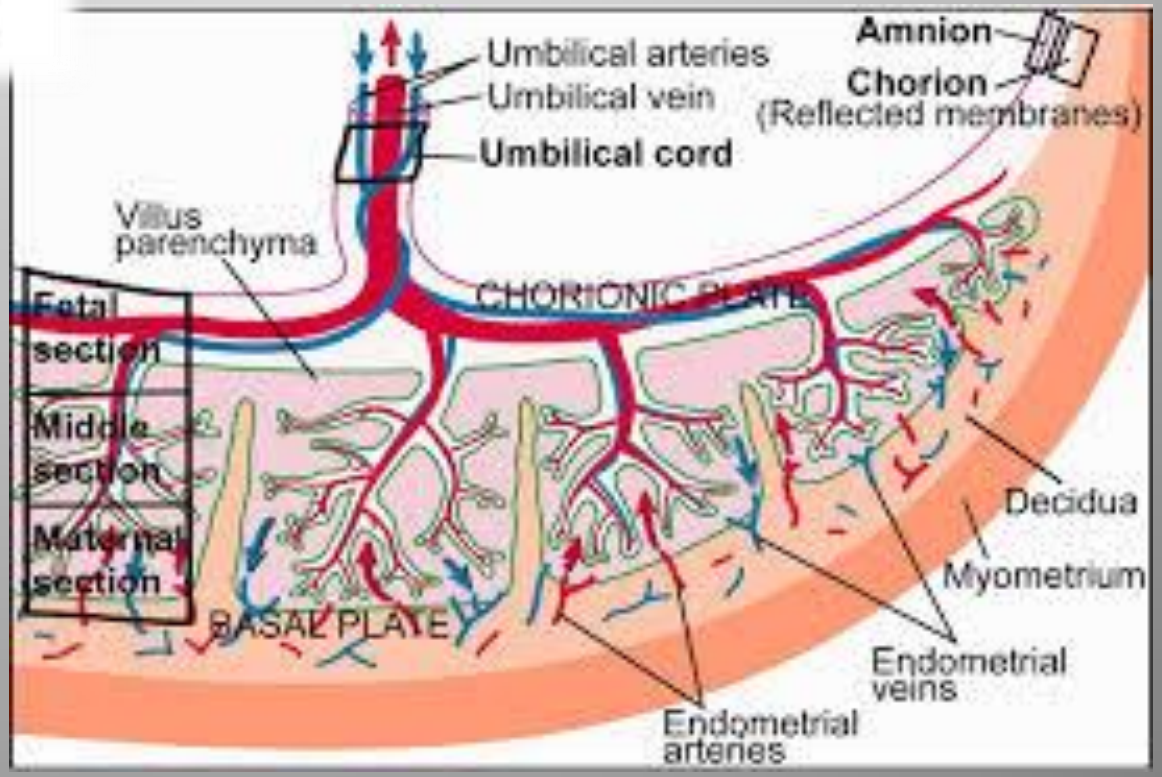
The **placenta** attaches to the wall of your uterus, and foetus's umbilical cord arises from it.

Placentation:

The **formation or arrangement of a placenta** or **placentae** in a maternal uterus.

Structure of Placenta



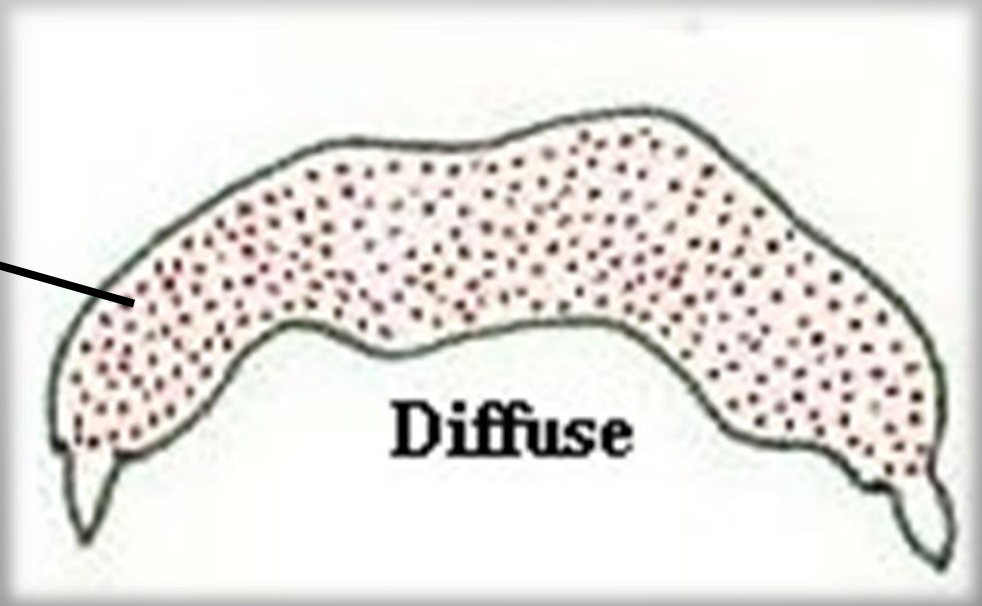


Classification Based on Placental Shape and Contact Points:

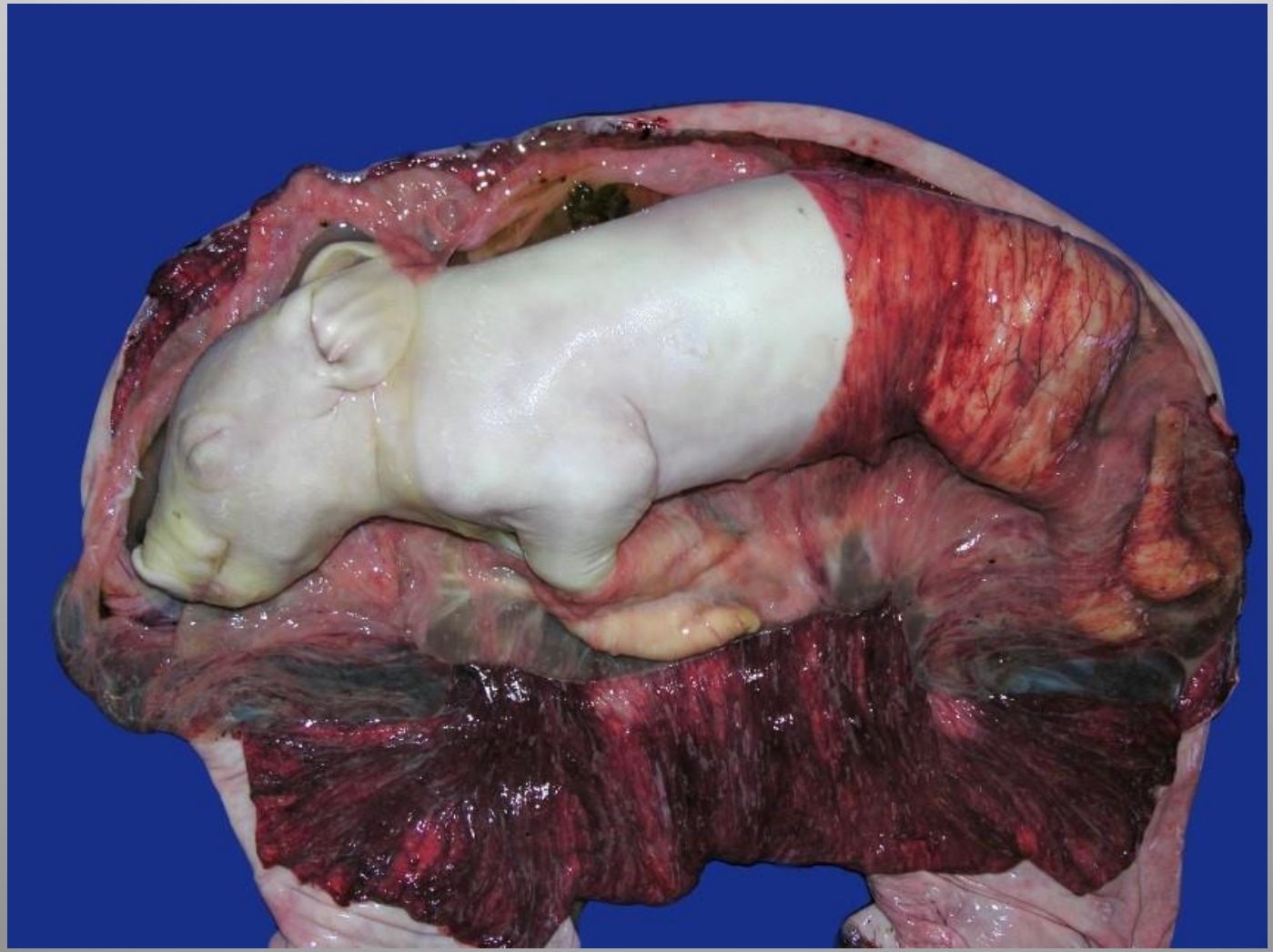
Different species reveals striking differences in their shape and the area of contact between foetal and maternal tissue:

Diffuse: Almost the **entire surface of the allantochorion is involved in formation of the placenta**.

Eg: **horses and pigs**.



Placenta in Pig -Diffuse cotyledon



Cotyledonary:

Multiple, discrete areas of attachment called **cotyledons** are formed by interaction of patches of **allantochorion (chorioallantois.) with endometrium**.

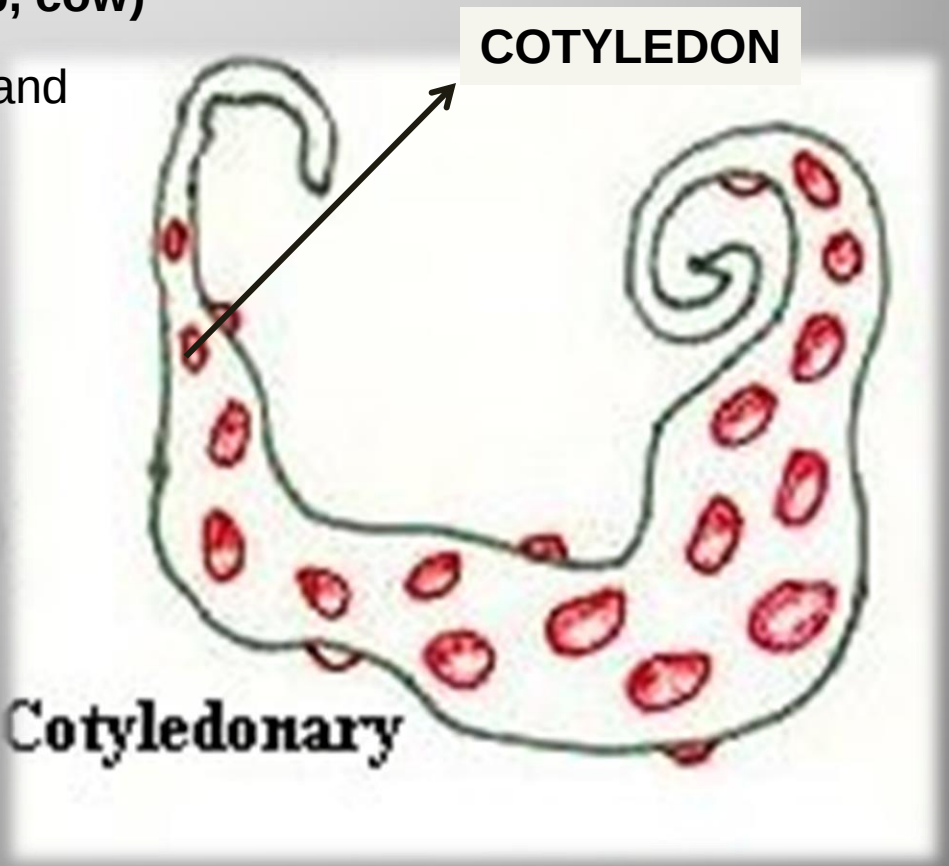
The fetal portions of this type of placenta are called cotyledons, the **maternal contact sites (caruncles**), and the cotyledon-caruncle complex a **placentome**.

Eg: ruminants (Sheep, cow)

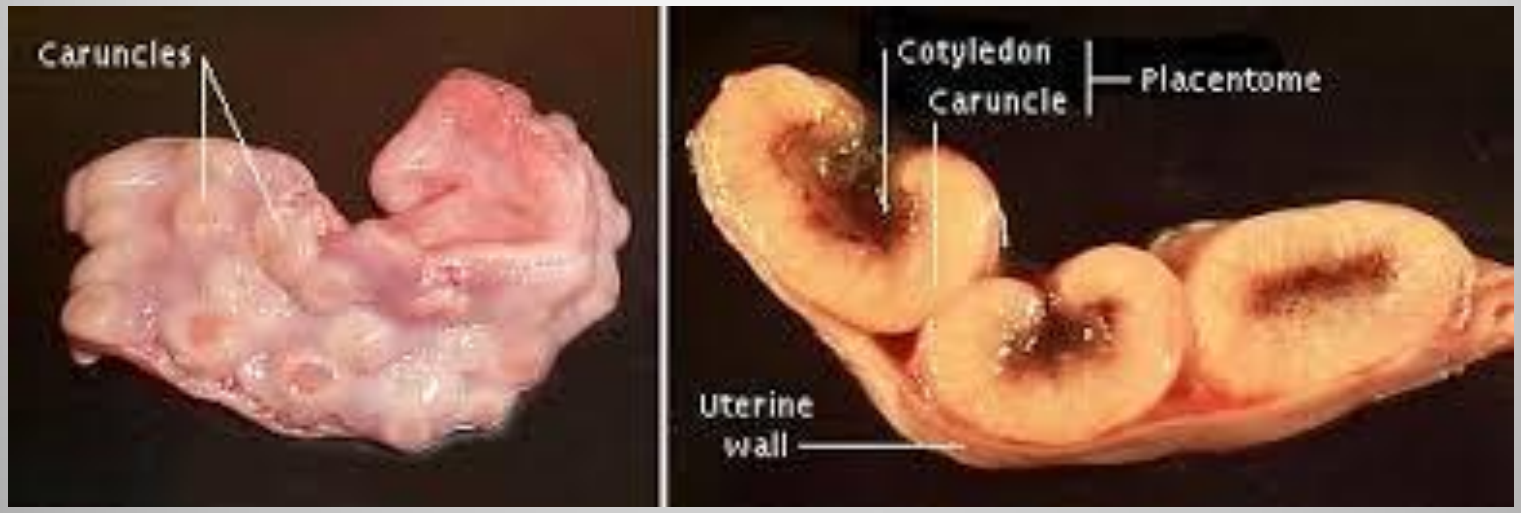
Milky looking membrane covering and present between placentomes is called **chorioallantois**.

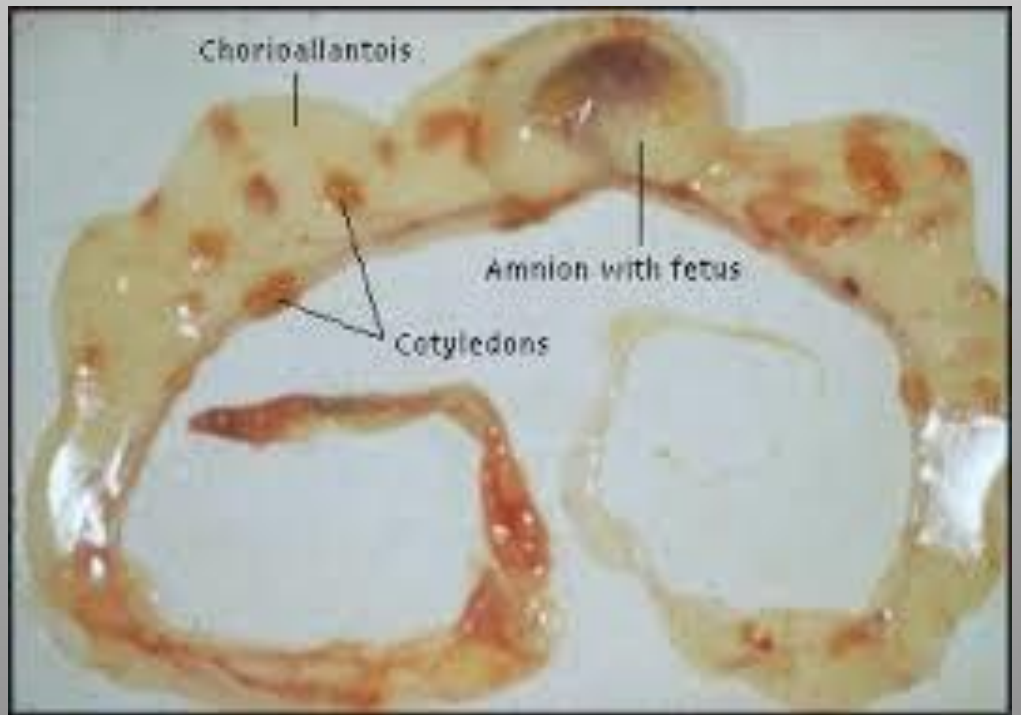
Chorioallantois is a vascular membrane found in eggs of some **amniotes**, such as birds and reptiles.

It is formed by the **fusion of chorion and allantois.**



Palcenta in Sheep – Cotyledonary placenta

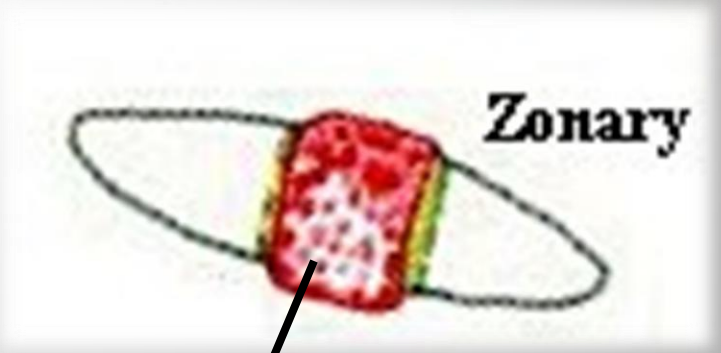


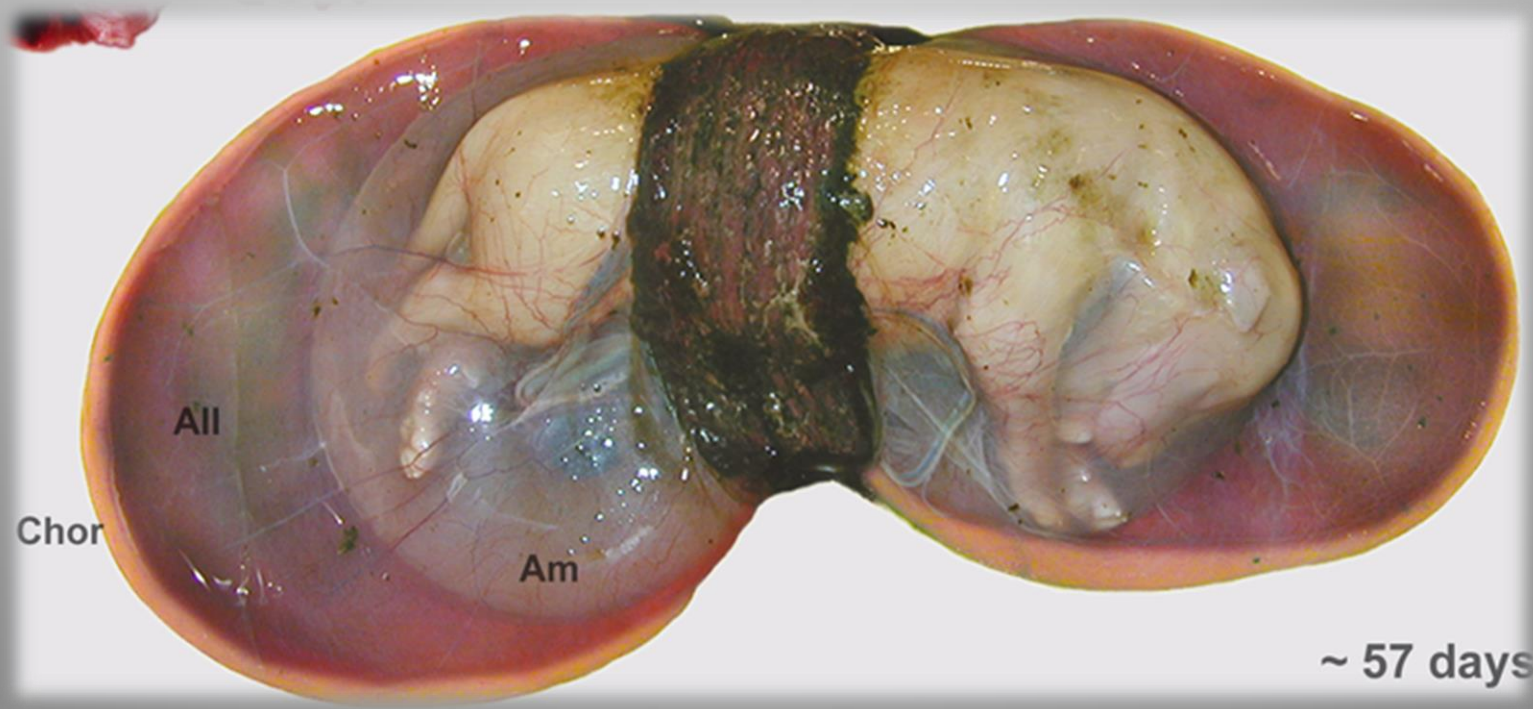


Zonary:

The placenta takes the form of a **complete or incomplete band of tissue (villi) surrounding the fetus**.

