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وزارة التعليم العالي والبحث العلمي
People's Democratic Republic of Algeria
Ministry of Higher Education and Scientific Research

University Ferhat Abbas Setif 1
Faculty of Nature and Life Sciences

جامعة فرحات عباس
كلية علوم الطبيعة سطيف والحياة

Embryonic development

Realized by Dr. Benchikh Fatima

intended for third-year Animal Biology and Physiology students.

2024/2025

Summary

List of Figures

Overview for "Embryonic Development" 📚

Subject Name: Embryonic Development

Credits: 7

Coefficient: 3

Teaching Objectives 🎯

In this course, students will explore the intricate process of embryonic development across various species, including bacteria, birds, and mammals. The key focus will be on understanding the cellular and molecular mechanisms that govern the establishment of different tissues during the key stages of development. By the end of the course, students will be equipped with the knowledge to explain how primordial tissues form and give rise to the complex structures of living organisms.

Through lectures, hands-on sessions, and case studies, students will:

- Recall and compare embryonic development across different species.
- Understand the molecular pathways involved in tissue formation.
- Analyse the role of gene expression and cell differentiation in early embryogenesis.
- Study cell signalling, tissue interactions, and other molecular mechanisms responsible for tissue establishment.

Recommended Prior Knowledge 💡

To get the most out of this course, students should have a solid understanding of:

- Animal Embryology: Basic concepts and stages.
- Primary Tissues: Knowledge of epithelial, connective, muscle, and nervous tissues.
- Establishment of Primordial Tissues: How initial tissues form during early developmental stages.

Key Concepts 📘

- Cellular Differentiation: Mechanisms that lead to the formation of specialized cells.
- Molecular Signaling: How signals guide cells during tissue formation.
- Tissue Interactions: Communication between developing tissues that ensures proper organization

I. Introduction

Embryonic development is a meticulously coordinated process in which a single fertilized egg undergoes a series of transformations to form a complex, multicellular organism. This progression involves distinct stages, each governed by specific cellular movements, molecular signaling, and genetic regulation. From the initial fertilization to the formation of tissues and organs, developmental biology provides a detailed map of how life begins and evolves at the earliest stages.The journey starts with the fusion of gametes, where protective envelopes and reserves within the egg play essential roles in ensuring successful fertilization and subsequent development. Following this, the fertilized egg undergoes segmentation, a process where rapid cell division transforms it into a multicellular structure. Key molecular interactions and regulatory mechanisms then guide the process of gastrulation, positioning the three primary germ layers—ectoderm, mesoderm, and endoderm—necessary for forming all tissues and organs. As development proceeds, neurulation leads to the establishment of the neural tube, which becomes the central nervous system. This is followed by organogenesis, where specialized organs form, and morphogenesis, which shapes the embryonic appendages like limbs in birds and mammals. Genetic control mechanisms, such as the expression of Hox genes, regulate these complex processes, ensuring that the developmental plan is executed correctly.

A crucial element in mammalian development is the formation of the placenta, an organ that supports the growing embryo by facilitating nutrient and waste exchange between mother and fetus. Throughout this process, precise cellular interactions and regulatory genes orchestrate the emergence of life from a single cell into a fully formed organism. This understanding of embryonic development is foundational for fields ranging from developmental biology to medicine, providing insights into both normal growth and congenital abnormality.

II. Main Characteristics of Development of Some Fundamental Types

In the biological context of embryonic development, the formation of foundational structures such as tissues, organs, and systems are guided by key principles: stability, adaptability, precise regulation, and temporal coordination. Initially, the embryo undergoes gastrulation, where the three primary germ layers ectoderm, mesoderm, and endoderm are established. These layers provide the structural stability necessary for further development. The ectoderm forms the nervous system and skin, the mesoderm gives rise to muscles, bones, and the circulatory system, while the endoderm develops into internal organs like the digestive and respiratory systems. This structural foundation is crucial for the proper growth and organization of tissues throughout development.

Adaptability is another vital principle, allowing cells within these layers to respond dynamically to molecular signals and environmental cues that shape their fate. Signaling pathways such as Sonic hedgehog (Shh) and Bone Morphogenetic Proteins (BMPs) create morphogen gradients that provide spatial information, guiding cells to differentiate into specific tissue types. The ability of these pluripotent cells to interpret positional information and adapt accordingly is essential for the correct development of specialized tissues and organs.

Equally important is the precise regulation of developmental processes. Genetic networks and signaling cascades such as the Wnt, Notch, and TGF-β pathways must be tightly controlled to ensure that cells differentiate at the appropriate times and locations. For example, Hox genes offer positional instructions along the body axis, ensuring that structures like limbs, vertebrae, or organs develop in the correct anatomical positions. This regulatory control operates at various levels, including transcriptional, post-transcriptional, and epigenetic modifications, all of which fine-tune gene expression. Lastly, temporal coordination ensures that embryonic events follow the correct developmental timeline. Processes such as neurulation (neural tube

formation) and organogenesis (organ formation) occur in a synchronized sequence, with disruptions in timing leading to developmental abnormalities. Together, these principles stability, adaptability, precise regulation, and temporal coordination ensure that embryonic development progresses in an organized, efficient manner, transforming a single cell into a complex, multicellular organism.

II.2. Development of Amphibians

Amphibian, (class Amphibia), any member of the group of vertebrate animals characterized by their ability to exploit both aquatic and terrestrial habitats. The name amphibian, derived from the Greek amphibios meaning “living a double life,” reflects this dual life strategy though some species are permanent land dwellers, while other species have a completely aquatic mode of existence(*Amphibian | Characteristics, Life Cycle, & Facts | Britannica*, n.d.-a).