Eg: dogs and cats, seals, bears, and elephants.

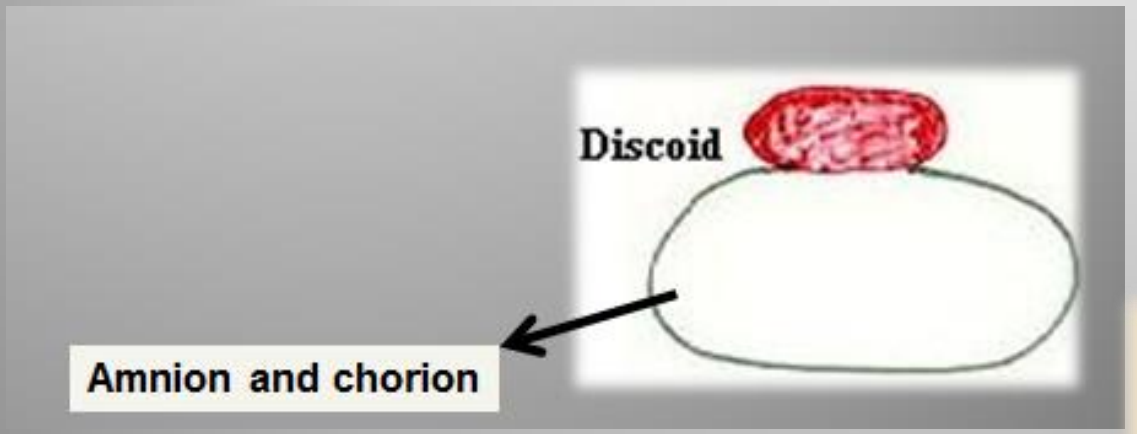


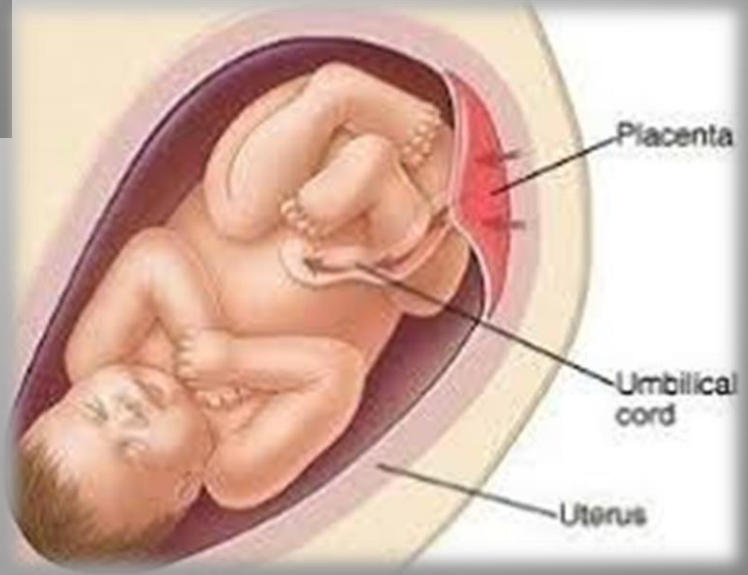


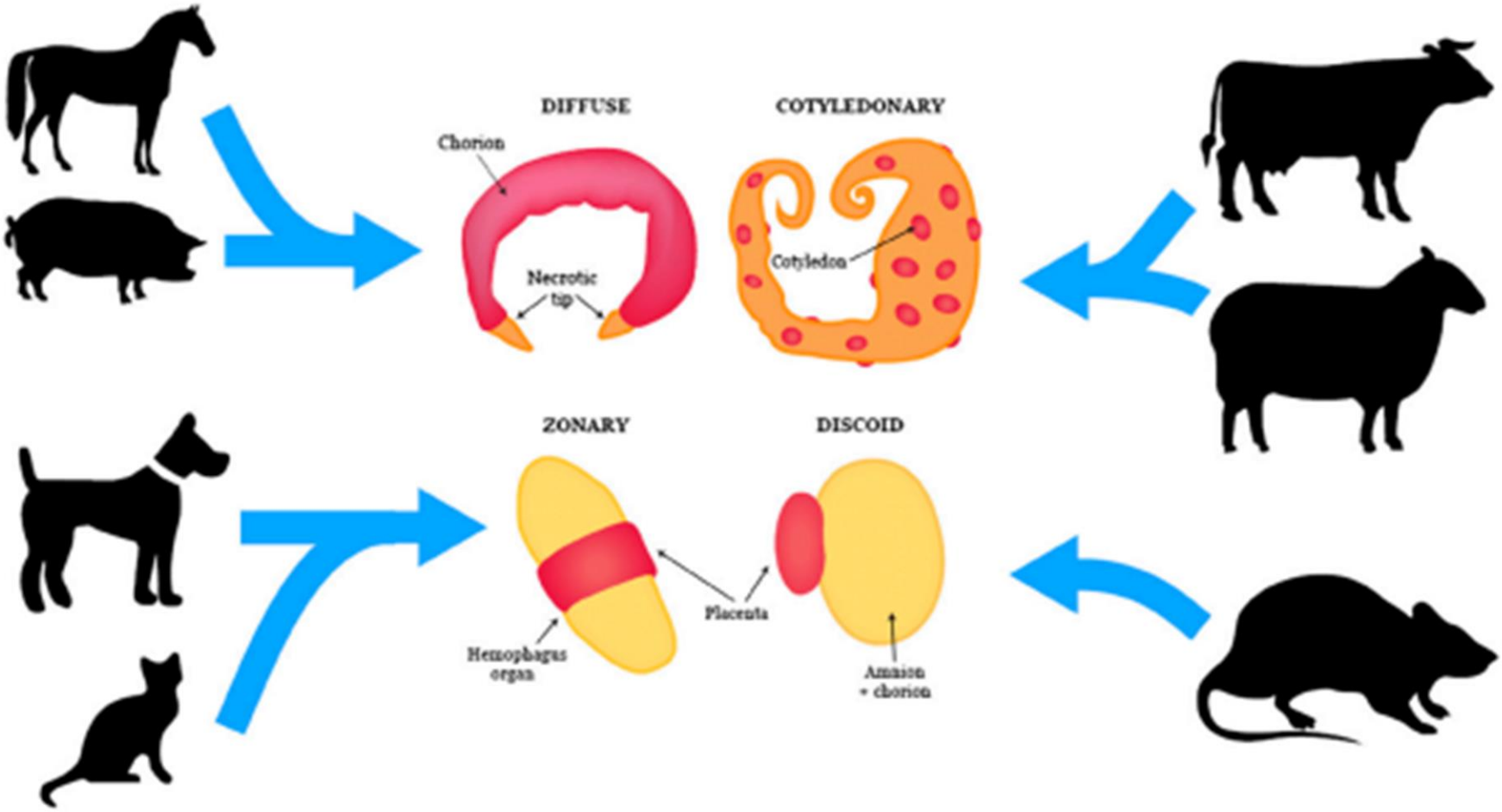
Discoid:

A **single placenta** is formed and is **discoid in shape** and it has **disc-shaped villous area called monodiscoidal placenta**.

Eg: Giraffee and other primates, rodents.

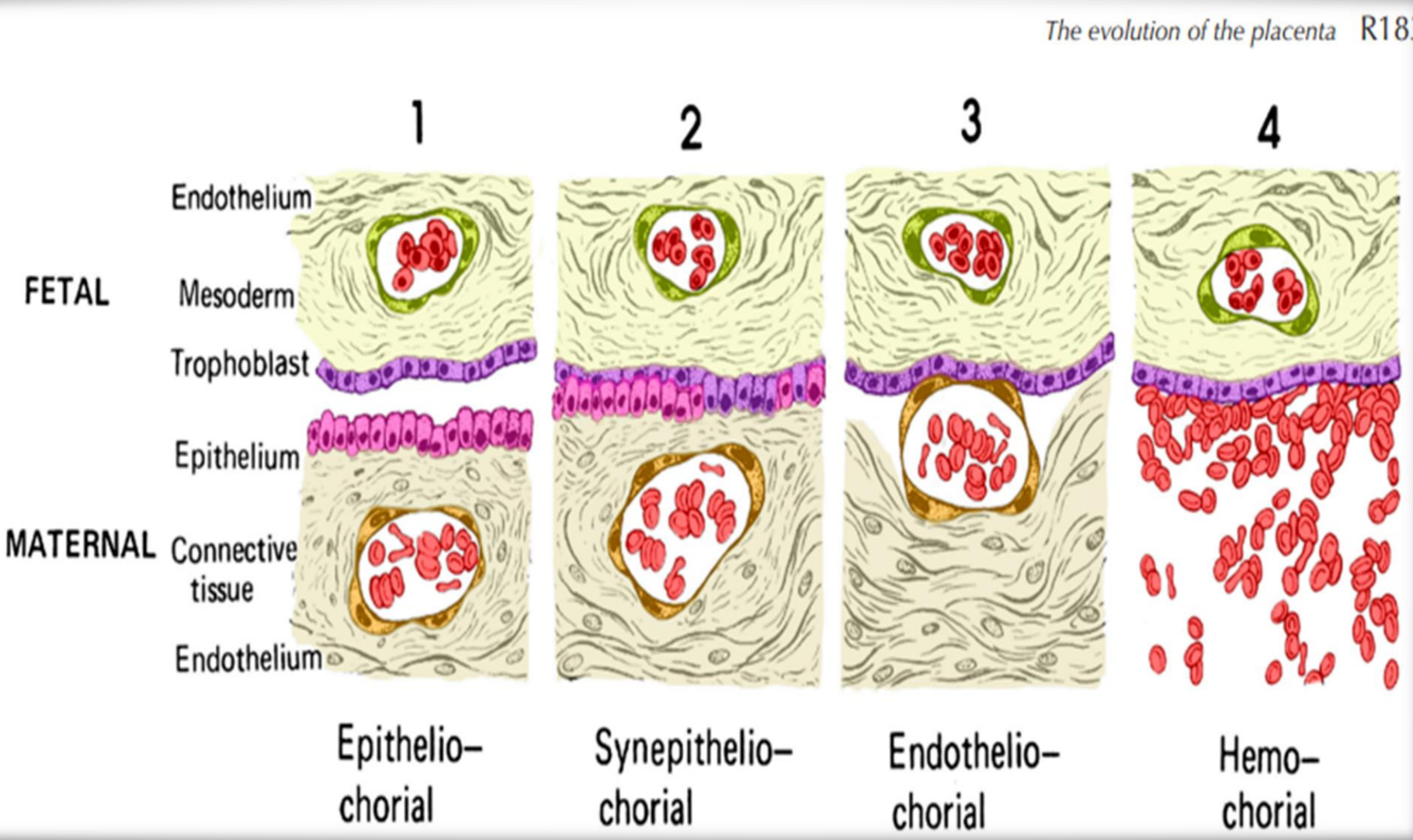






Reproduction (2016) **152** R179–R189

Classification according to the numbers and kinds of cell layers that separate the bloodstreams of the mother and conceptus



Diffuse: **Cotyledonary**: **Zonary** **Discoid**:

REPRODUCTION

REVIEW

The evolution of the placenta

R Michael Roberts[1,2], Jonathan A Green[2] and Laura C Schulz[3]

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Abstract

The very apt definition of a placenta is coined by Mossman, namely apposition or fusion of the fetal membranes to the uterine mucosa for physiological exchange. As such, it is a specialized organ whose purpose is to provide continuing support to the developing young. By this definition, placentas have evolved within every vertebrate class other than birds. They have evolved on multiple occasions, often within quite narrow taxonomic groups. As the placenta and the maternal system associate more intimately, such that the conceptus relies extensively on maternal support, the relationship leads to increased conflict that drives adaptive changes on both sides. The story of vertebrate placentation, therefore, is one of convergent evolution at both the macromolecular and molecular levels. In this short review, we first describe the emergence of placental-like structures in nonmammalian vertebrates and then transition to mammals themselves. We close the review by discussing the mechanisms that might have favored diversity and hence evolution of the morphology and physiology of the placentas of eutherian mammals.

Reproduction (2016) **152** R179–R189

J Toxicol Pathol 2014; 27: 11–18

Concise Review

A Comparison of the Histological Structure of the Placenta in Experimental Animals

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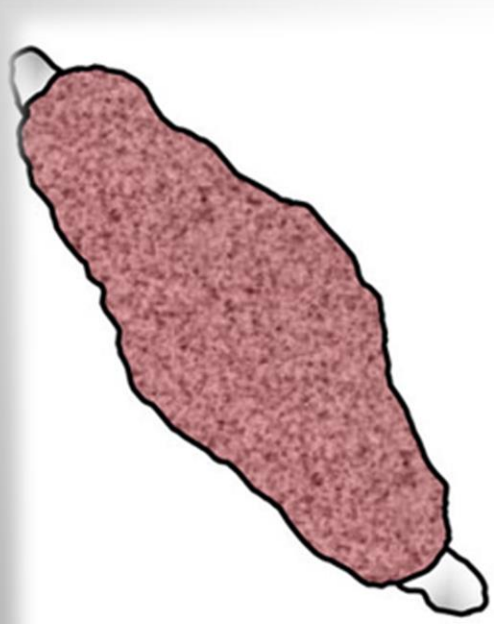
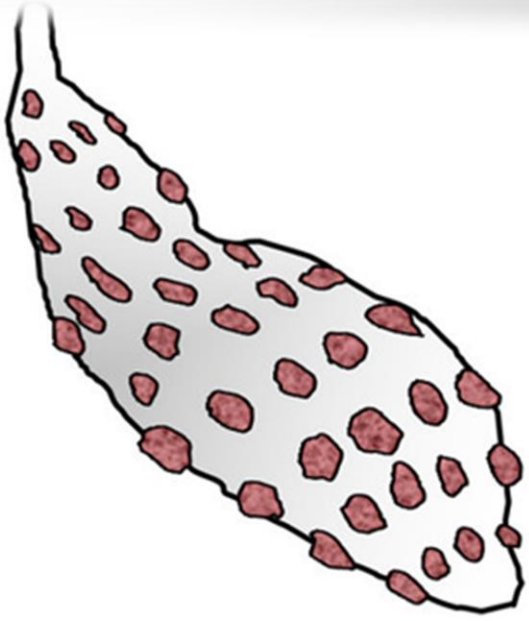
[2] Courses of Veterinary Laboratory Medicine, School of Veterinary Medicine, Faculty of Agriculture, Tottori University, 4-101 Koyama-cho Minami, Tottori 680-8553, Japan

Abstract: The primary function of the placenta is to act as an interface between the dam and fetus. The anatomic structure of the chorioallantoic placenta in eutherian mammals varies between different animal species. The placental types in eutherian mammals are classified from various standpoints based on the gross shape, the histological structure of the materno-fetal interface, the type of materno-fetal interdigitation, etc. Particularly, the histological structure is generally considered one of the most useful and instructive classifications for functionally describing placental type. In this system, three main types are recognized according to the cell layers comprising the interhemal area: (1) epitheliochorial type (horses, pigs and ruminants), (2) endotheliochorial type (carnivores) and (3) hemochorial type (primates, rodents and rabbits). The number of cell layers in the interhemal area is considered to modify the transfer of nutrients between maternal and fetal blood and is one of the important factors with respect to the difference in placental permeability between animal species. Therefore, in reproductive and developmental toxicity studies, careful attention should be paid to the histological structure of the interhemal area when extrapolating information concerning placental transfer characteristics to different animal species. (DOI: 10.1293/tox.27.2013-0060; J Toxicol Pathol 2014; 27: 11–18)

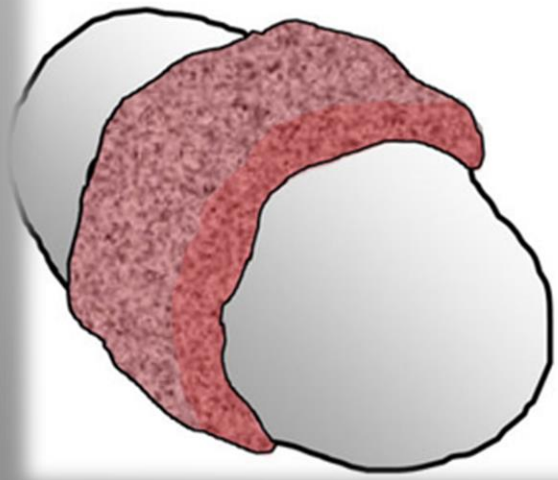
Key words: cynomolgus monkey, dog, minipig, placenta, rabbit, rat

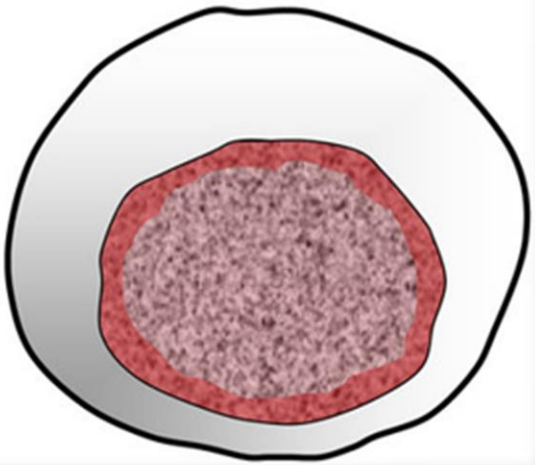
Find?



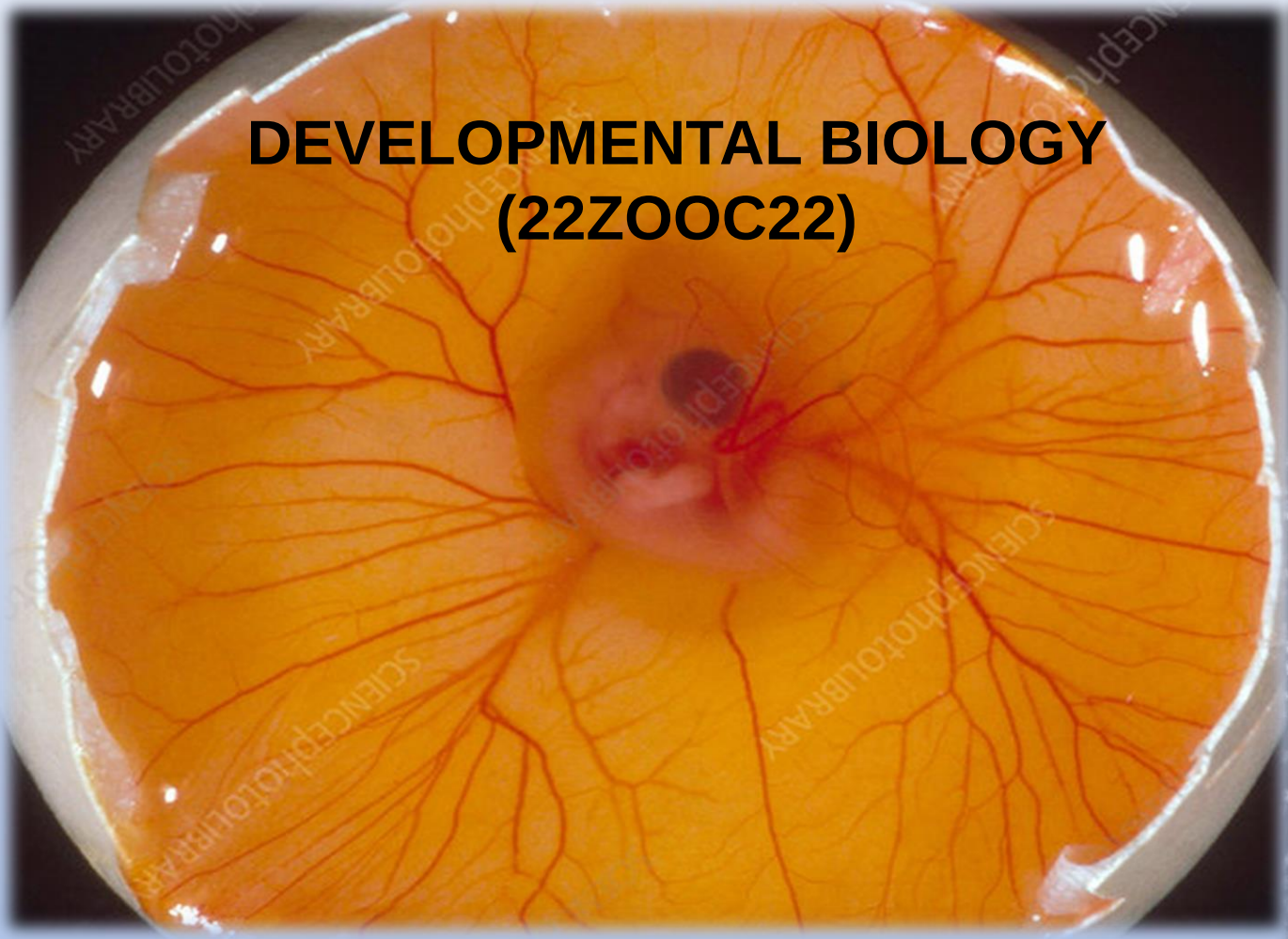


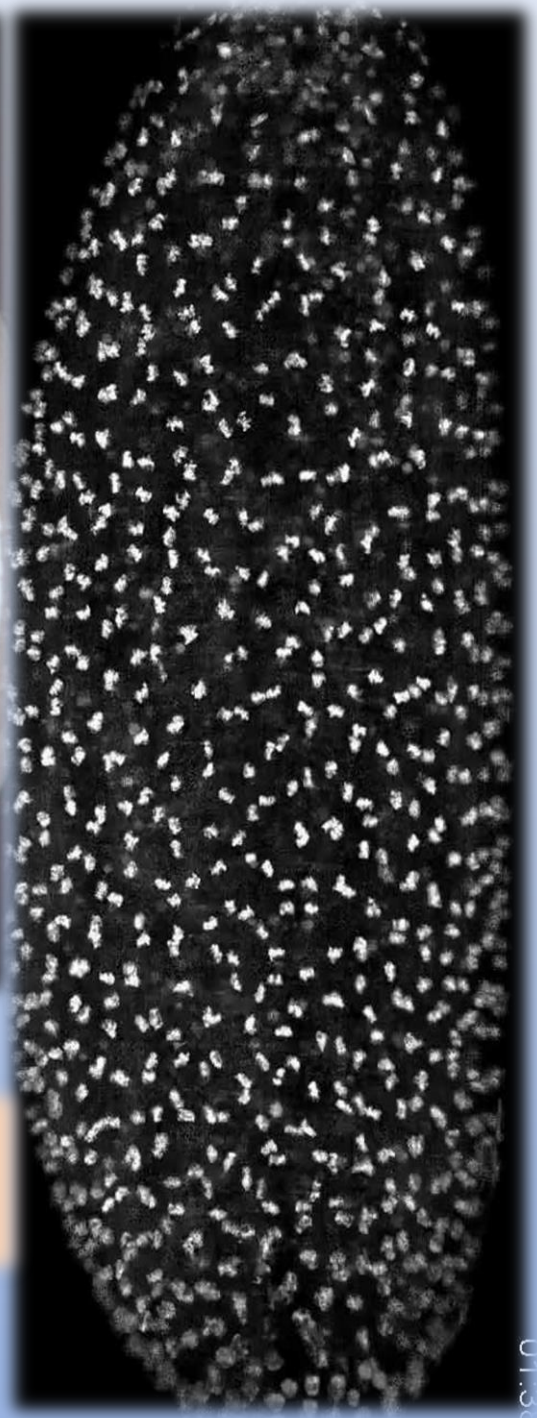
Classification by placental gross shape





DEVELOPMENTAL BIOLOGY
(22ZOOC22)





AXES AND PATTERN FORMATION
IN
CHICK EMBRYO

Introduction:

The body plan of organisms can be defined by three major axes patterned during embryogenesis:

- Anteroposterior (A-P)
- Dorsoventral (D-V)
- Left-right (L-R) axes

- Axis formation is regulated by signaling molecules such as members of the BMP, Wnt, transforming growth factor-beta (TGF-beta) and fibroblast growth factor (FGF) families.

- A biological reaction-diffusion system are thought to maintain morphogen gradients across cell fields.

- Feedback mechanisms, local activation and long-range inhibition, allow cells near the growth factor source to receive high levels of signal.

- While a consistent gradient ensures that distant cells experience little to no signaling.

1. FGF Family - cell proliferation, growth and differentiation.
   - Regulate diverse physiologic functions: Angiogenesis, cell growth, pattern formation, embryonic development, metabolic regulation, cell migration, neurotrophic effects, and tissue repair.
   - Its activities are mediated by **receptor tyrosine kinases** and are facilitated by heparan sulfate.
   - Family members have been implicated in several disorders of bone growth, as well as in tumor formation and progression.

2. Hedgehog Family - The *Hedgehog* gene was initially identified in *Drosophila* (morphogen) - segment polarity.

In vertebrates, three members:

- Desert hedgehog (Dhh),
- Indian hedgehog (Ihh), and
- Sonic hedgehog (Shh).

Hedgehog signaling - two proteins:

i. Patched (Ptc) - twelve-pass transmembrane protein that binds to the Hedgehog ligand, and
ii. Smoothened (Smo) - seven-pass transmembrane protein that transmits a downstream signal.

Shh signaling in vertebrates is involved in diverse areas of development, including neurogenesis, hematopoiesis, bone formation, and gonad development.

3. Notch Family - Organisms have four Notch receptors (Notch 1-4).

- Activation of Notch receptors requires direct cell-cell interactions between the extracellular portion of the receptor and transmembrane ligands.
- Notch signaling is highly conserved in multicellular organisms.
- Specifies cell fates, regulating pattern formation, and defining boundaries between different cell types during early development.
- Required for multiple developmental processes - vasculogenesis, angiogenesis, hematopoiesis, somatogenesis, myogenesis, and neurogenesis and has been implicated in cancer biology.

4. TGF-beta Superfamily – This superfamily consists of

- TGF-beta proteins,
- Bone Morphogenetic Proteins (BMPs),
- Growth Differentiation Factors (GDFs),
- Glial-derived Neurotrophic Factors (GDNFs),
- Activins,
- Inhibins,
- Nodal,
- Lefty and Mülllerian Inhibiting Substance (MIS).
- Ligands of the TGF-beta superfamily initiates a Smad-dependent signaling cascade that induces or represses transcriptional activity.
- TGF-beta family are required for D-V patterning, mesoderm induction & patterning, limb bud formation, bone and cartilage formation, neuron differentiation, and the development of a variety of tissues and organs.

5. Wnt Family –

- *Wingless*, the *Drosophila melanogaster* (segment-polarity gene), and *Integrase-1*, the vertebrate homologue.
- A highly conserved signal transduction cascade that has a central role in embryonic development, tissue regeneration, and a host of other biological processes.

There are three established Wnt signaling pathways:

1) Canonical pathway, involving beta-Catenin,
2) Planar cell polarity (PCP) pathway,
3) Wnt-Ca^{2+} pathway.

- Target cell populations respond to secreted Wnt morphogens in a concentration dependent manner → gene expression and cellular differentiation.
- Centralize cell proliferation, survival, and differentiation.
- Deregulation of the Wnt pathway results in cancer and other disease conditions.
- Recent studies have shown that Wnt molecules also play a role in the immune system → regulate T cell development and activation, and dendritic cell maturation.

6. Other Morphogens –

- TBX family of transcription factors (tf) - heart and limb development,
- Twist basic helix-loop-helix tf - skeletal development,
- Protogenin and LRRTM proteins – CNS
- Epimorphin - a protein associated with branching morphogenesis.

AXIS AND PATTERN FORMATION – CHICK EMBRYO

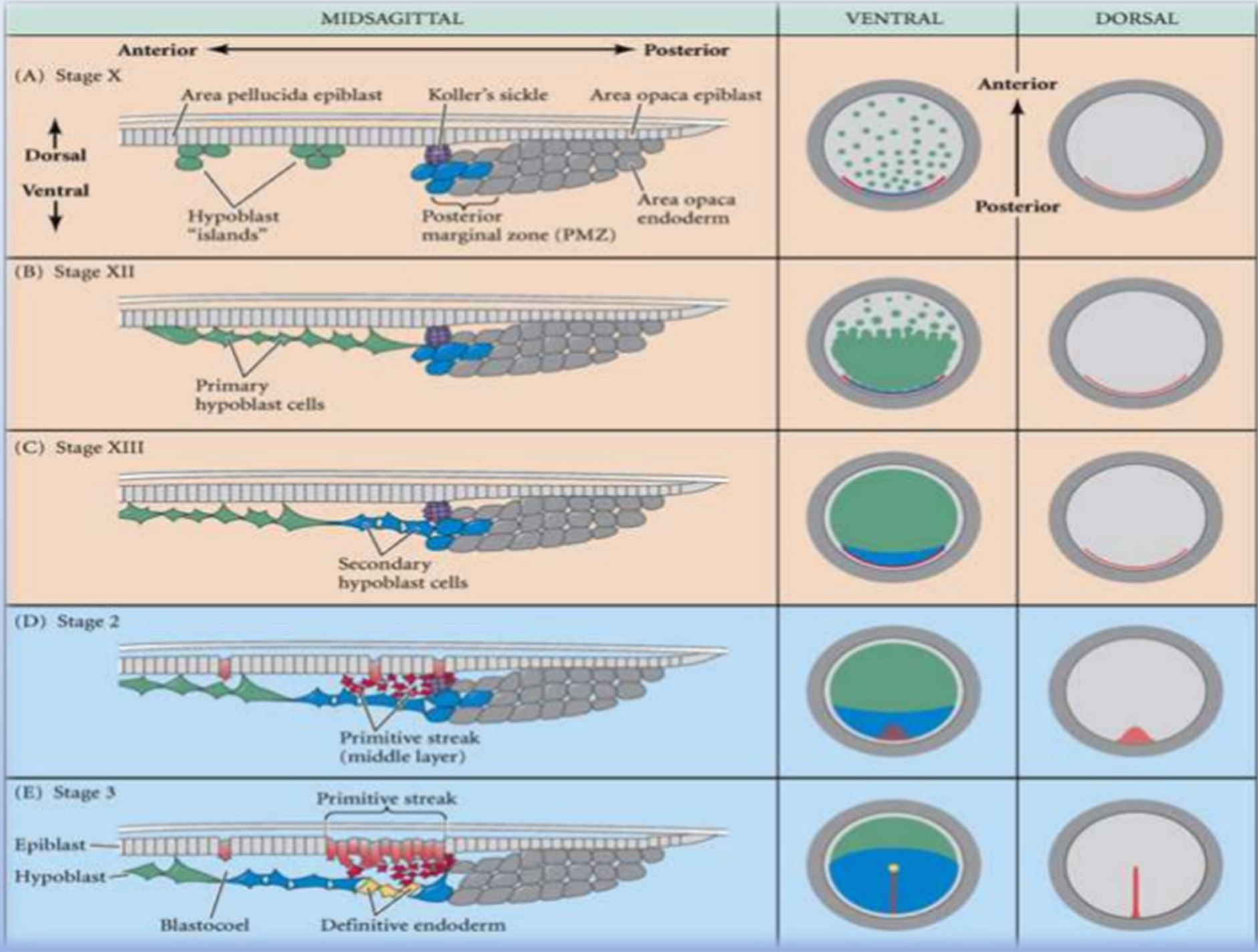
- Development involves cell division, body axis formation, tissue and organ development, and cell differentiation (gaining a final cell type identity).
- Amniotic Eggs is found in birds , reptiles and mammals.
- Meroblastic cleavage - Blastodisc seen.
- First cleavage furrow appears in the blastodisc.
- Other cleavage form the single layered blastoderm.
- Equatorial and vertical cleavages: five- six layered blastoderm.

- After 16 hrs of incubation, the streak acquires a definite shape and this stage is known as **primitive streak stage**.
- A groove appears within the streak **cephalo-caudally** known as the **primitive groove**.
- At the anterior end the streak cells condense and the region becomes very thick and this region is known as the **Hensen's node** or **primitive knot**.
- It acts a **organizer for gastrulation** and starts regressing when formation of head starts.

- Migration of cells from primitive streak: after the formation of primitive streak the epiblast cells migrate through it into the blastocoel.

- In this way the continuous flow of migratory cells through the node down into the blastocoel and migrate anteriorly forming the endoderm, the notochord and cephalic mesoderm.

- After cell migration the primitive streak is almost disappeared and a portion of it is seen in tail bud and partly with the cloaca.


MIDSAGITTAL
VENTRAL
DORSAL
Anterior
Posterior
(A) Stage X
Area pellucida epiblast
Koller's sickle
Area opaca epiblast
Dorsal
Ventral
Hypoblast "islands"
Posterior marginal zone (PMZ)
Area opaca endoderm
Anterior
Posterior
(B) Stage XII
Primary hypoblast cells
(C) Stage XIII
Secondary hypoblast cells
(D) Stage 2
Primitive streak (middle layer)
(E) Stage 3
Primitive streak
Epiblast
Hypoblast
Blastocoel
Definitive endoderm

Axis formation in Chick embryo

The role of pH in forming the dorsal-ventral axis:

- Dorsal Ventral axis is critical to the formation of the hypoblast and to the further development.

- This axis is established when the cleaving cells of the blastoderm establish a barrier between the basic **(pH9.5)** albumin above the blastodisc and acidic **(pH-6.5)** subgerminal space below it.

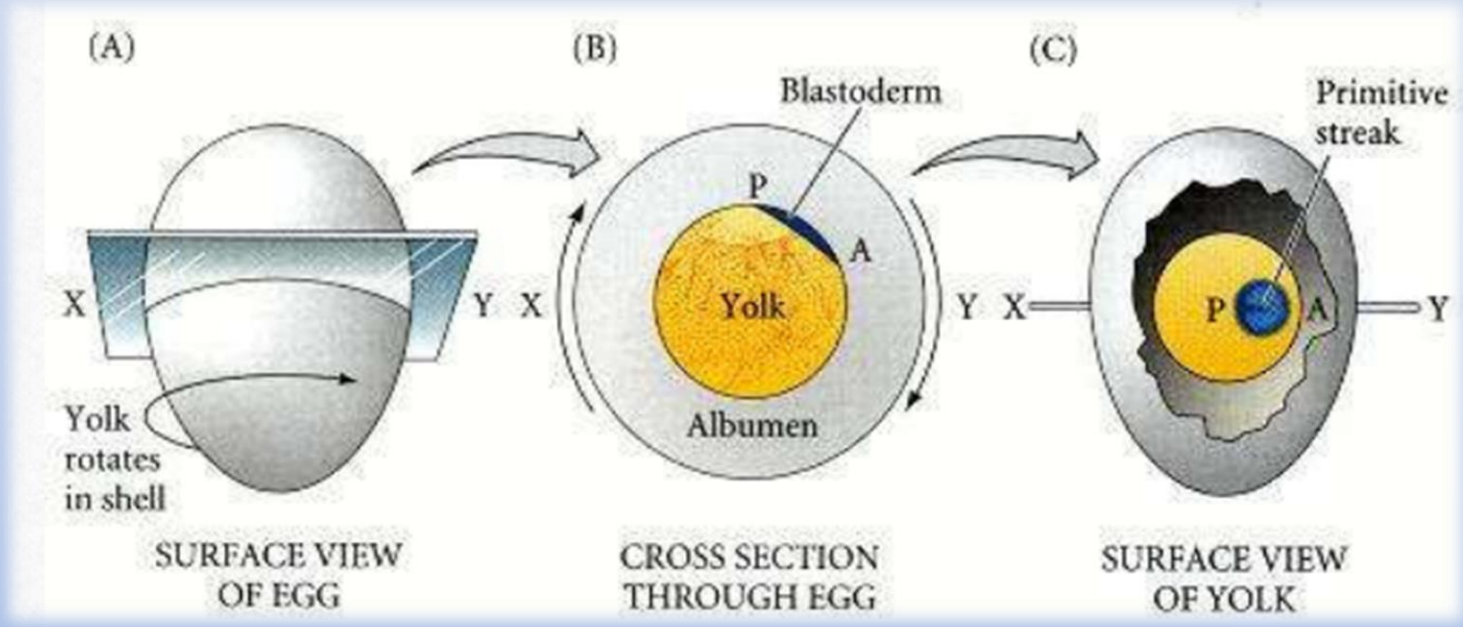
- A potential difference of **25mV** across the epiblast cell layer due to the transportation of water and sodium ions from the albumin to the subgerminal cavity.