Approximately 8,100 species of living amphibians are known. First appearing about 340 million years ago during the Middle Mississippian Epoch(Glasspool et al., 2015), they were one of the earliest groups to diverge from ancestral fish-tetrapod stock during the evolution of animals from strictly aquatic forms to terrestrial types(*Evolution | Definition, History, Types, & Examples | Britannica*, n.d.). Today amphibians are represented by frogs and toads (order Anura), newts and salamanders (order Caudata), and caecilians (order Gymnophiona). These three orders of living amphibians are thought to derive from a single radiation of ancient amphibians, and although strikingly different in body form, they are probably the closest relatives to one another. As a group, the three orders make up subclass Lissamphibia. Neither the lissamphibians nor any of the extinct groups of amphibians were the ancestors of the group of tetrapods that gave rise to reptiles. Though some aspects of the biology and anatomy of the various amphibian groups might demonstrate features possessed by reptilian ancestors,

amphibians are not the intermediate step in the evolution of reptiles from fishes.(*Amphibian Pictures & Facts*, n.d.)

Modern amphibians are united by several unique traits as shown in Figure 1. They typically have a moist skin and rely heavily on cutaneous (skin-surface) respiration. They possess a double channelled hearing system, green rods in their retinas to discriminate hues, and pedicellate (two-part) teeth. Some of these traits may have also existed in extinct groups.(*Amphibian | Characteristics, Life Cycle, & Facts | Britannica*, n.d.-a; *Amphibian Pictures & Facts*, n.d.)

Members of the three extant orders differ markedly in their structural appearance. Frogs and toads are tailless and somewhat squat with long, powerful hind limbs modified for leaping. In contrast, caecilians are limbless, wormlike, and highly adapted for a burrowing existence. Salamanders and newts have tails and two pairs of limbs of roughly the same size; however, they are somewhat less specialized in body form than the other two orders.

Figure 1:LIST OF AMPHIBIANS | LIST OF AMPHIBIANS WITH PICTURES

(LIST OF AMPHIBIANS | LIST OF AMPHIBIANS WITH PICTURES - Vocabulary Point, n.d

i General features

- **Size Range and Structural Diversity**

The three extant orders of amphibians display significant variation in size and structure. Salamanders and newts (order Caudata) are generally characterized by a long tail and two pairs of limbs of similar size. However, in the family Sirenidae, members have lost their hind limbs, giving them an eel-like appearance. Salamanders vary widely in size, with species like Thorius from Mexico measuring only 25 to 30 mm (1 to 1.2 inches), while the giant aquatic salamanders of the genus Andrias, native to China and Japan, can grow over 1.5 meters (5 feet) long.

Frogs and toads (order Anura) are easily recognized by their long hind limbs, which are adapted for jumping, and the absence of a tail. The largest species in this group, the West African goliath frog (Conraua goliath), can grow up to 30 cm (12 inches) and weigh up to 3.3 kg (7.3 pounds). On the other hand, some of the smallest anurans, such as certain species of brachycephalids and microhylids from South America, are only 9 to 12 mm (0.4 to 0.5 inch) in length.

Caecilians (order Gymnophiona) are limbless, elongated amphibians adapted to a burrowing lifestyle. Their bodies are segmented by annular grooves, and they possess a short, blunt tail. Some caecilians can grow over 1 meter (3 feet) long, with the largest species, Caecilia thompsoni, reaching up to 1.5 meters (5 feet), while the smallest species, Idiocranium russeli, measures only 90 to 114 mm (3.5 to 5 inches).

- **Distribution and Abundance**

Amphibians are found throughout the world, including regions as far north as the Arctic Circle in Eurasia. However, they are absent in Antarctica, remote oceanic islands, and extremely dry deserts. Frogs and toads show the greatest diversity in tropical, humid environments, while salamanders are most commonly found in the Northern Hemisphere, especially in cool, moist montane forests. Lungless salamanders (family Plethodontidae), which breathe entirely through their skin, are notably diverse in the montane forests of Mexico, Central

America, and northwestern South America. Caecilians inhabit various wet tropical regions across Africa, the Americas, and Asia. In recent decades, habitat destruction has severely impacted amphibian populations. Since the 1980s, a marked decline in frog species has been observed globally. Contributing factors include acid rain, global warming, and ozone depletion. However, the parasitic fungus Batrachochytrium dendrobatidis (amphibian chytrid) has been identified as a primary cause of widespread frog die-offs, particularly in parts of Australia, Central America, North America, and Europe.

- **Economic Importance**

Amphibians, especially frogs (order Anura), play an important economic role by helping to control insect populations that can damage crops or spread diseases. Additionally, frogs are harvested for food, with thousands of tons of frog legs consumed or exported each year. The skin secretions of certain tropical anurans have been found to possess hallucinogenic properties, as well as effects on the central nervous and respiratory systems in humans. Some of these secretions, such as magainin, act as natural antibiotics, while others contain toxins that show potential as anesthetics or painkillers. Scientists are currently studying these substances for their possible medicinal applications.

ii Natural history

1. Reproduction in Amphibians

Amphibians, comprising frogs and toads (Anura), salamanders and newts (Caudata), and caecilians (Gymnophiona), exhibit diverse reproductive strategies that reflect their evolutionary lineages. Despite these differences, a common trend is the evolution of direct development, a process where the aquatic egg and free-swimming larval stages are bypassed. In direct development, embryos grow entirely within the egg capsule, and the young hatch as smaller versions of the adult. This mode of reproduction is seen in many lungless salamanders (family Plethodontidae), some species of caecilians, and numerous anurans. Additionally, viviparity

(live birth) occurs in certain caecilians, anurans, and salamanders, where young are born fully developed rather than hatching from eggs. As shown in Figure 2, the reproduction process of amphibians involves distinct stages that are closely tied to their natural habitat