This distinguishes the **two sides of epiblast:**

1. The **dorsal side** (side facing the negative and basic albumin)
2. The **ventral side** (side facing the positive and the acidic subgerminal space fluid).

The role of gravity in forming the anterior posterior axis:

The conversion of the radially symmetrical blastoderm into a bilaterally symmetrical structure is determination by gravity.



Left-right axis formation

The distinction between left and right sides is regulated by two proteins:

i. the paracrine factor **Nodal** and
ii. the transcription factor **Pitx2**.

Right side:

- The transcription of sonic hedgehog (Shh) gene ceases due to the expression activin on the right side of the embryo.
- This in turn activates the expression of fgf8 which in turn prevents the transcription of the caronte gene.
- In the **absence of caronte**, bone morphogenetic proteins (BMP's) which block the expression of the nodal and lefty-2.
- This activates the snail gene (cSNR) that is characteristics of the **right side of the avain embryonic organs.**

Left side:

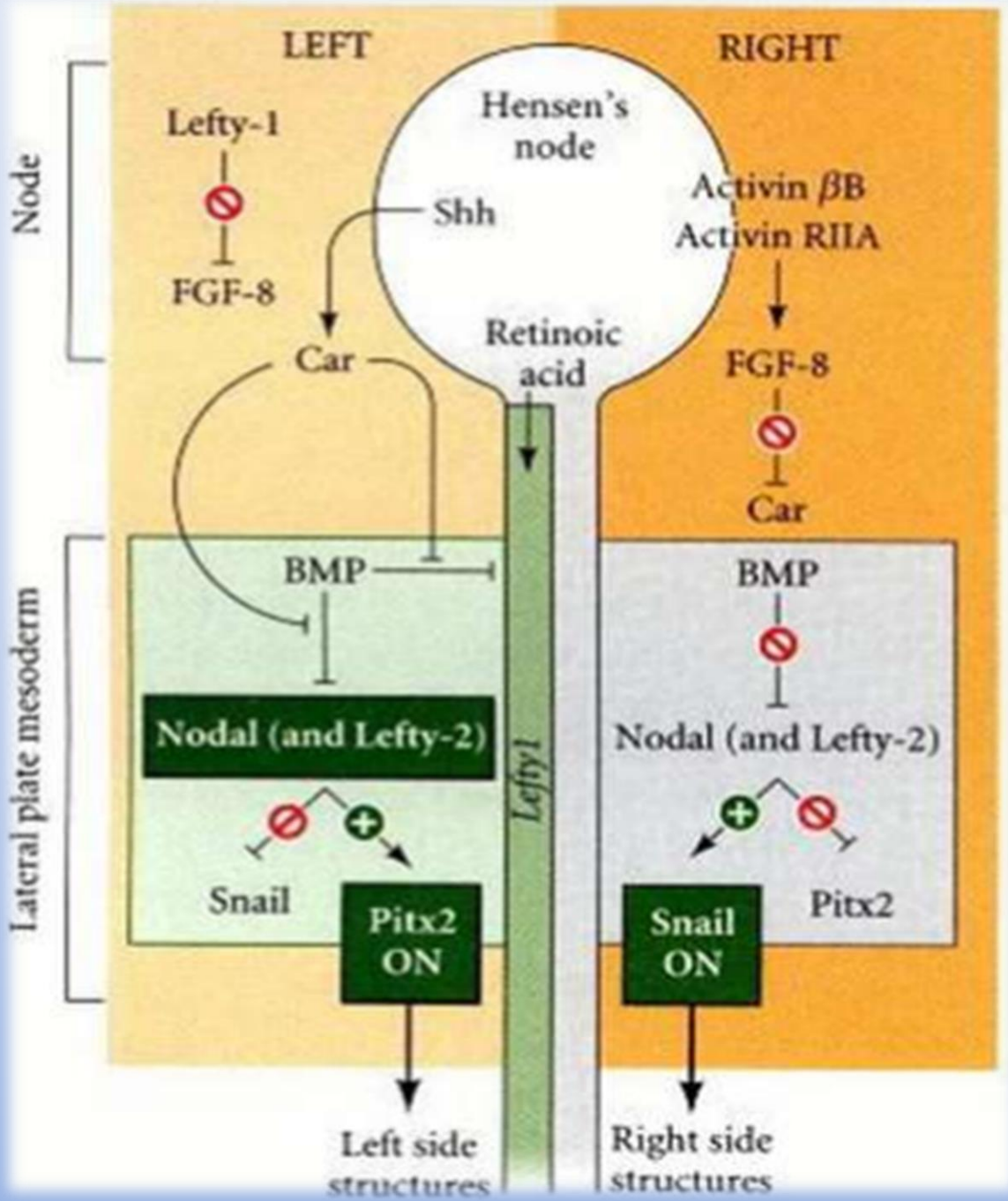
- The **lefty-I protein** blocks the expression of **fgf8** while **Shh activates caronte**.

- Caronte is a **paracrine factor** that prevents BMPs from expressing the nodal and lefty-2 genes.

- It also inhibits BMPs from blocking the expressin of lefty-1 on the ventral midline structures.

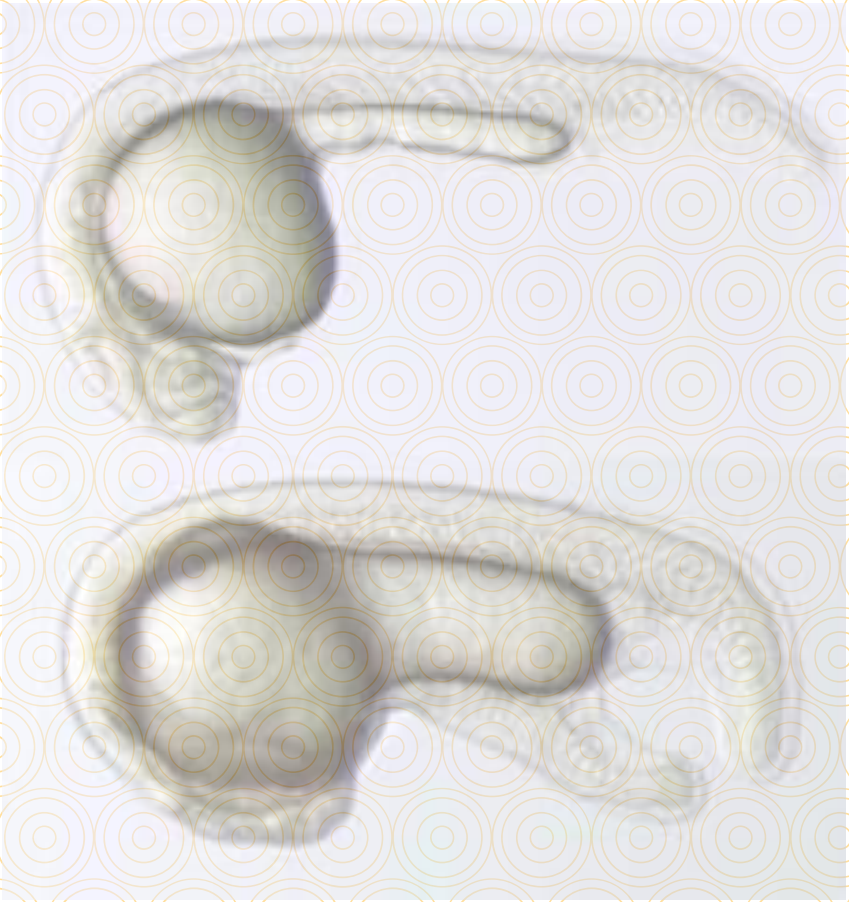
- Nodal and Lefty-2 activate Pitx2 and repress snail (cSNR) Pitx2 is crucial in directing the asymmetry of embryonic structures.

- The left side structures starts forming.


LEFT
RIGHT
Node
Lateral plate mesoderm
Hensen's node
Lefty-1
FGF-8
Shh
Activin βB
Activin RIIA
Car
Retinoic acid
FGF-8
Car
BMP
BMP
Nodal (and Lefty-2)
Nodal (and Lefty-2)
Lefty1
Snail
Pitx2 ON
Pitx2
Snail ON
Left side structures
Right side structures

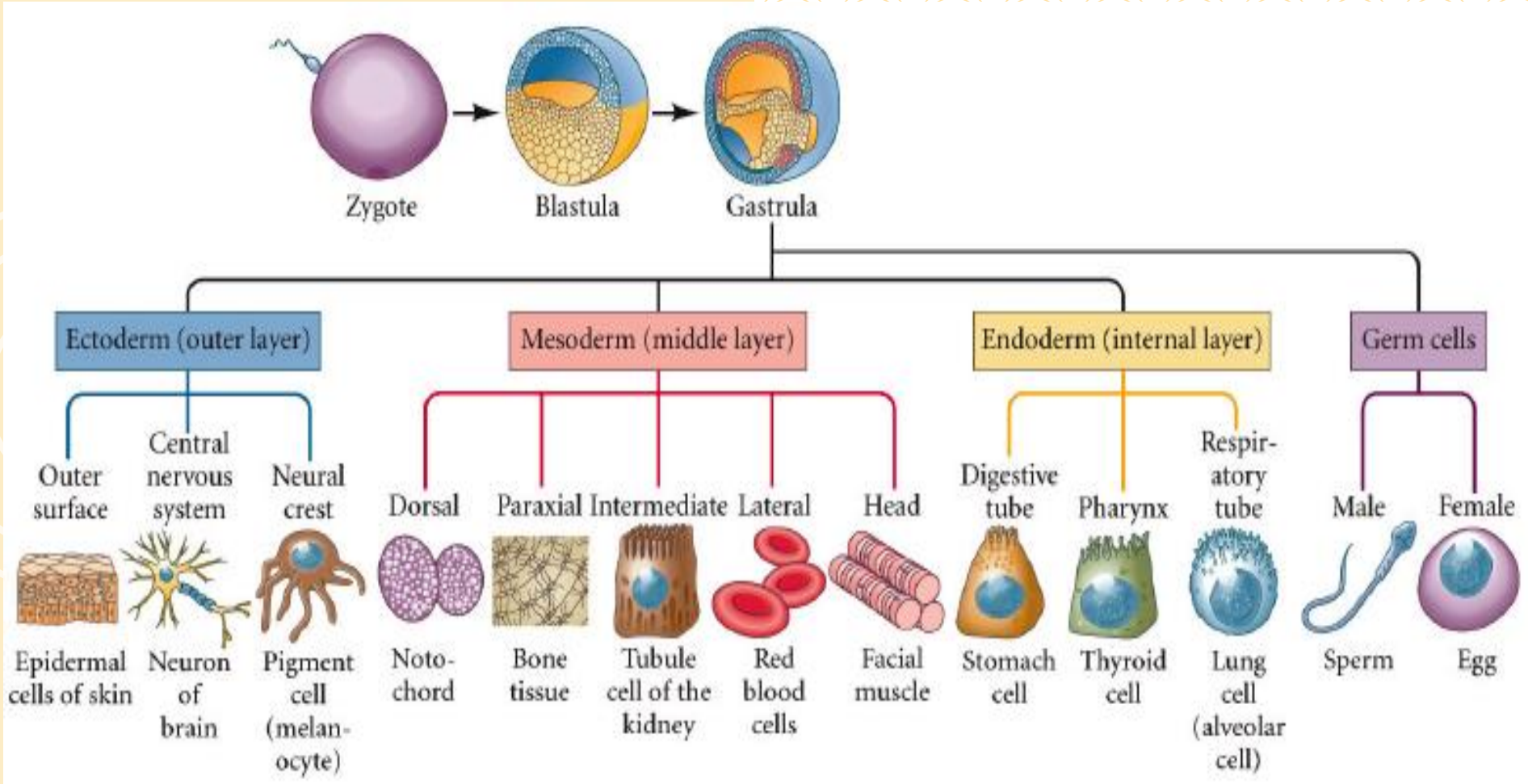
DEVELOPMENTAL BIOLOGY (22ZOOC22)

ORGANOGENESIS



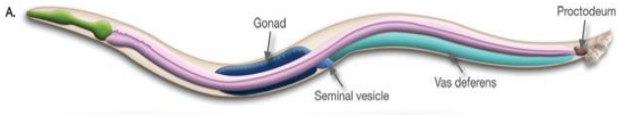
Introduction

- The early stages of embryonic development begin with fertilization, a tightly controlled process and the zygote undergoes cleavage to form the blastula.
- The blastula undergoes a process called gastrulation.
- Gastrulation process of animal embryogenesis produces cellular rearrangements giving rise to three primary germ layers called ectoderm, mesoderm and endoderm. The ectoderm, the outermost layer is the first layer to appear.
- The lower phyla like Porifera , Ctenophora ,Cnidaria contain two primary layers that give rise to all the tissues and organs of the animals making them diploblastic.

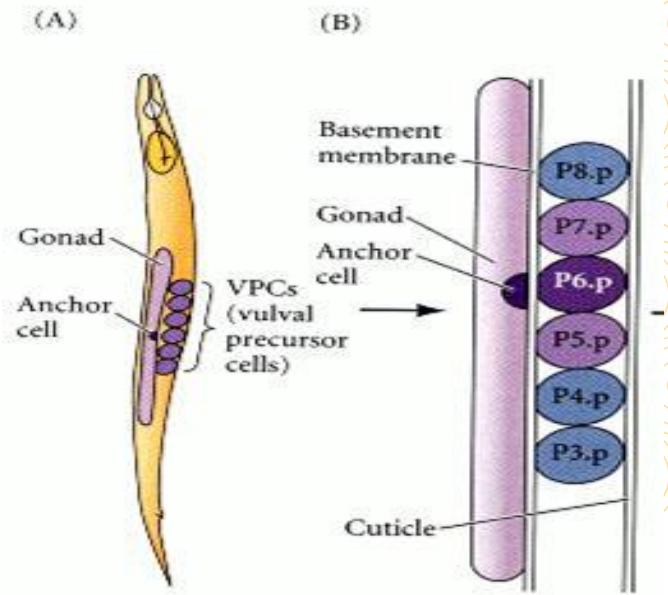

Zygote
Blastula
Gastrula
Ectoderm (outer layer)
Mesoderm (middle layer)
Endoderm (internal layer)
Germ cells
Outer surface
Central nervous system
Neural crest
Dorsal
Paraxial
Intermediate
Lateral
Head
Digestive tube
Pharynx
Respir-atory tube
Male
Female
Epidermal cells of skin
Neuron of brain
Pigment cell (melan-ocyte)
Noto-chord
Bone tissue
Tubule cell of the kidney
Red blood cells
Facial muscle
Stomach cell
Thyroid cell
Lung cell (alveolar cell)
Sperm
Egg

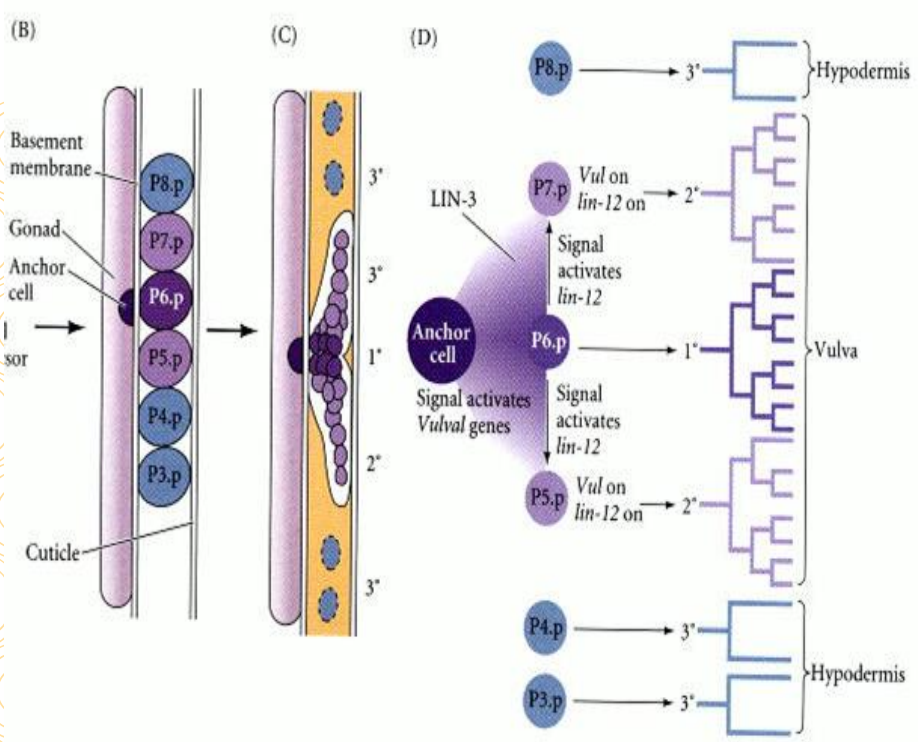
Vulval Induction in *Caenorhabditis elegans*

- The *C. elegans* vulva is a hermaphrodite-specific ectodermal organ that develops post-embryonically (larval stage).
- It functions to connect the internal reproductive system with the external environment.
- The vulva is required for mating, as males inject sperm through it, and for deposition of embryos after internal fertilization.
- Vulval development has emerged as a paradigm of morphogenesis because it offers a simple model of tissue rearrangement in which a tube forms from an epithelial sheet of only 22 cells.
- As most *C. elegans* are hermaphrodites, in their early development, they are male, and the gonad produces sperm, which is stored for later use.
- As they grow older, they develop ovaries.
- The eggs "roll" through the region of sperm storage, are fertilized inside the nematode, and then pass out of the body through the vulva.
- The vulva of *C. elegans* represents a case in which one inductive signal generates a variety of cell types.



- ★ This organ forms during the larval stage from six cells called the vulval precursor cells (VPCs).
- ★ The cell connecting the overlying gonad to the vulval precursor cells is called the anchor cell.
- ★ The anchor cell secretes the LIN-3 protein, a relative of epidermal growth factor (EGF) and the Boss protein.
- ★ If the anchor cell is destroyed (or lin-3 gene mutated), the VPCs will not form a vulva; they will instead become part of the hypodermis (skin).
- ★ The six VPCs influenced by the anchor cell form an equivalence group.
- ★ Each member of this group is competent to become induced by the anchor cell and can assume any of three fates, depending on its proximity to the anchor cell.





★ The LIN-3 protein is received by the LET-23 receptor tyrosine kinase on the VPCs.

★ The signal is transferred to the nucleus through the RTK-Ras pathway.

★ The target is the LIN-31 protein.

★ Its phosphorylated in the nucleus, loses its inhibitory protein partner and is able to function as a transcription factor, promoting vulval cell fates.

Two major mechanisms coordinate the formation of the vulva through this induction

1. The LIN-3 protein forms a concentration gradient:

   The VPC closest to the anchor cell (P6.p cell) receives the highest concentration of LIN-3 protein and generates the central vulval cells.

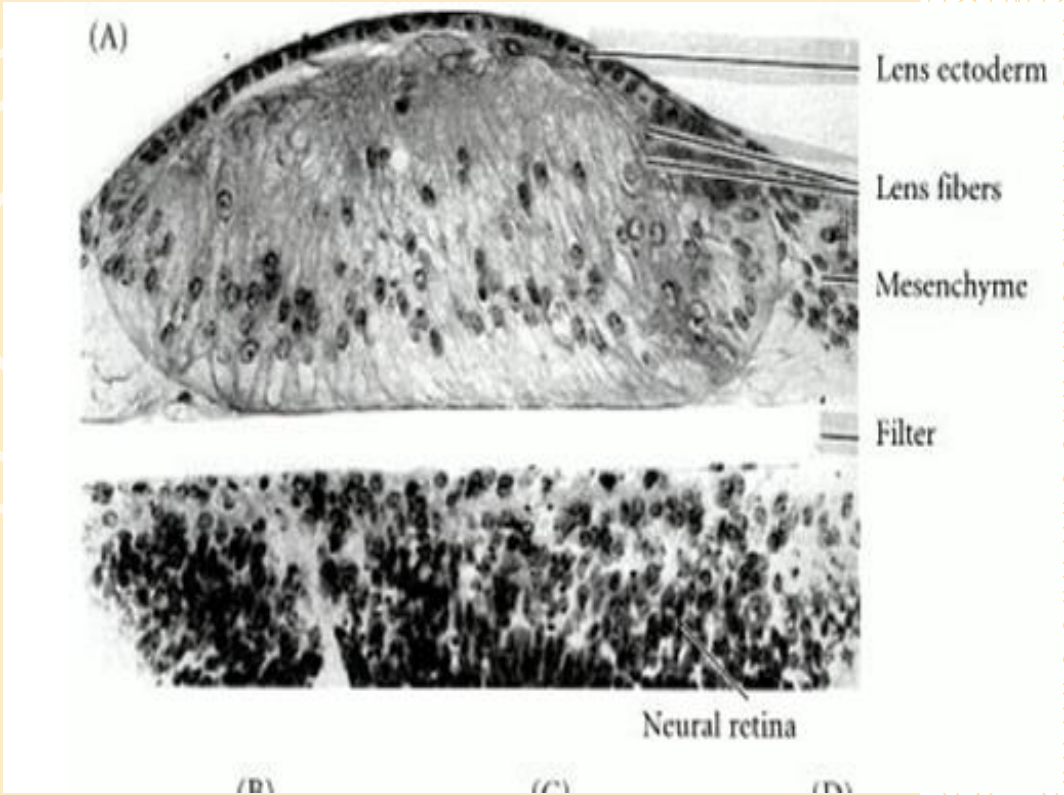
   The two VPCs adjacent to it (P5.p and P7.p) receive a lower amount of LIN-3 and become lateral vulval cells.

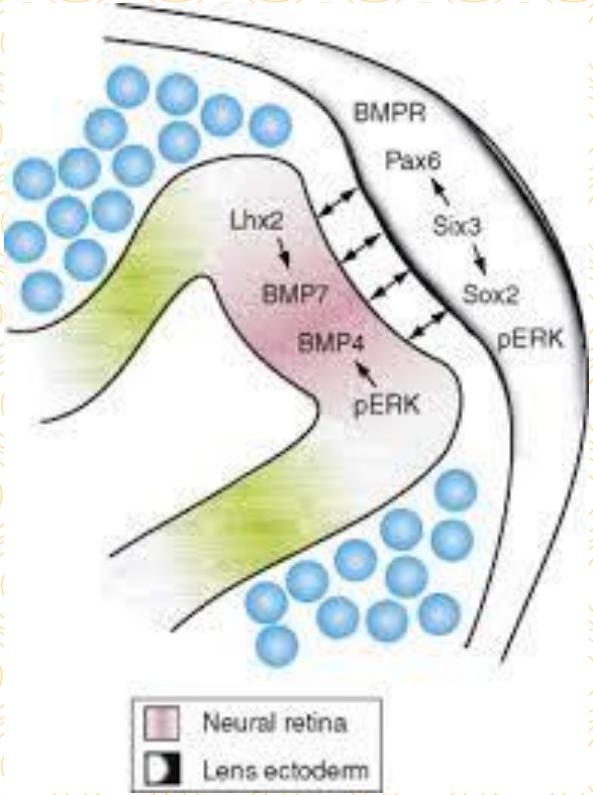
   The VPCs farther away from the anchor cell (P3.p, P4.p and P8.p) do not receive enough LIN-3 to have an effect, so they become hypodermis

2. In addition to forming the central vulval lineage, the VPC closest to the anchor cell also signals laterally to the two adjacent cells and instructs them to generate the lateral vulval lineages:

This lateral inhibition of the "secondary" vulval precursor cells by the "primary" VPC is accomplished through the LIN-12 proteins.

EYE LENS INDUCTION





★ Coordination in the construction of organs is accomplished by one group of cells changing the behavior of an adjacent set of cells, thereby causing them to
change their shape, mitotic rate, or fate.

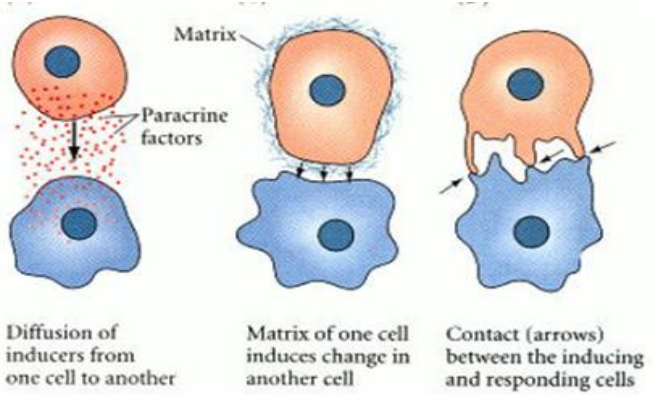
This kind of interaction at close range between two or more cells or tissues of different history and properties is called proximate interaction, or Induction.

★ There are at least two components to every inductive interaction.
★ Inducer: the tissue that produces a signal (or signals) that changes the cellular behavior of the other tissue.
Responder: the tissue being induced.

Not all tissues can respond to the signal being produced by the inducer.

Eg: If the optic vesicle (presumptive retina) of *Xenopus laevis* is placed in an ectopic location underneath the head ectoderm, it will induce that ectoderm to form lens tissue.

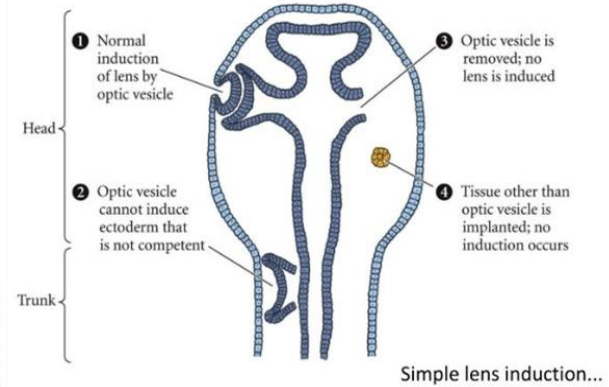
Paracrine factors or
Growth and differentiation factors (GDFs)



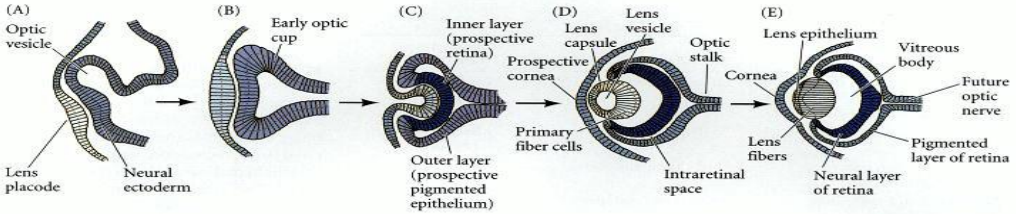
This ability to respond to a specific inductive signal is called competence.
Eg: Pax6 expression is seen in the
head ectoderm, which can respond to the optic vesicle by forming lenses.

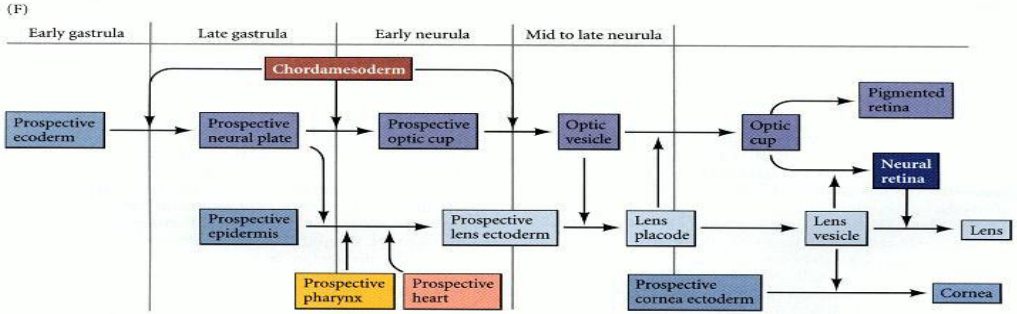
Reciprocal Induction

Eye formation is a classically studied cascade of induction



Simple lens induction...

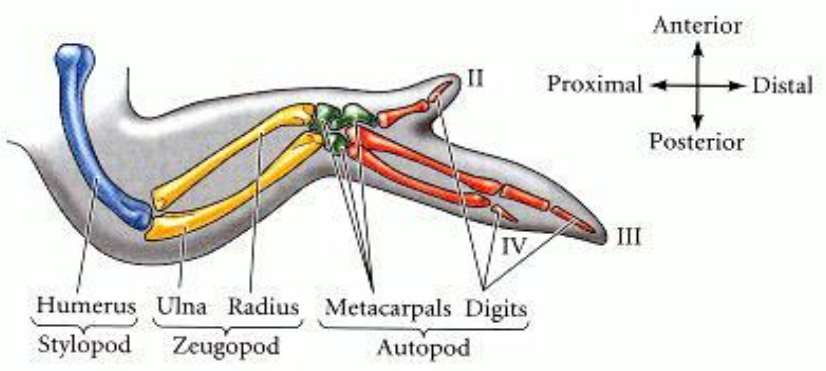




- Optic vesicle secretes.....
  - BMP 4
  - Fgf 8
- Head ectoderm expresses.....
  - Sox 2
  - L-Maf
  - Pax 6
- Lens genes turned on.....
  - crystallin
  - others

LIMB DEVELOPMENT

Pattern formation is the process by which embryonic cells form ordered spatial arrangements of differentiated tissues.



- The vertebrate limb is an extremely complex organ with an asymmetrical arrangement of parts.
- Originally, these structures are cartilaginous, but eventually, most of the cartilage is replaced by bone.
- The basic "morphogenetic rules" for forming a limb appear to be the same in all tetrapods.
- Particular proteins have been identified that play a role in the formation of each of these limb axes.
  - Proximal-distal axis - regulated by the fibroblast growth factor (FGF) family.
  - Anterior-posterior axis - regulated by the Shh protein.
  - Dorsal-ventral axis is regulated - Wnt7a.
- The interactions of these proteins determine the differentiation of the cell types and also mutually support one another.

Formation of the Limb Bud: Specification of the limb fields: Hox genes and retinoic acid:

- ★ In all land vertebrates, there are only four limb buds per embryo, and they are always opposite each other with respect to the midline.
- ★ Retinoic acid appears to be critical for the initiation of limb bud outgrowth.
- ★ A gradient of retinoic acid along the anterior-posterior axis might activate certain homeotic genes (Hox) in particular cells (limb field).
- ★ The source of this retinoic acid is probably Hensen's node.

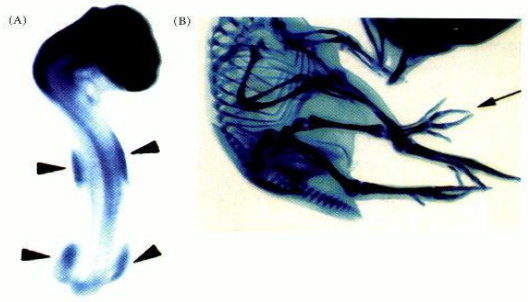
Eg:

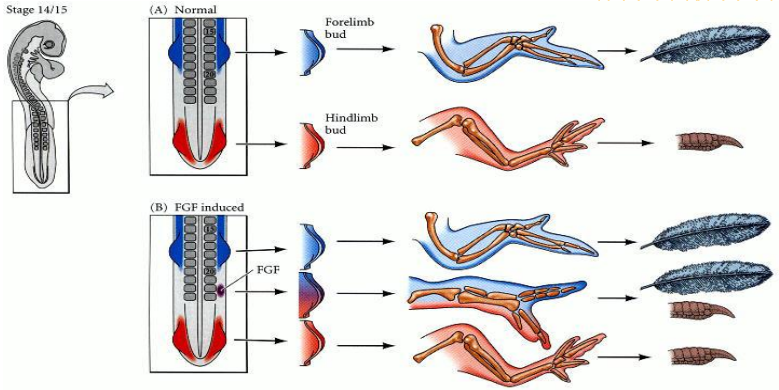
When the tails of tadpoles were amputated and the stumps exposed to retinoic acid during the first days of regeneration, the tadpoles regenerated several legs from the tail stump.

Induction of the early limb bud: Fibroblast growth factors:

★ Mesenchyme cells proliferate from the somatic layer of the limb field lateral plate mesoderm (limb skeletal precursors) and from the somites (limb muscle precursors).

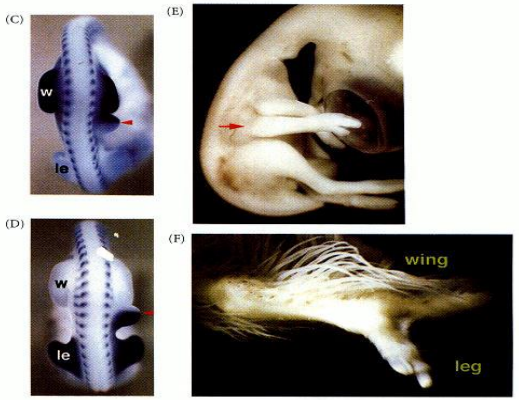
These cells accumulate under the epidermal tissue to create a circular bulge called a limb bud.



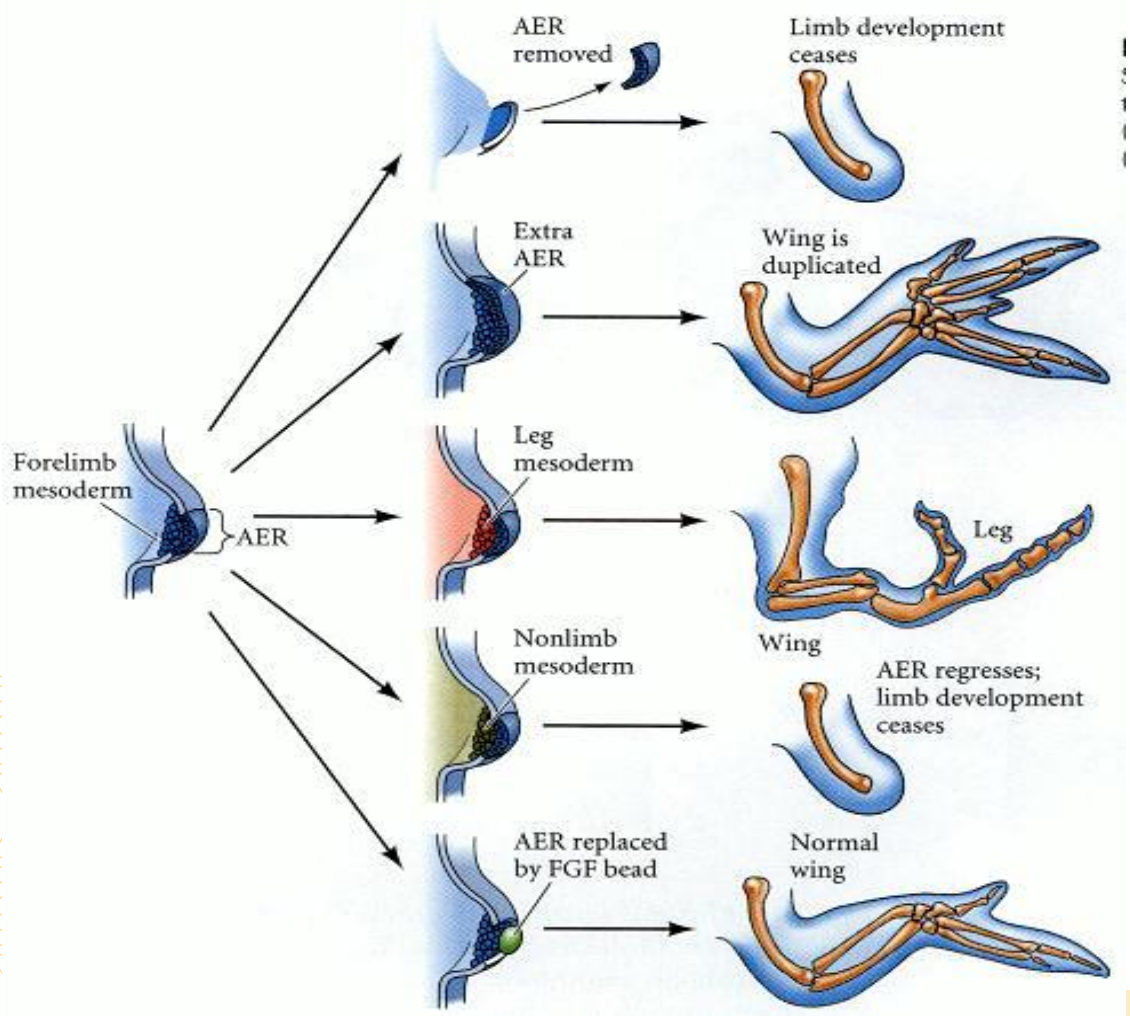


These cells secrete the paracrine factor FGF10.

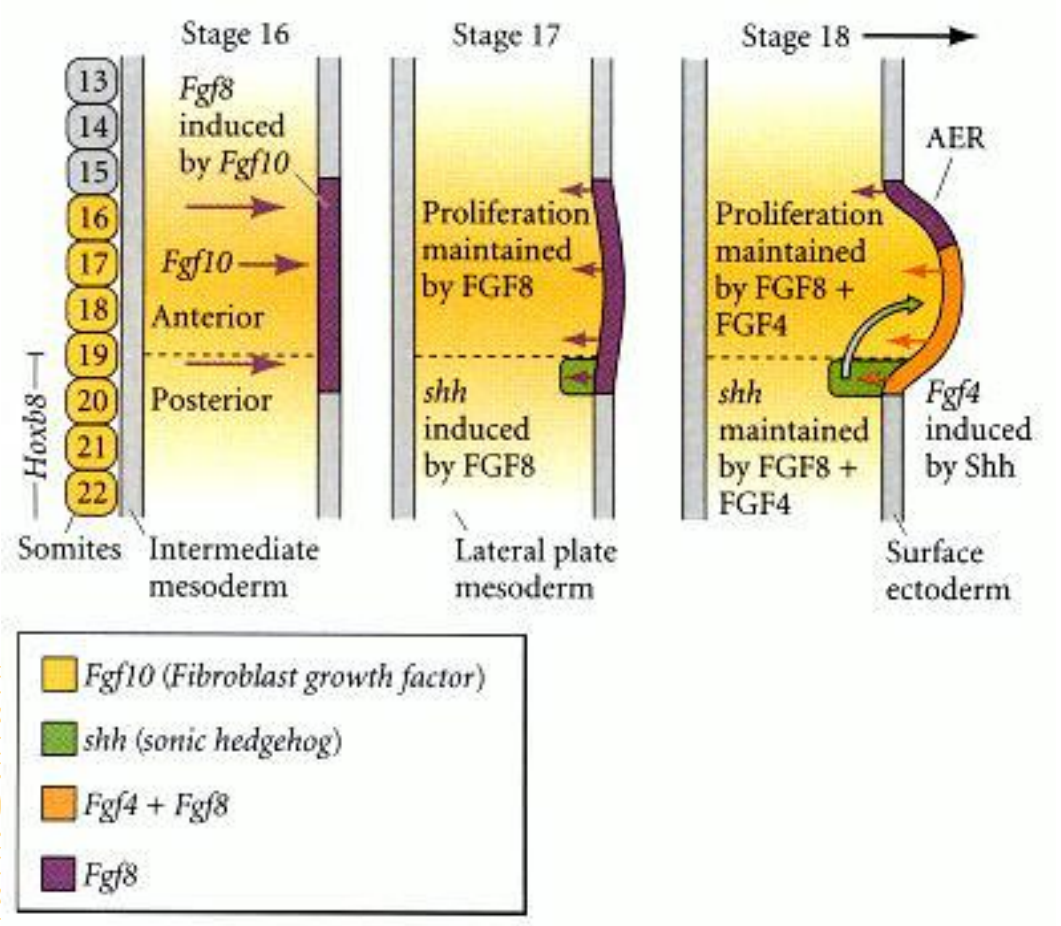
FGF10 is capable of initiating the limb-forming interactions between the ectoderm and the mesoderm.