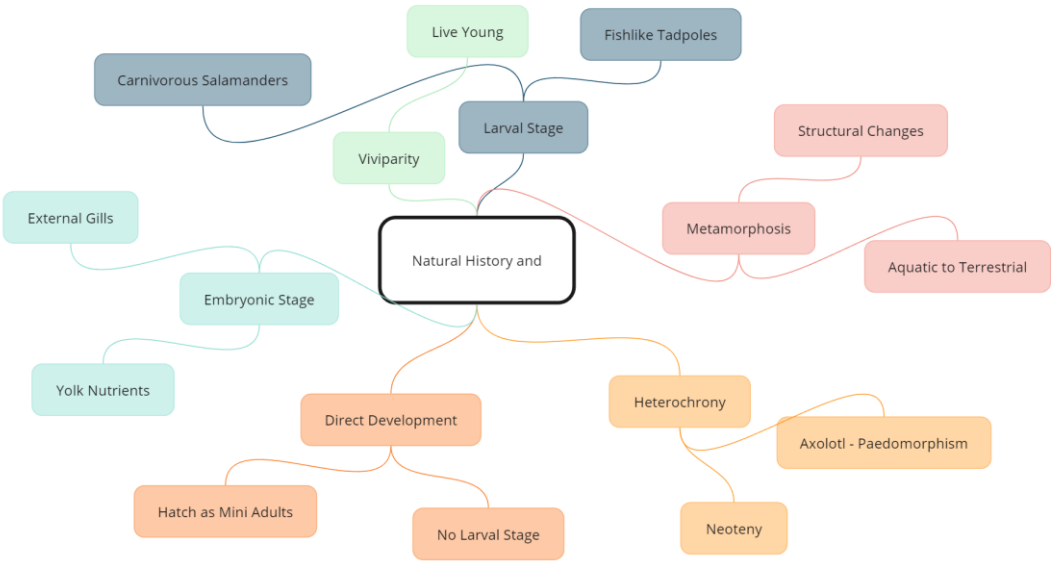

Figure 2:Natural History and Reproduction of Amphibians

2. Anuran Reproductive Strategies

Anurans (frogs and toads) demonstrate a wide variety of life histories. Some species, such as those in the families Centrolenidae and Phyllomedusine hylids, lay their eggs on vegetation overhanging streams or ponds. After hatching, tadpoles fall into the water, where they continue their development. Other species, such as those in the Leptodactylidae and Rhacophoridae families, build foam nests for their eggs in aquatic, terrestrial, or arboreal environments. Once hatched, these tadpoles usually develop in water. Some species, such as those in the Dendrobatidae family, lay their eggs on land and then transport the young to water for the tadpole stage. Marsupial frogs (Hylid) carry eggs in a pouch on their backs, while a few species of anurans carry tadpoles exposed on their backs before depositing them in ponds after they hatch. As depicted in Figure 3, amplexus involves a male grasping a female by the head, waist,

or armpits for external fertilization (a), while copulexus involves inguinal clasping for internal fertilization (b). The figure further distinguishes monogamy, where pairs mate exclusively (c), from polyandry, where a female mates with multiple males (d)

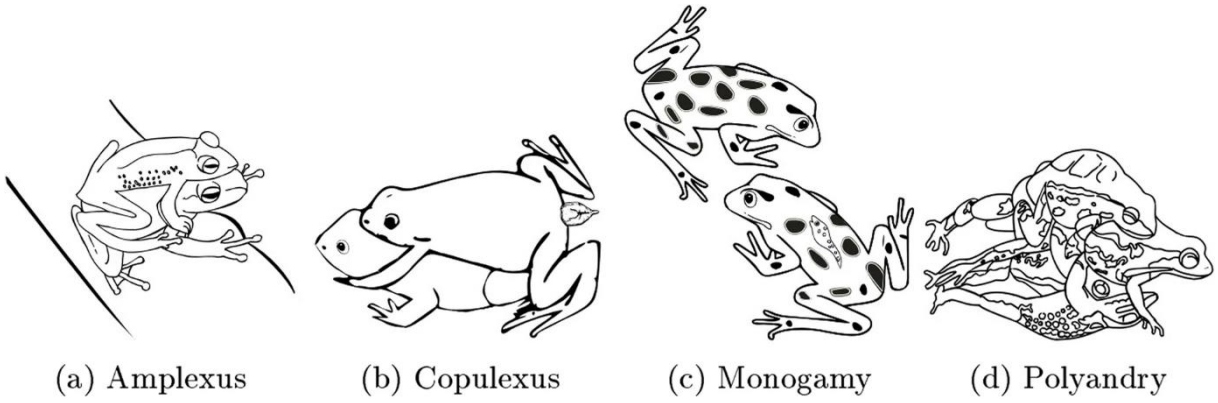

*Figure 3:Mating systems and modes in frogs. Amplexus; a male can grasp a female with his front legs by the female's head, waist, or armpits .This reproductive mode occurs in externally fertilizing species (a). Copulexus; a male clasps the female inguinally rather than axillary to insert its tail. This reproductive mode occurs in internally fertilizing species (b). Monogamy is a mating system where females and males select each other exclusively to couple (c). In contrast, polyandry is when a female mates with two or more different males (d)(*Amphibian | Characteristics, Life Cycle, & Facts | Britannica, *n.d.-a)*

The amphibians have developed a diverse range of reproductive modes, i.e., strategies related to reproductive behaviour and oviposition. Therefore, the oviposition site, oocyte characteristics, clutch-type, size of hatchlings, and parental care are associated with reproductive mode.(O'Connell, 2020). In addition to vocalizations, male frogs may use visual signals or secretion of pheromones to attract females (Pearl et al., 2000). Most males vocalize once they reach sexual maturity; they emit calls to attract mates and maintain territorial boundaries. Meanwhile, adult females emit sounds to alert males about threats or courtship during copulation. In some species, the vocal sac has been coopted temporarily as a gestational organ to incubate eggs and larvae.("The Reproductive Biology of Amphibians," 1977; Wells, 1977).