Specification of forelimb or hindlimb:
Tbx4 and Tbx5



Although the mesenchyme cells induce and sustain the AER and determine the type of limb to be formed, the AER is responsible for the sustained outgrowth and development of the limb.



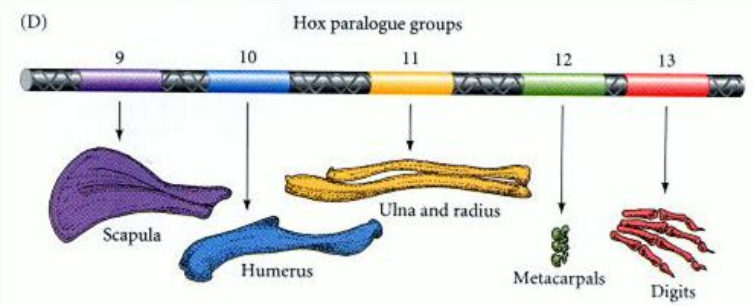
The limb bud elongates by means of the proliferation of the mesenchyme cells underneath the AER.

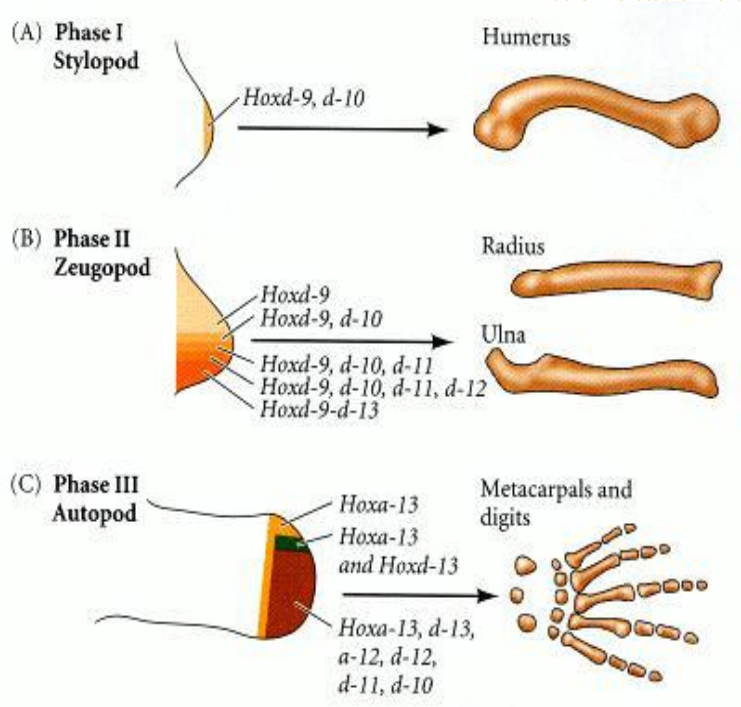
This region of cell division is called the progress zone.

FGF10 secretion by the mesenchyme cells induces the AER, and it also induces the AER to express FGF8.

FGF8 secreted by AER maintain the mitotic activity of the progress zone mesenchyme cells.

Hox genes and the specification of the proximal-distal axis





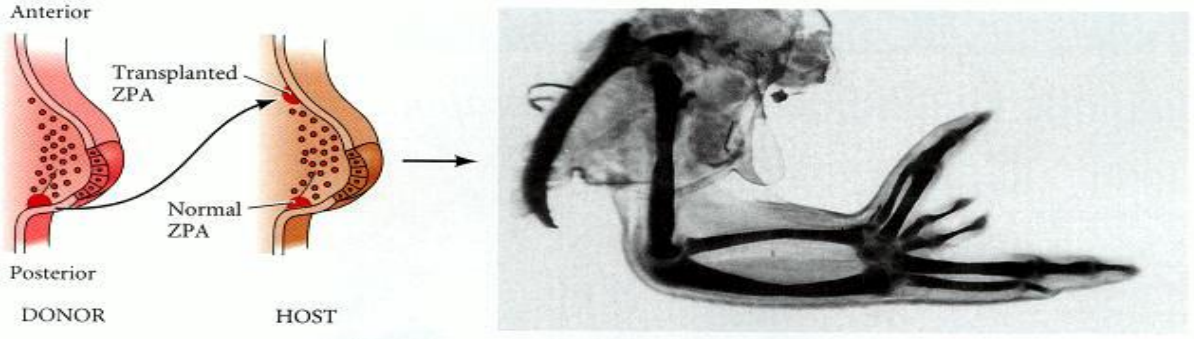
As the limb grows outward, the pattern of Hox gene expression changes.

Specification of the Anterior-Posterior Limb Axis
The zone of polarizing activity

The specification of the anterior-posterior axis of the limb is the earliest change from the pluripotent condition.

In chicks, this axis is specified shortly before a limb bud is recognizable.

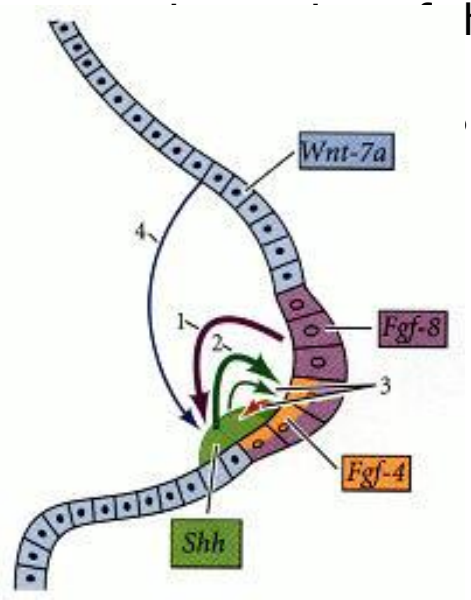
Hamburger (1938) showed that as early (16-somite) stage, prospective wing mesoderm transplanted to the flank area develops into a limb with the anterior-posterior and dorsal-ventral polarities of the donor graft, not those of the host tissue.



The three axes of the tetrapod limb are all interrelated and coordinated:

The molecules that define one of these axes are often used to maintain another axis.

★ Sonic hedgehog in the ZPA activates the expression of the Fgf4 gene in the he AER.



Fgf4 expression is important in recruiting mesenchyme cells into the progress zone, and it is also in maintaining the expression of Shh in the ZPA.

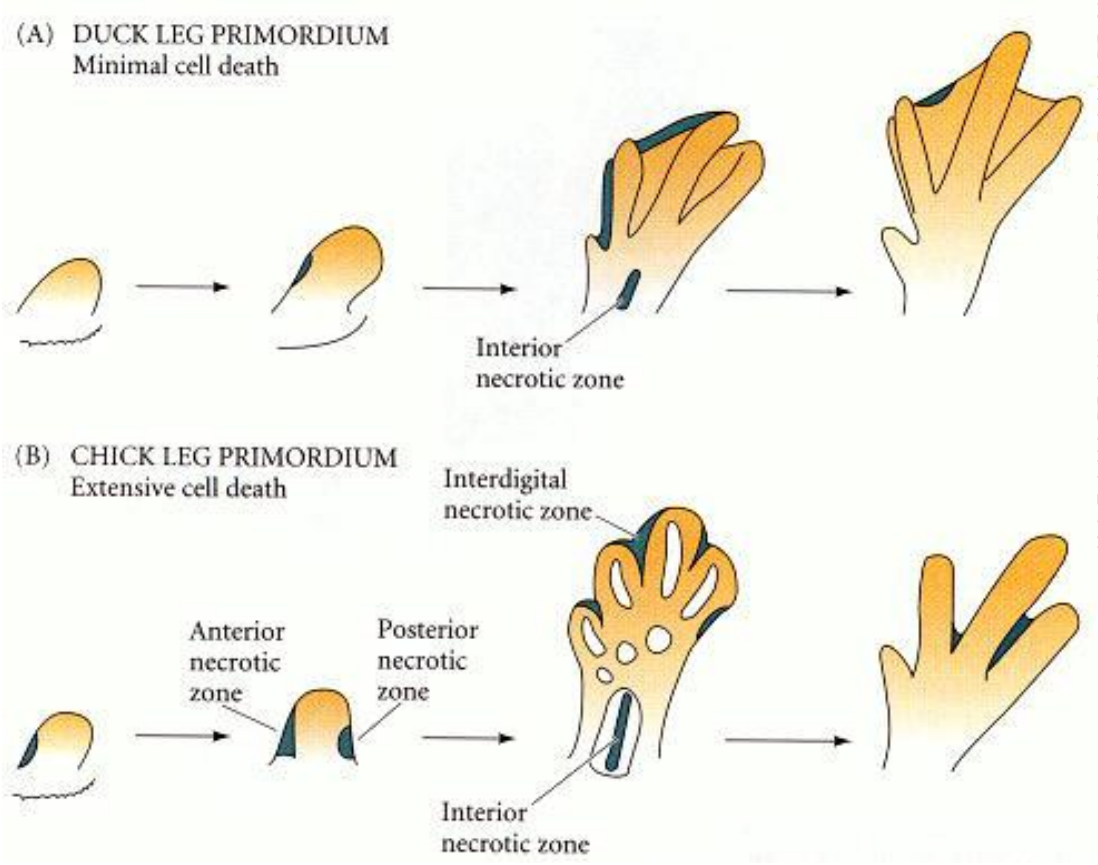
Therefore, the AER and the ZPA mutually support each Other through the positive loop of Sonic hedgehog and FGF4

Webbing and joints formation:

Cell death in the limb is necessary for the formation of digits and joints. It is mediated by BMPs, regulated by the Noggin protein.

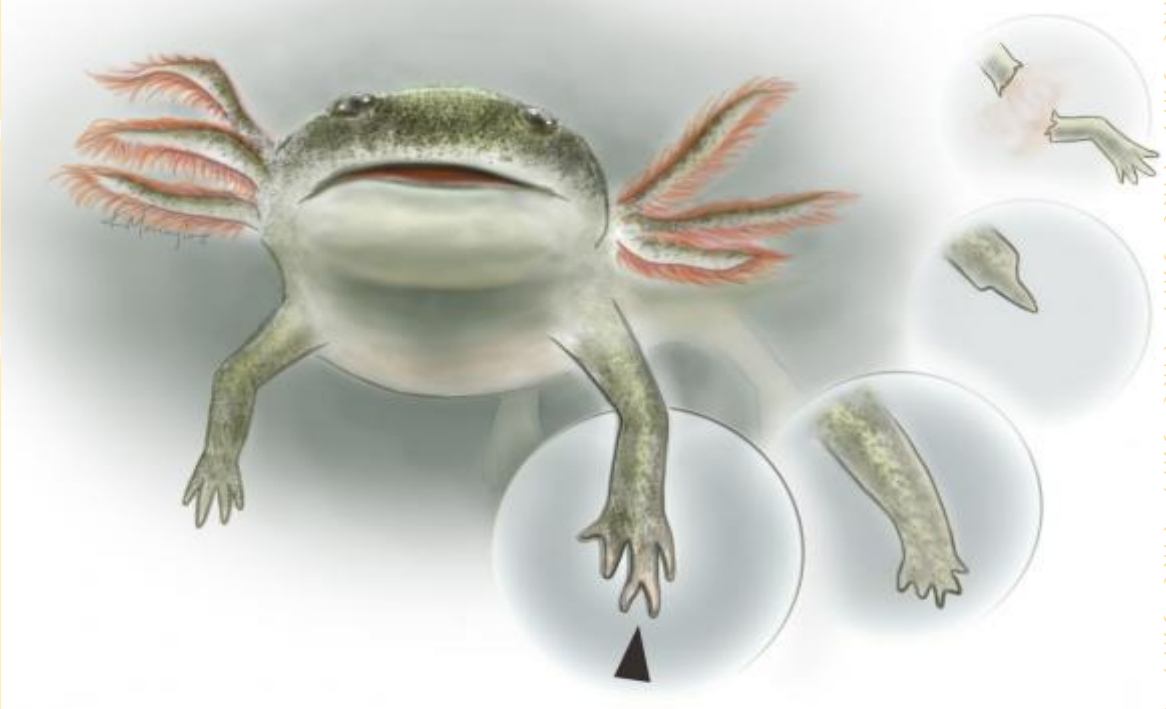
BMPs can be involved both in inducing apoptosis and in differentiating the mesenchymal cells into cartilage.

Two BMP proteins, BMP2 and GDF5, are expressed at the regions between the bones, where joints will form.



DEVELOPMENTAL BIOLOGY
(22ZOOC22)

REGENERATION IN VERTEBRATES

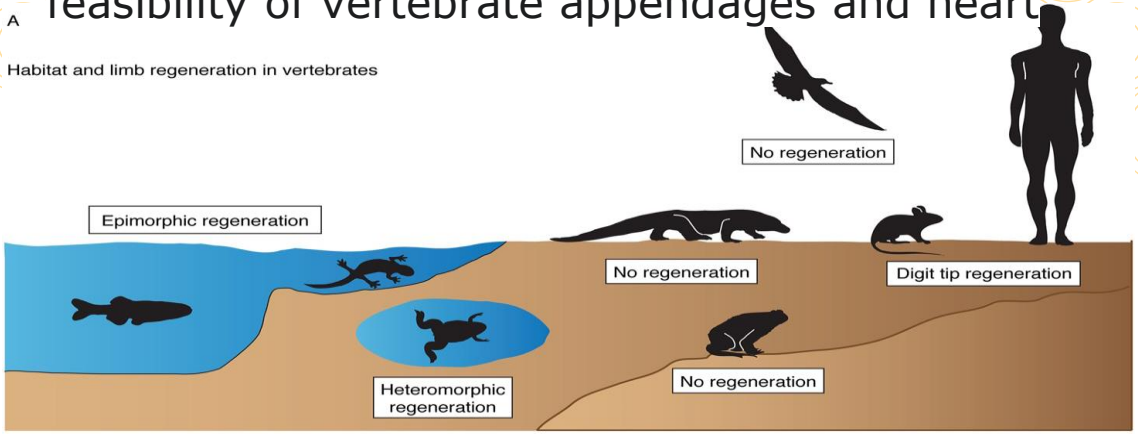


Regeneration faded as most four leg vertebrates evolved…….

Potential impact of external and internal environment on the regenerative feasibility of vertebrate appendages and heart

A

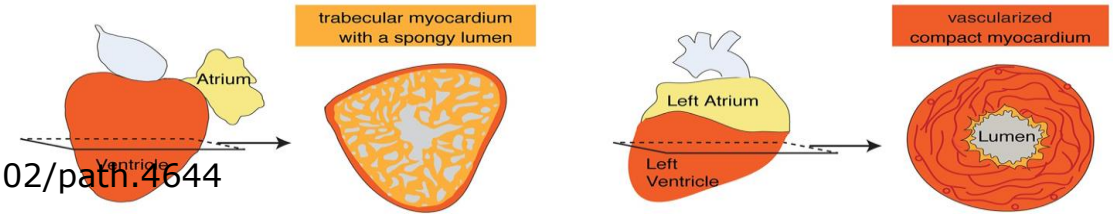
Habitat and limb regeneration in vertebrates



B

Thermoregulation and anatomy of the myocardial wall in adult vertebrates

poikilothermic vertebrates: fish, amphibians, reptiles		homeothermic vertebrates: birds, mammals
low	metabolic state	high
low	cardiac workload	high
low	dependency on coronary circulation	high
zebrafish, newt, axolotl	regenerative species	none



Ref: https://doi.org/10.1002/path.4644

(A) The transition onto land coincides with the reduction of limb-regenerative capability.

Fish and aquatic urodeles → completely and efficiently reproduce amputated appendages.

Post-metamorphic terrestrial frogs and toads → completely lack.

Xenopus froglets → **heteromorphic regeneration** (cartilaginous 'spike') from the amputation plane.

Amniotic vertebrates (reptiles, birds and mammals) - adapted to life and reproduction on the land → no limb regeneration (digit tip regeneration in mice and humans)

(B) The rise in cardiac workload in endothermic vertebrates correlates with compaction of the myocardial wall and elaboration of the coronary vasculature; Heart anatomy with a transverse section of the ventricle in fish and mammals

★ Poikilothermic vertebrates (fish, amphibians and reptiles) – the ventricle is a trabecular, sponge-like chamber.

★ Zebrafish, newts and axolotls display heart regeneration.

★ In homeothermic mammals and birds oxygenation of cardiomyocytes is dependent on coronary circulation.

★ This architecture has been associated with no regenerative capabilities.

★ Regeneration the reactivation of development in later life to restore missing tissues is so "unhuman" that it has been a source of fascination to humans since the beginnings of biological science.

★ The regeneration experiments of Tremblay (hydras), Réaumur (crustaceans), and Spallanzani (salamanders) set the standard for experimental research and for the intelligent discussion.

"One should not become disheartened by want of success,
but should try anew whatever has failed.
It is even good to repeat successful experiments a number of times.
All that is possible to see is not discovered, and often cannot be discovered,
the first time."

Susan Bryant (1999)

"a regeneration renaissance" (rebirth - embryonic state)

Mechanism of regeneration (3):

1. Epimorphosis:

   The dedifferentiation of adult structures to form an undifferentiated mass of cells that then becomes respecified.
   It is the characteristic of regenerating limbs.

1. Morphallaxis:

   Regeneration occurs through the repatterning of existing tissues, and there is little new growth. Such regeneration is seen in hydras.

1. Compensatory regeneration/ Intermediate:

   The cells divide, but maintain their differentiated functions.

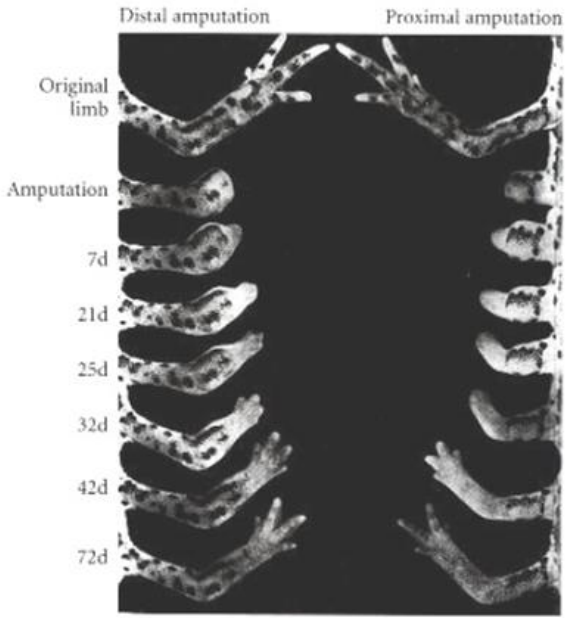
   They produce cells similar to themselves and do not form a mass of undifferentiated tissue.

   It is the characteristic of the mammalian liver.

Epimorphic Regeneration of Salamander Limbs:

When an adult salamander limb is amputated, the remaining cells are able to reconstruct a complete limb, with all its differentiated cells arranged in the proper Order.

Thus the new cells c tructures and no more (proximal-distal axis).



Formation of the apical ectodermal cap and regeneration blastema (dedifferentiation and respecification)

Limb amputation

↓

Plasma clot

↓

Wound epidermis (within 6 to 12 hours)
(Single-layered structure is required for regeneration of limb)

↓

Proliferates

↓

Apical ectodermal cap

- ★ No scar forms (contrast to mammals) and the dermis does not move with the epidermis to cover the site of amputation.
- ★ The nerves innervating the limb degenerate for a short distance proximal to the plane of amputation.
- ★ During the next 4 days, the cells beneath ectodermal cap undergo dedifferentiation:
  - bone cells,
  - cartilage cells,
  - fibroblasts,
  - myocytes, and
  - neural cells lose their differentiated characteristics and become detached from one another.

- ★ Genes that are expressed in differentiated tissues (MRF4 and myf5 in muscle cells) are downregulated.
- ★ Dramatic increase in the expression of genes (msx1) - proliferating progress zone mesenchyme of the embryonic limb.
- ★ This dedifferentiated cell mass is called the regeneration blastema.
- ★ Blastema cells will continue to proliferate, and eventually redifferentiate to form the new structures of the limb.
- ★ Macrophages are released into wound site secrete metalloproteinases that digest the extracellular matrices holding epithelial cells together.

Proliferation of the blastema cells: the requirement for nerves:

- ★ Regeneration blastema is dependent on a minimum number of nerve fibers for regeneration to take place.
- ★ Neural-derived mitotic factors : Glial growth factor (GGF) is present in the blastema, and is lost upon denervation.
- ★ When this peptide is added to a denervated blastema, the mitotically arrested cells are able to divide again.
- ★ FGFs are infused into denervated blastemas - able to restore mitosis.
- ★ Transferrin - iron-transport protein that is necessary for mitosis in all dividing cells (neural cell cycle regulator).

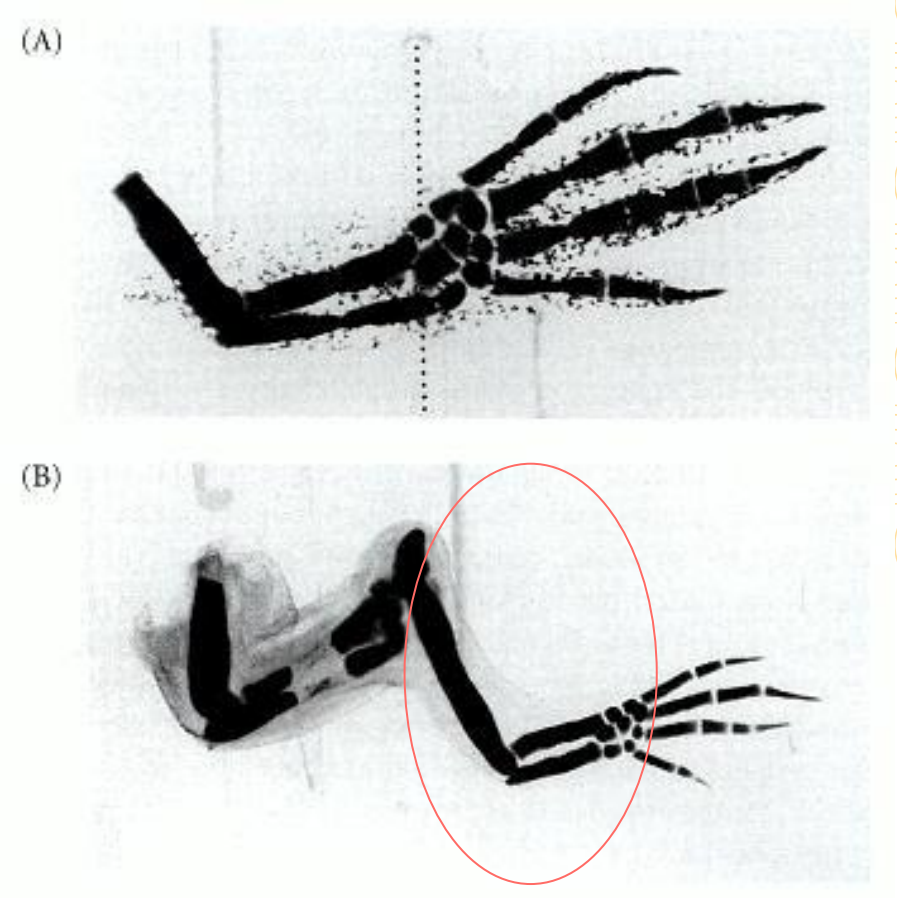
Pattern formation in the regeneration blastema:

- ★ The dorsal-ventral and anterior-posterior axes between the stump and the regenerating tissue are conserved.
- ★ Cellular and molecular studies have confirmed that the patterning mechanisms of developing and regenerating limbs are very similar.
- ★ The blastema cells could respond to limb bud signals and contribute to the developing limb. Sonic hedgehog is seen in the early posterior regeneration blastema.
- ★ A nested pattern of Hoxa and Hoxd gene expression - characteristic of limb development is established as the limb regenerates.

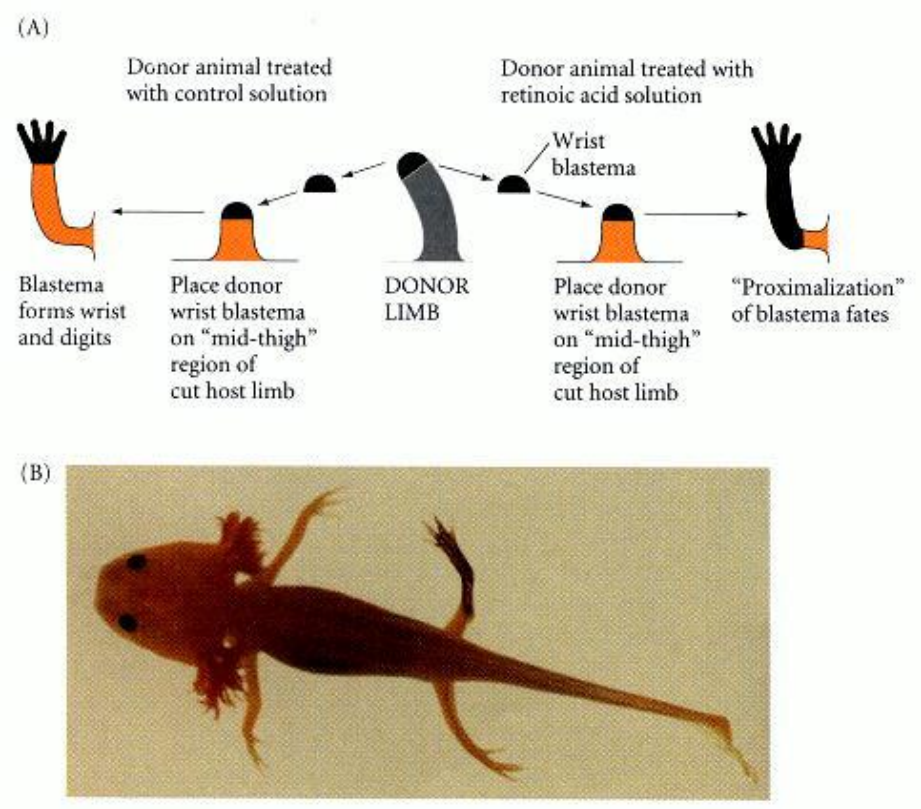
- ★ Retinoic acid appears to play an important role - dedifferentiation (blastema) and respecification processes as the cells Redifferentiate.

- ★ It causes the cells to be respecified to a more at proximal position.

Retinoic acid is synthesized in the regenerating limb wound epidermis and is seen to form a gradient along the proximal-distal axis of the blastema



Thus, in salamander limb regeneration, adult cells can go "back to the future," returning to an "embryonic" condition to begin the formation of the limb anew.



Developmental Biology (22ZOOC22)

Environmental regulation of Animal Development and Teratogens

- "Model systems" give one the erroneous impression that "DNA provides the programme which controls the development of the embryo" or that everything needed to form the embryo is within the fertilized egg.

- When focussing the loss of organismal diversity and about the effects of environmental pollutants, there is renewed interest in the regulation of development by the environment.

- Present focus on

How organisms use environmental cues in the course of their their normal development?

How exogenous compounds found in the environment can divert development from its usual path and cause congenital abnormalities?

Environmental Cues and Normal Development

1. LARVAL SETTLEMENT:

★ It occurs during the settling of marine larvae.

★ These cues need to be part of the environment if further development is to occur.

★ A free-swimming marine larva often needs to settle near a source of food or on a firm substrate on which it can metamorphose.

★ Thus, if **prey or substrates give off** soluble molecules, these molecules can be used by the larvae as cues to settle and begin metamorphosis.

Eg: Red abalone, *Haliotis rufescens* larvae only settle when they physically contact coralline red algae.

Red abalone, larvae only settle when they physically contact coralline red algae

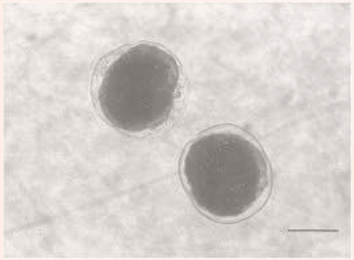


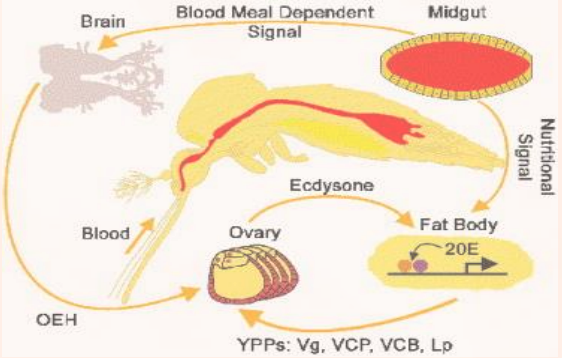
Table 21.1. Specific settlement substrates of molluscan larvae

Molluscan species	Substrate
Gastropoda (snails, nudibranchs)	
Nassarius obsoletus	Mud from adult habitat
Philippia radiata	*Porites lobata* (a cnidarian)
Adalaria proxima	*Electra pilosa* (a bryozoan)
Doridella obscura	*Electra crustulenta* (a bryozoan)
Phestilla sibogae	*Porites compressa* (a cnidarian)
Rostanga pulchra	*Ophlitaspongia pennata* (a sponge)
Trinchesia aurantia	*Tubularia indivisa* (a cnidarian)
Elysia chlorotica	Primary film of microorganisms from adult habitat
Haminoea solitaria	Primary film of microorganisms from adult habitat
Aplysia californica	*Laurencia pacifica* (a red alga)
Aplysia juliana	*Ulva spp.* (green algae)
Aplysia parvula	*Chondrococcus hornemanni* (a red alga)
Stylocheilus longicauda	Lyngbya majuscula (a cyanobacterium)
Onchidoris bilamellata	Living barnacles
Amphineura (chitons)	
Tonicella lineata	*Lithophyllum sp.* and *Lithothamnion sp.* (red algae)
Lamellibranchia (bivalves)	
Teredo sp.	Wood
Bankia gouldi	Wood
Mercenaria mercenaria	Clam liquor; sand
Placopecten magellanicus	Adult shell; sand; etc.
Mytilus edulis	Filamentous algae; other nonbiological silk material
Crassostrea virginica	Shell liquor; body extract; "shellfish glycogen"

2. BLOOD MEALS:

- ★ In many mosquitoes, egg production is triggered by a blood meal.
- ★ Only female mosquitoes bite, and prior to a blood meal they make no vitellogenin yolk protein.
- ★ In *Aedes aegypti*, the digested products of the blood meal stimulate the brain to secrete egg development neurosecretory hormone (EDNH / ovarian ecdysteroidogenic hormone, or OEH).
- ★ It stimulates the ovary to make ecdysteroids, which instruct the fat body cells to make vitellogenin for the oocytes. Vitellogenin is critical for egg production.

Thus, without the blood meal, there is no vitellogenin and no eggs.



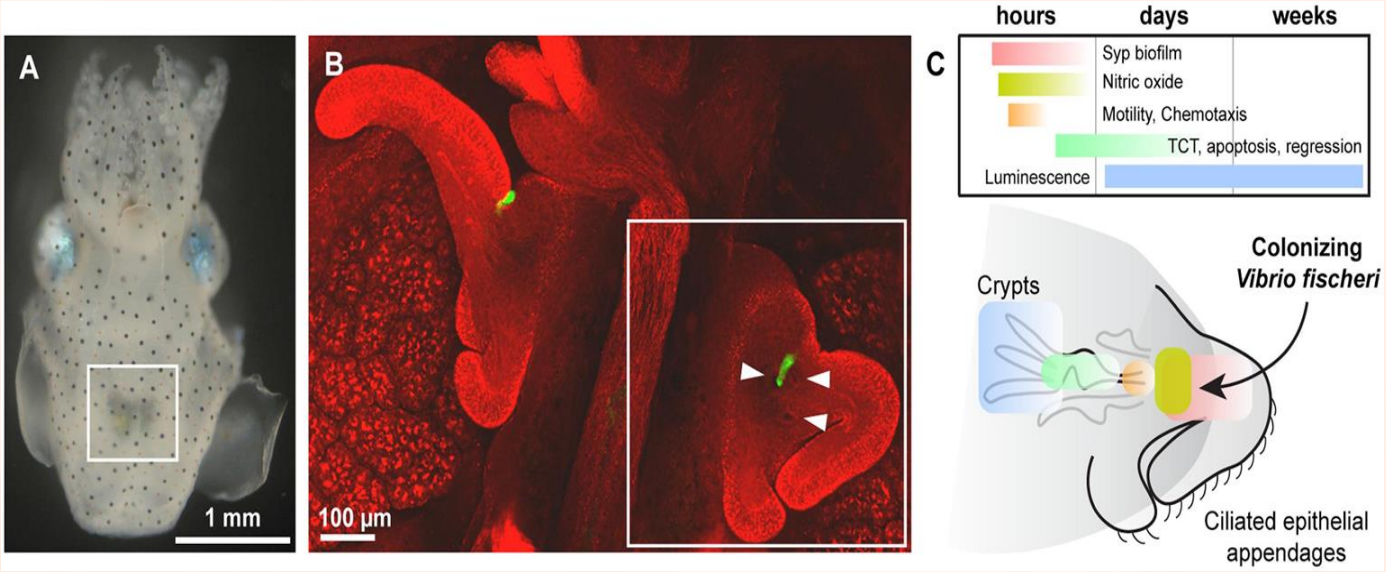
Blood free meal

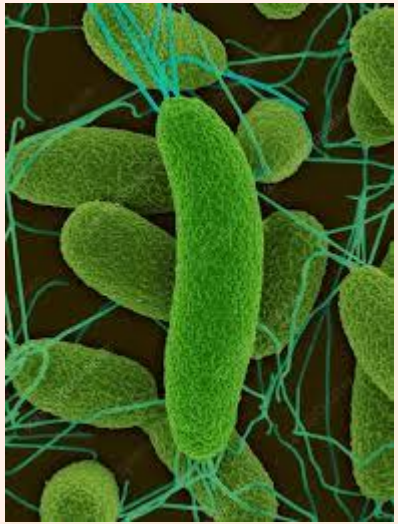
logenic carboxypeptidase (**VCP**) and **vitellogenic** ca- thepsin B (**VCB**)

3. DEVELOPMENTAL SYMBIOSIS:

The symbionts become so tightly integrated into the host organism that the host cannot develop without them.

★ The adult squid *Euprymna scolopes* is equipped with a light organ composed of sacs containing the luminous bacteria *Vibrio fischeri*.



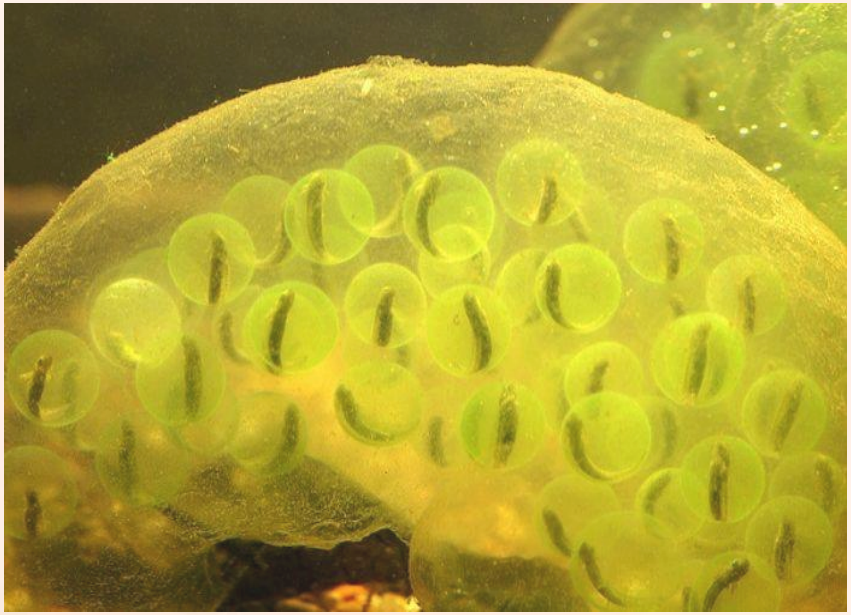


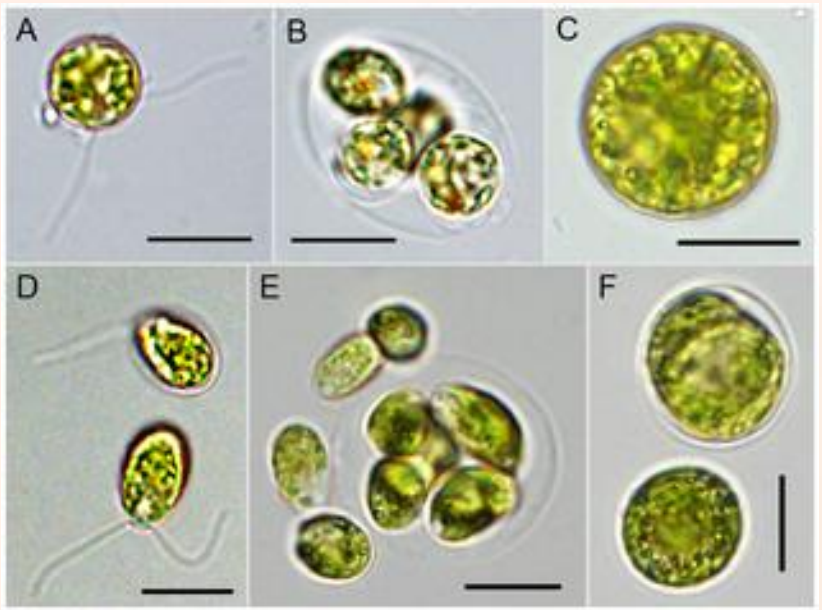
- ★ The juvenile squid, however, does not contain these light-emitting symbionts; nor does it have a structure to house them.
- ★ It acquires the bacteria from the seawater pumped through its mantle cavity.
- ★ The bacteria induce the apoptotic death of these epithelial cells, their replacement by a nonciliated epithelium.
- ★ The differentiation of the surrounding epithelial cells into storage sacs for the bacteria.