3. Embryonic Stage

Amphibian embryos develop within gelatinous egg capsules and are suspended in perivitelline fluid, a substance that surrounds both the embryo and yolk. Tadpoles hatch by dissolving these egg capsules with enzymes secreted from specialized glands on their snouts. Throughout development, the yolk provides all the necessary nutrients. Different nutrients are utilized at various developmental stages: early development relies on fats, during gastrulation (when the embryo consists of two cell layers), carbohydrates are the primary energy source, and during later stages, proteins are consumed as morphological structures form. By the neurula stage, when nervous tissue develops, cilia appear on the embryo's surface, allowing it to rotate gracefully within the perivitelline fluid.(Graham & Morgan, 1966; Schoch, 2009)

In direct-developing and live-bearing species, external gills press against the egg capsule, facilitating gas exchange (oxygen and carbon dioxide) with the surrounding environment or maternal tissues. During this time, nitrogenous waste in the form of ammonia is diluted by the constant diffusion of water in the perivitelline fluid.(Schoch, 2009)

Cleavage in most frog and salamander embryos is radially symmetrical and holoblastic, just like echinoderm cleavage. The amphibian egg, however, contains much more yolk. This yolk, which is concentrated in the vegetal hemisphere, is an impediment to cleavage. Thus, the first division begins at the animal pole and slowly extends down into the vegetal region the developmental timeline of amphibian embryos, depicted in Figure 4, highlights key milestones from fertilization to the formation of the neural tube, emphasizing rapid cellular differentiation and organization.

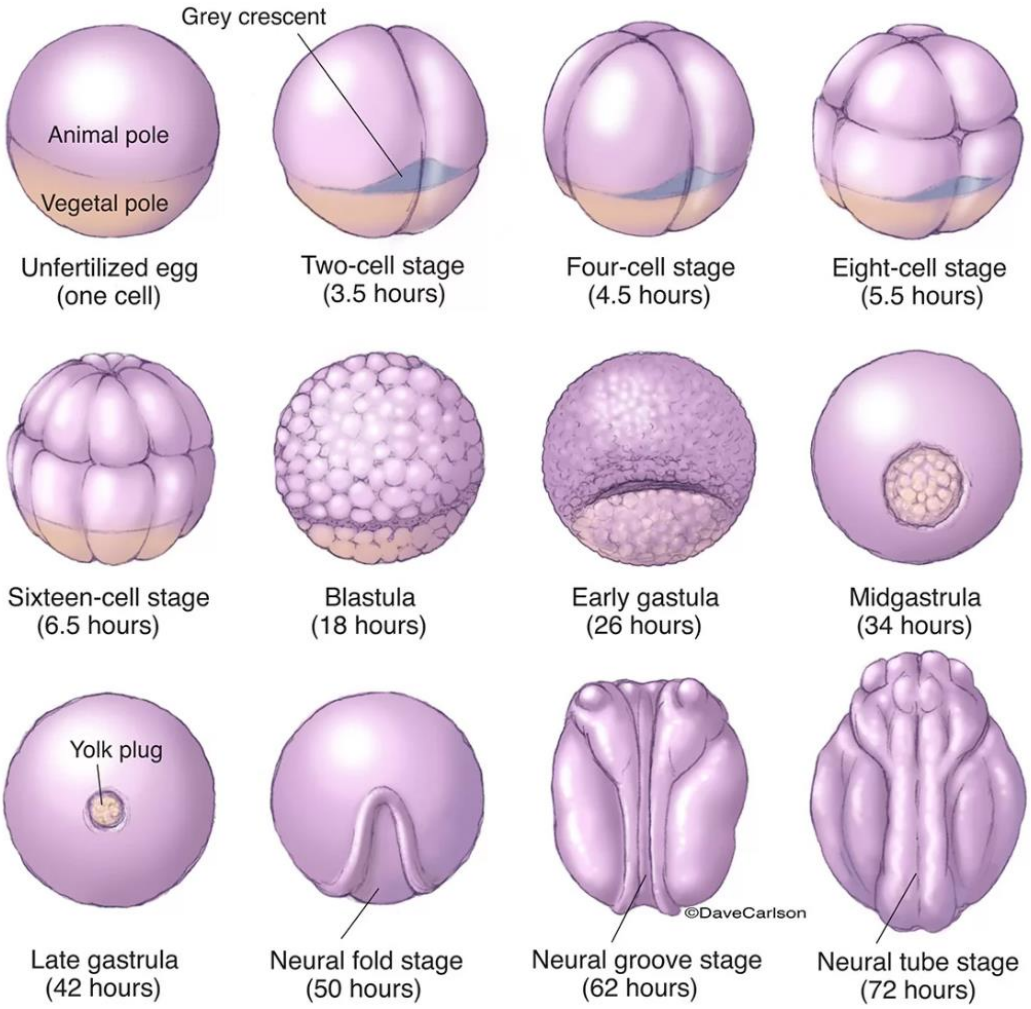

*Figure 4: Illustration of typical amphibian embryonic development from single cell (unfertilized egg) to neural tube stage (72 hrs). Image © copyright by Dave Carlson.(*Evolution | Definition, History, Types, & Examples | Britannica, *n.d.)*

4. Development of Limbs and Balancers

The development of limbs in aquatic salamander embryos begins at the head and progresses down the body. Salamanders that deposit their eggs in streams develop both sets of limbs before hatching, while those in still water develop only forelimbs before hatching(*Formation of the Limb Bud - Developmental Biology - NCBI Bookshelf*, n.d.). Shortly after the appearance of forelimbs, pond-dwelling salamanders grow rodlike projections called balancers on each side of their heads. These structures, which emerge from the mandibular arch, contain nerves and capillaries, secrete a sticky substance, and help larvae maintain balance. Once forelimbs fully develop, the balancers degenerate. Limb regeneration provides a valuable model for studying organ reconstruction along the proximodistal (PD), anteroposterior (AP), and dorsoventral

(DV) axes. The re-expression of genes linked to axial patterning is essential for achieving full regeneration. Positional information within the limb blastema is thought to be crucial in guiding this gene re-expression. Recent research highlights the significant role of epigenetic mechanisms in influencing both development and regenerative processes. This review examines how epigenetic mechanisms contribute to the preservation of positional information and regulate gene re-expression during limb regeneration (Yakushiji et al., 2009).