Ref: https://www.yumpu.com/en/document/view/28355665/hawaiian-bobtail-squid-university-of-wisconsin-madison

4. SYMBIOSIS BETWEEN EGG MASS AND PHOTOSYNTHETIC ALGAE:

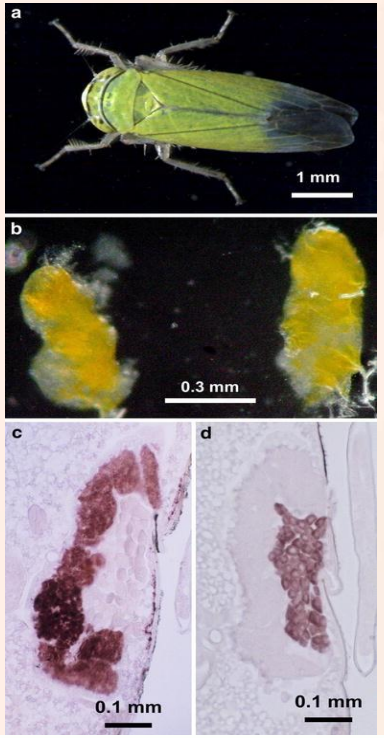
★ In clutches of amphibian (salamanders) and snail eggs, photosynthesis from algal "fouling" enables net oxygen production in the light, while respiration exceeds photosynthesis in the dark.



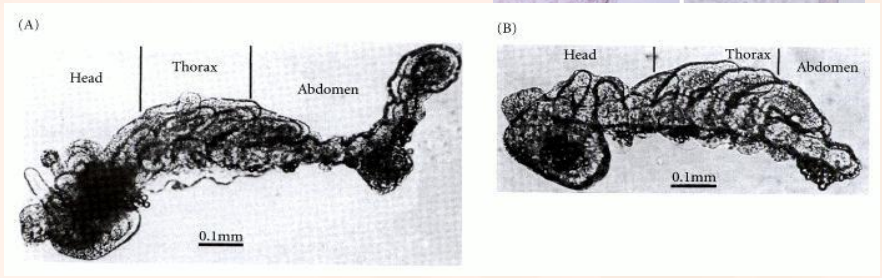


5. MORPHOGENESIS AND SYMBIOSIS:

- ★ It is exemplified by the leafhopper, *Euscelis incisus*.
- ★ Symbiosis occurs within the egg.
- ★ Symbiotic bacteria are found within the egg cytoplasm and are transferred through the generations, (like mitochondria).
- ★ These bacteria have become so specialized that they can multiply only inside the leafhopper's cytoplasm, and the host has become so dependent on the bacteria.
- ★ It cannot complete embryogenesis without them as bacterial symbionts are essential for the formation of the embryonic gut.



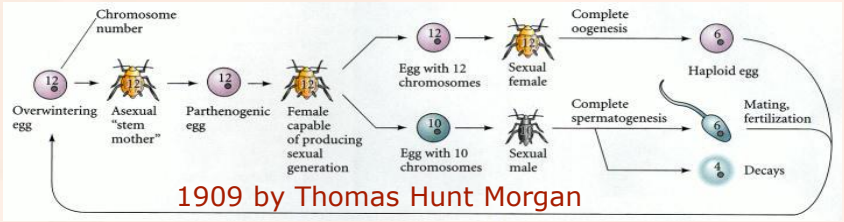
If the bacteria are surgically or metabolically removed from the eggs (by feeding antibiotics to larvae or adults), these symbiont-free oocytes develop into embryos that lack an abdomen.



Predictable Environmental Differences as Cues for Development

- The **environment** contains predictable components (gravity) or predictable changes (seasons) that **become part of the development of the organism**.
- The use of **temperature and daylight length** is used by numerous species to adjust their development to a changing environment.
- The **stresses of gravitational pressure** also play a role in the development of some organisms.

SEASONALITY AND SEX IN APHIDS:



1909 by Thomas Hunt Morgan

- Several species of aphids have a **fascinating life cycle** wherein
  - **an egg hatched in the spring** gives rise to several generations of **parthenogenetically (asexually) reproducing females**.
  - During the **autumn**, a type of female's eggs can give rise to **both males and sexual females**. These sexual forms mate, and their eggs are able to survive thewinter.
  - When the **overwintering eggs** hatch, each one gives rise to an **asexual female**.

How the autumn weather regulates whether the female reproduces sexually or parthenogenetically ?

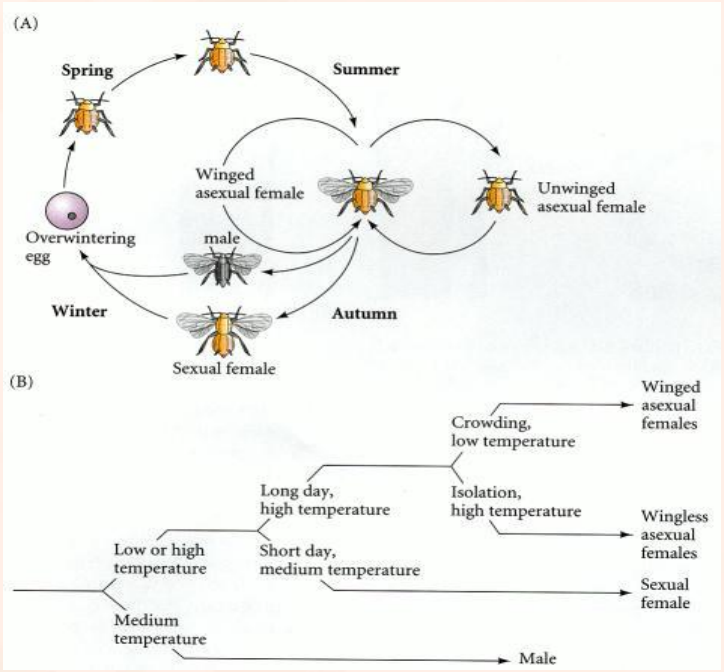
2. Temperature determines the sex early in development

- ★ In *Megoura viciae*, (with extreme temperatures favoring the production of females).

- ★ In female development, day length and temperature determine whether the female will reproduce sexually or parthenogenetically.

- ★ A combination of temperature and population density determine whether she will be winged or wingless.
  Juvenile hormone controls parthenogenetic/sexual switch and inhibits the formation of wings.

Megoura viciae



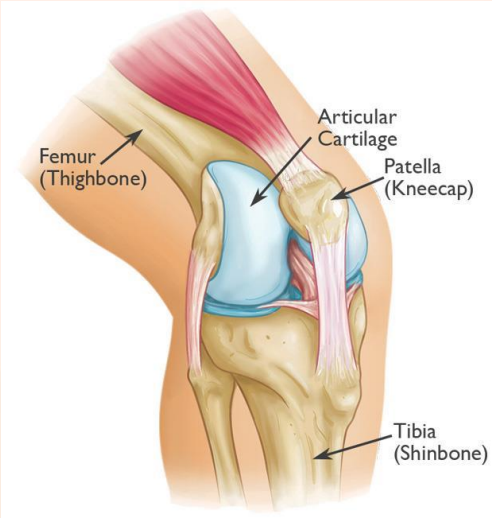
3. GRAVITY AND PRESSURE:

★ Gravity and movement are environmental constants that aids in normal development.

Eg: Gravity was critical for frog and chick axis formation.

★ Several bone formation is dependent on stresses occasioned by the movement of the embryo.

Eg: Formation of the human patella (kneecap) after birth.



Phenotypic Plasticity: Polyphenism and Reaction Norms

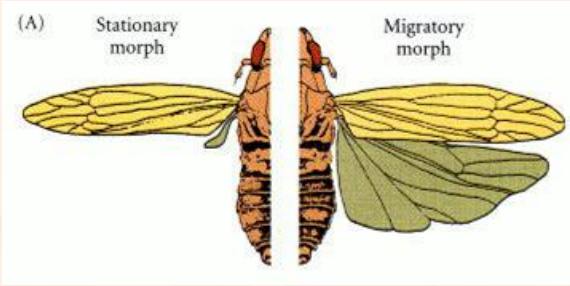
The ability of an individual to express one phenotype under one set of circumstances and another phenotype under another set is called phenotypic plasticity.

- ★ There are two main types of phenotypic plasticity:
  - ○ Polyphenism and
  - ○ Reaction norms.

- ★ Polyphenism → "discontinuous phenotypes elicited by environment"
  Eg: Migratory locusts

It exist in two mutually exclusive forms:

i. a short-winged, uniformly colored solitary phase
ii. a long-winged, brightly colored gregarious phase.



✓ At a time when India is struggling with the **DEADLY CORONAVIRUS**, huge swarms of locusts in many states has bought nightmares to the farmers.

✓ Experts warn of extensive crop losses if authorities fail to curb the fast-spreading swarms by June when monsoon rains spur rice, cane, corn, cotton, and soybean sowing.

✓ Locusts entered India after traveling from Africa through Yemen, Iran and Pakistan.

✓ After massive devastation in Pakistan, the swarms of locusts entered India through Rajasthan and Gujarat.

Locusts attack

https://zeenews.india.com/india/locusts-attack-threatens-summer-crop-in-india-farmers-across-several-states-fear-massive-losses-2286317.html

Migratory locusts

The genome encodes a range of potential phenotypes, and the environment selects the phenotype that is usually the most adaptive.

LOCUSTS BY NAME, NOT BY NATURE

➤ Spotted coffee grasshopper (aularches miliaris) belongs to the family Pyrgomorphidae while locusts which have infested parts of NE and central India belong to schistocerca gregaria and are commonly called as 'desert locust'

➤ Locusts belong to the family acrididae and meet two criteria – 1) They form dense groups which migrate 2) They are polyphenic in the sense that individuals living separately differ in many characteristics from those living in groups

➤ Aularches miliaris grasshoppers satisfy the first criteria of forming dense groups alone

➤ Though grasshoppers were spotted in parts of Pulpally forest fringes earlier too, this year, the insect which usually lays eggs in egg pods in Sep-Oct which hatch in April has been spotted gregariously

➤ Experts say mono cropping farming practices and soil remaining unattended partly due to the lockdown could have helped in emergence and survival of large number of nymphs

➤ They also said chemical pesticide application is unwarranted as the insect is only a minor pest and listed as near-threatened for south India in the regional conservation status assessment

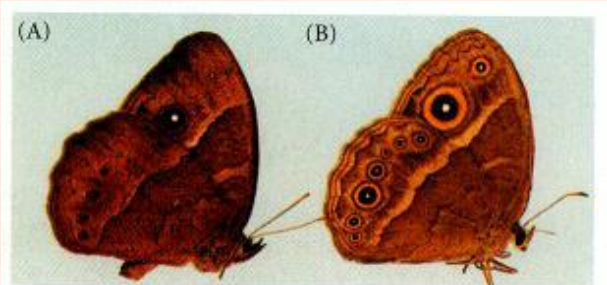
➤ Also grasshoppers are indicator species and have important role in food cycle, pollination and nutrient cycling

→ **Dhaneesh Bhaskar, researcher and member of the Species Survival Commission (Grasshopper specialist group) of IUCN.**

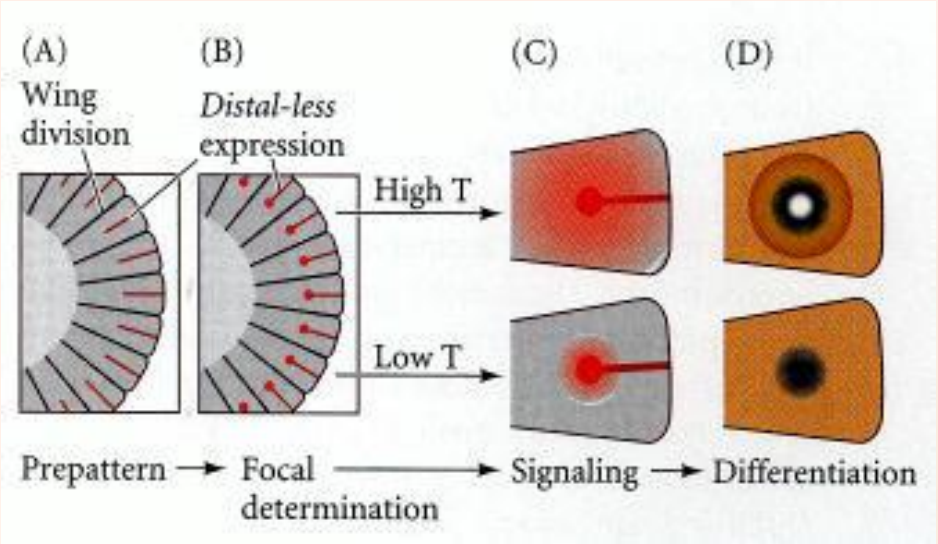
May 28, 2020

https://timesofindia.indiatimes.com/city/kozhikode/hopper-bands-found-in-wayanad-arentlocusts-pesticides-not-needed-experts/articleshow/76047386.cms

1. SEASONAL POLYPHENISM IN BUTTERFLIES:



Malawian butterfly *Bicyclus anynana*



2. NUTRITIONAL POLYPHENISM:

Bees:

★ The size of the female larva at its metamorphic molt determines whether the individual is to be a worker or a queen.

★ A larva fed nutrient-rich "royal jelly" retains the activity of her corpora allata Secreting JH delays pupation --> bee to emerge larger and (in some species) more specialized in her anatomy.

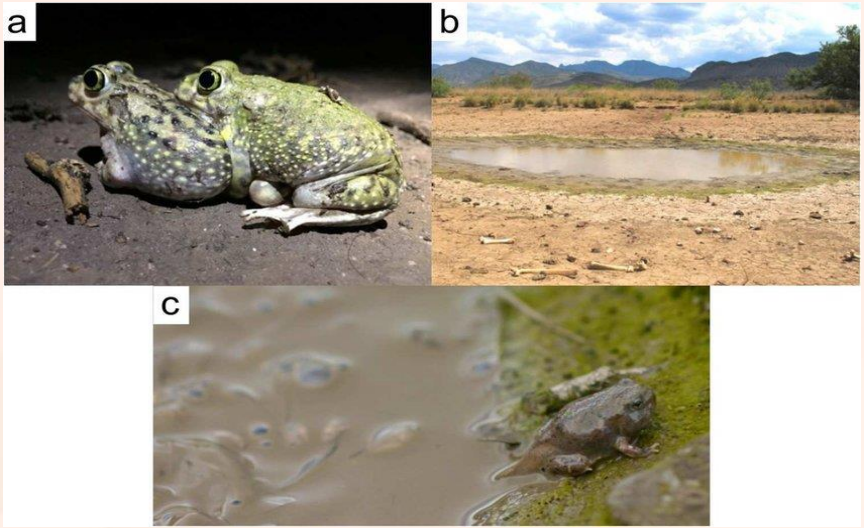
★ The JH level of larvae destined to become queens is 25 times that of larvae destined to become workers, and application of JH onto worker larvae can transform them into queens as well.

3. POLYPHENISMS FOR ALTERNATIVE CONDITIONS:

★ The developing animal also survive in its habitat, and its development must adapt to the conditions of its existence.

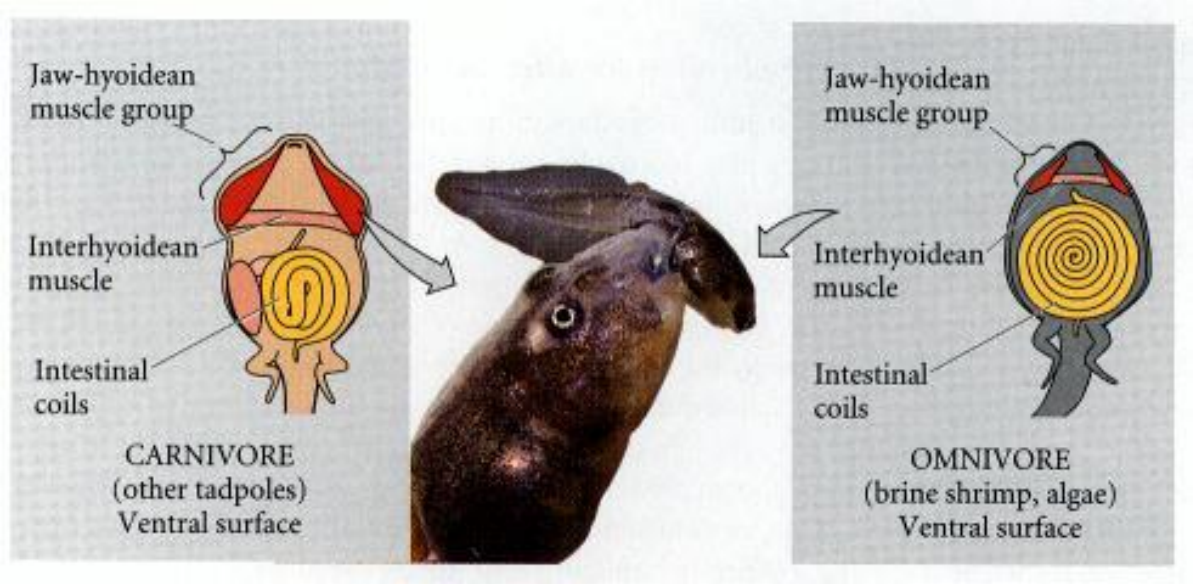
Eg:

The spadefoot toad, *Scaphiopus couchii*, has a remarkable strategy for coping with a very harsh environment.



Scaphiopus tadpoles - Desert ponds

Phenotypic plasticity gives an individual the ability to respond to different environmental conditions



Faster-developing carnivorous form is more fit in quickly drying ponds.
But the slower-developing tadpoles (which develop into larger, more robust toads) are more fit in wetter conditions.

Predator-Induced Defenses

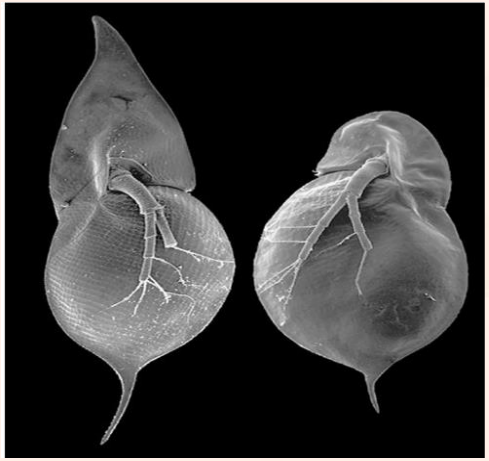
The development of the animal (embryos or juveniles) is changed by chemicals released by the predator, enabling a better escape those same predators.

Predator-induced defense, or predator-induced polyphenism

Eg:
Water flea (*Daphnia*)
Female *Daphnia* respond to chemical signals
From predators by growing protective helmets.

Offspring of helmeted Daphnia also born with helmets, even in absence of predator signals.

It continues to next generation, although helmet Size decreases.



Chemicals that are released by a predator and can induce defenses in the prey are called kairomones.