In anurans, limb development does not occur until after hatching. Newly hatched tadpoles often use adhesive organs, located at the base of the tongue, to attach themselves to the egg capsule or nearby vegetation. These adhesive organs secrete mucus, allowing the tadpole to remain stationary while it develops the ability to swim and feed. Once it becomes fully mobile, the organs degenerate.(Amphibian | Characteristics, Life Cycle, & Facts | Britannica, n.d.-b; Yakushiji et al., 2009). As shown in Figure 5 and 6 vertebrate limb development is divided into three distinct segments: the stylopod, forming the proximal limb structures; the zeugopod, constituting the middle segment; and the autopod, comprising the distal elements such as fingers or toes

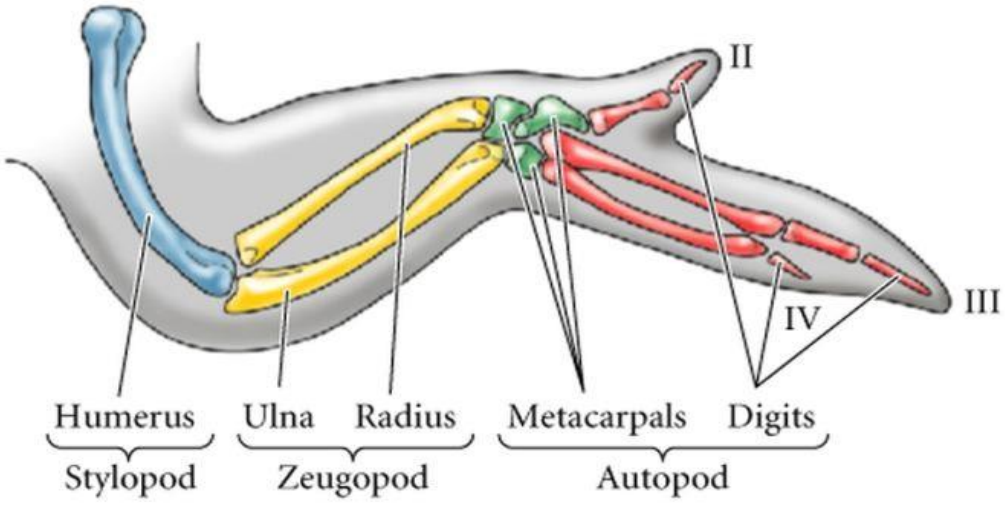

*Figure 5: Segmental Structure of Vertebrate Limb: Stylopod, Zeugopod, and Autopod(*Developmental Biology 3230, *n.d.)*

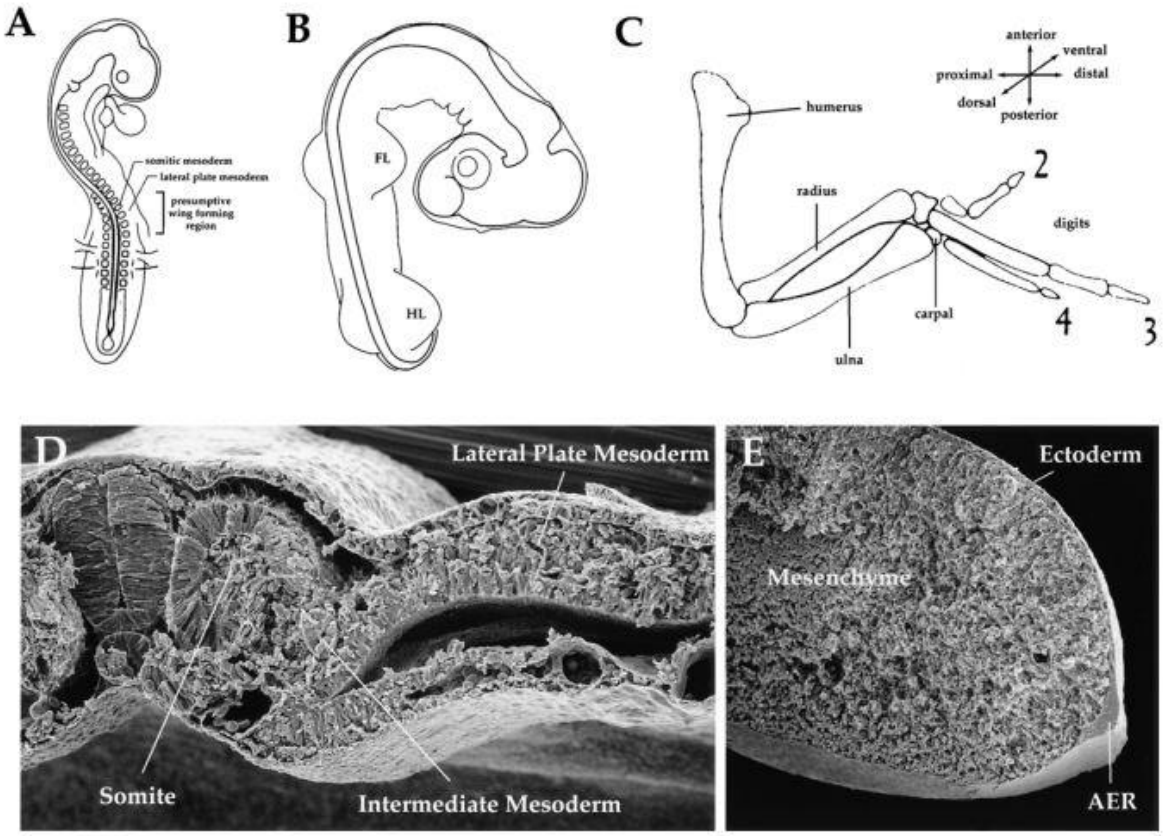

*Figure 6:Embryonic Limb Development Stages and Tissue Differentiation(*Developmental Biology 3230, *n.d.)*

5. Larval Stage

The larval stage represents a distinct developmental phase between embryo and adult in amphibians. Larvae are considered free-living embryos, as they must seek food, evade predators, and perform other survival tasks while they continue to develop. In salamanders and caecilians, larvae resemble miniature adults. For instance, larval salamanders possess fully developed limbs, external gills, tails, specialized larval teeth, a rudimentary tongue, and a lack of eyelids. These larvae are carnivorous and feed on small aquatic invertebrates shortly after emerging from their eggs(*Amphibians – Reproduction Study Guide - Inspirit*, n.d.). As shown in Figure 7 larval caecilians, also smaller versions of their adult forms, have external gills, a lateral-line system (sensory organs that detect movement), and thin skin.

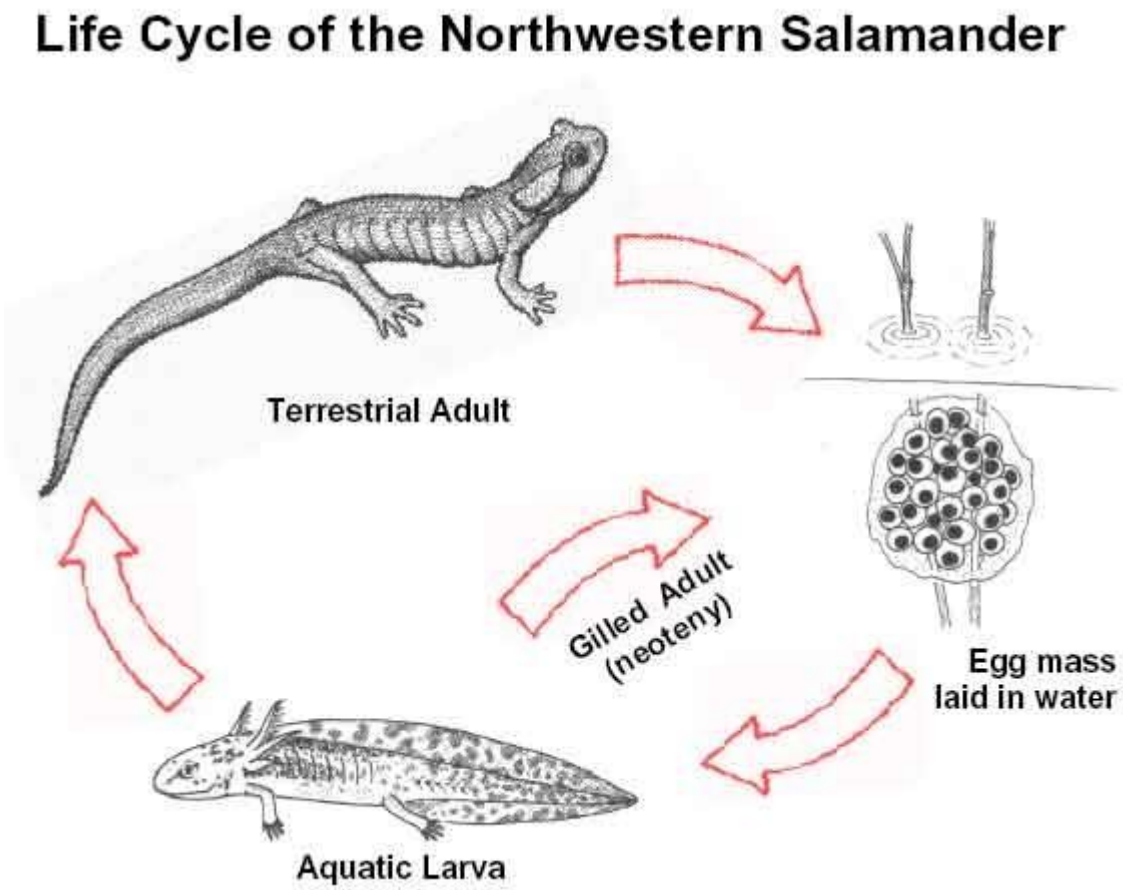

*Figure 7:Life Cycle of the Northwestern Salamander (Ambystoma gracile)(*Amphibians – Reproduction Study Guide - Inspirit, *n.d.)*

By contrast, anuran larvae (tadpoles) are more fish-like in appearance. Tadpoles have short, rounded bodies and long, laterally compressed tails with dorsal and ventral fins. Their mouths are often recessed and encircled by an oral disk fringed with papillae. Tadpoles feed primarily on bacteria and algae, which they scrape from aquatic plants using rows of keratinous denticles. Their gills are internal and covered by an operculum, with water passing over the gills and expelled through spiracles(Liedtke et al., 2022).

6. Metamorphosis

Metamorphosis marks the transition from larval to adult life in amphibians and involves dramatic changes in physiology, biochemistry, structure, and behaviour as shown in Figure 8 and 9 . This transformation, which is controlled by hormones, often includes a shift from an

aquatic to a terrestrial lifestyle. In salamanders, these changes are relatively subtle, including the resorption of gills, closure of gill slits, development of lungs, and the formation of eyelids and a maxillary bone. By contrast, anuran metamorphosis is much more radical. The limbs develop fully, the forelimbs break through the opercular wall, the tail shrinks and is resorbed, dermal glands develop, and the skin thickens. As the lungs become functional, gills and associated circulation are resorbed. The tadpole's mouthparts are replaced with adult mouthparts, and the digestive system is restructured for the adult diet. Eyelids form, the eyes grow larger, and sensory organs are modified to facilitate the shift to terrestrial life.

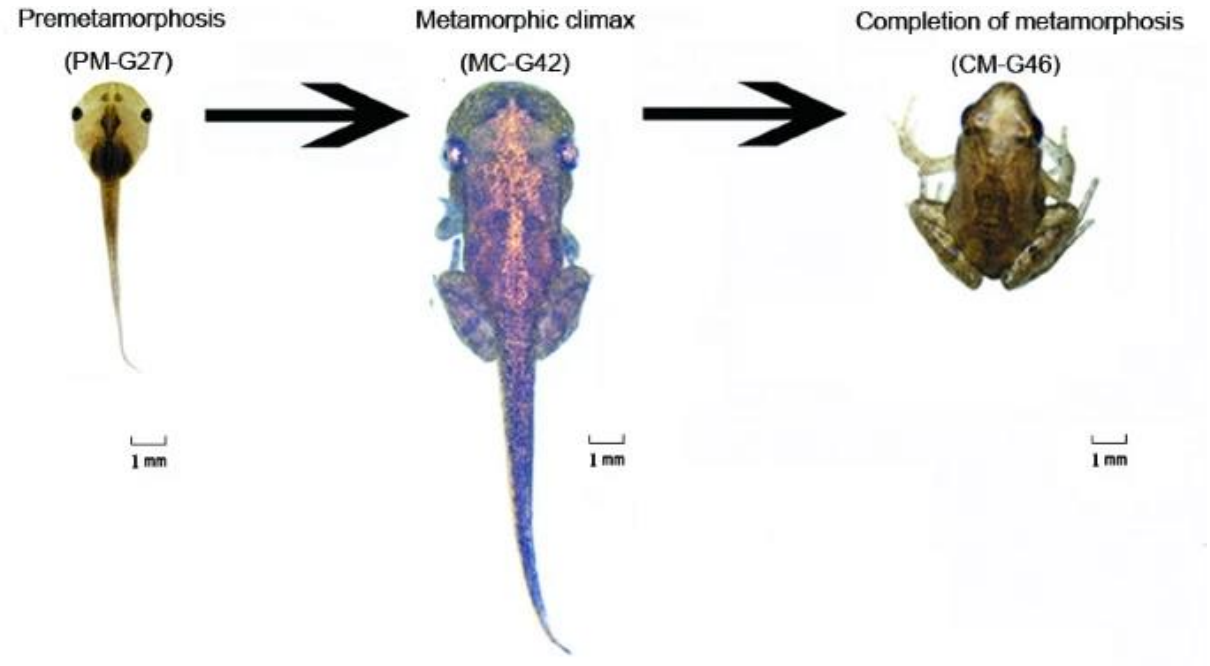

Figure 8:Stages of Frog Metamorphosis: From Premetamorphosis to Metamorphic Climax and Completion(Tata, 2006)

7. Heterochrony

Heterochrony refers to changes in the timing and rate of developmental events, and it is a common feature in amphibian evolution, especially in salamanders. One example is paedomorphosis, where larval traits are retained into adulthood(Reiss, 2002). The axolotl (Ambystoma mexicanum), for instance, retains its larval gills as an adult. In the mole salamander (Ambystoma talpoideum), some populations show delayed development of several

traits, allowing larvae to reach sexual maturity while still in their larval form. Heterochrony can also explain larval traits in adult salamanders from families such as Cryptobranchidae (hellbenders) and Proteidae (olms and mudpuppies)(Müller et al., 2007; Reiss, 2002).

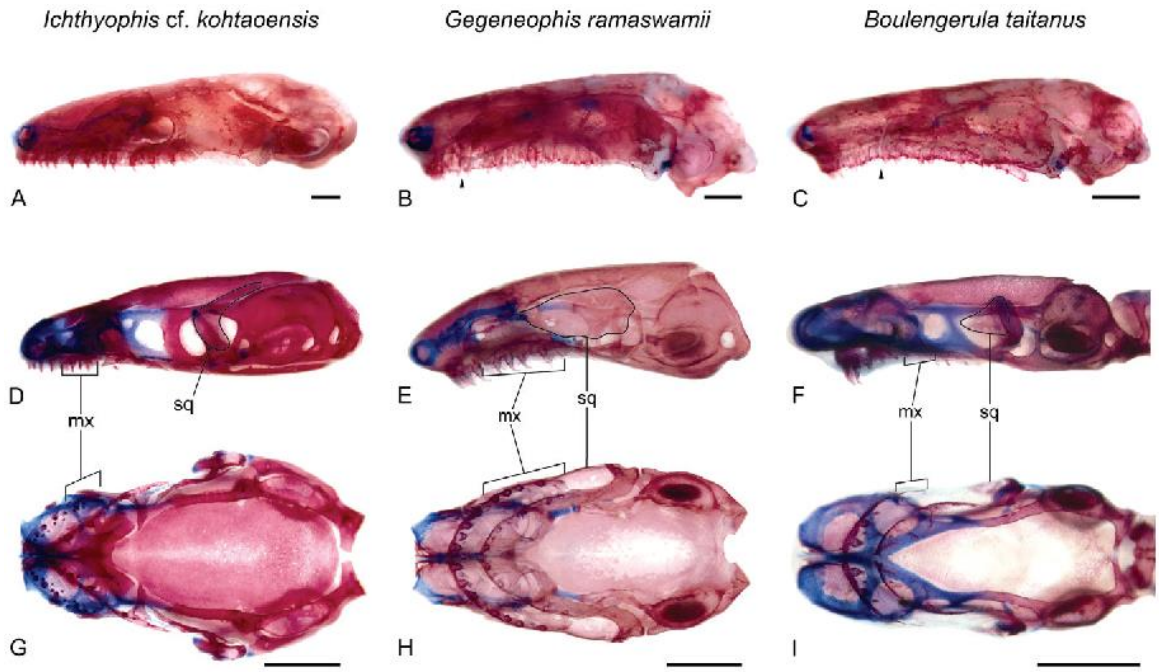

Figure 9:Comparative Cranial Morphology in Caecilian Species: Ichthyophis cf. kohtaoensis, Gegeneophis ramaswamii, and Boulengerula taitanus(Müller et al., 2007)

8. Life Cycle and Reproduction

Most amphibians follow a biphasic life cycle involving aquatic eggs and larvae that metamorphose into terrestrial or semi-aquatic juveniles and adults. Amphibians typically produce large clutches of eggs, with some species like the tiger salamander (Ambystoma tigrinum) laying over 5,000 eggs, while large anurans like the bullfrog (Lithobates catesbeianus) can lay up to 45,000 eggs. The size of the eggs and the temperature of the water greatly influence the development time, with warm water speeding up the process and cold water delaying it.

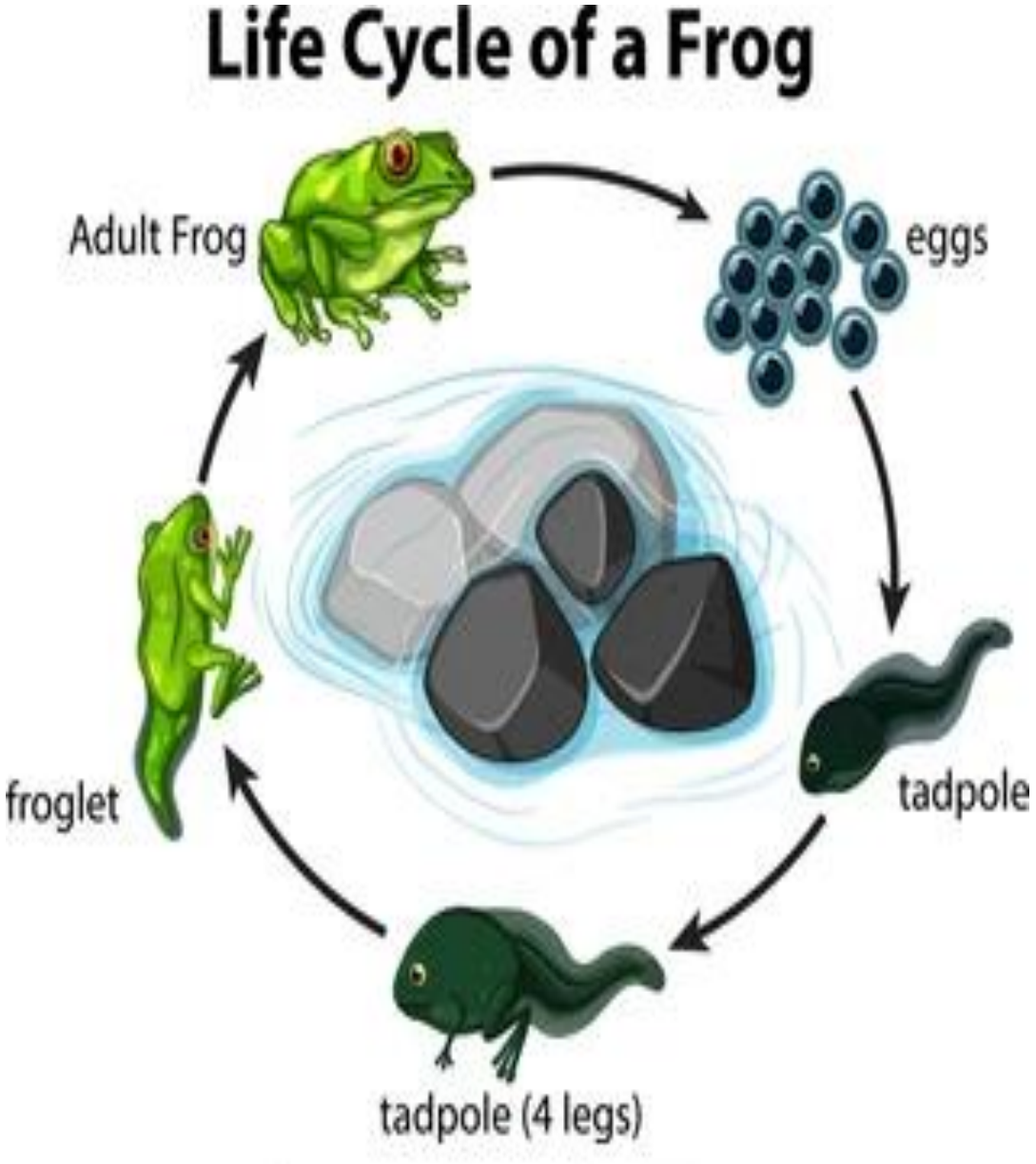

Figure 10:frog life cycle(Schoch, 2009)

The frog life cycle has five main stages:

- Eggs: Laid in clusters in water.
- Tadpole: Hatches from the egg, lives in water, breathes through gills.
- Tadpole with Legs: Begins developing legs, starting with hind legs.
- Froglet: Almost fully developed; tail starts to disappear.
- Adult Frog: Fully formed, with lungs for breathing air, ready to lay eggs.

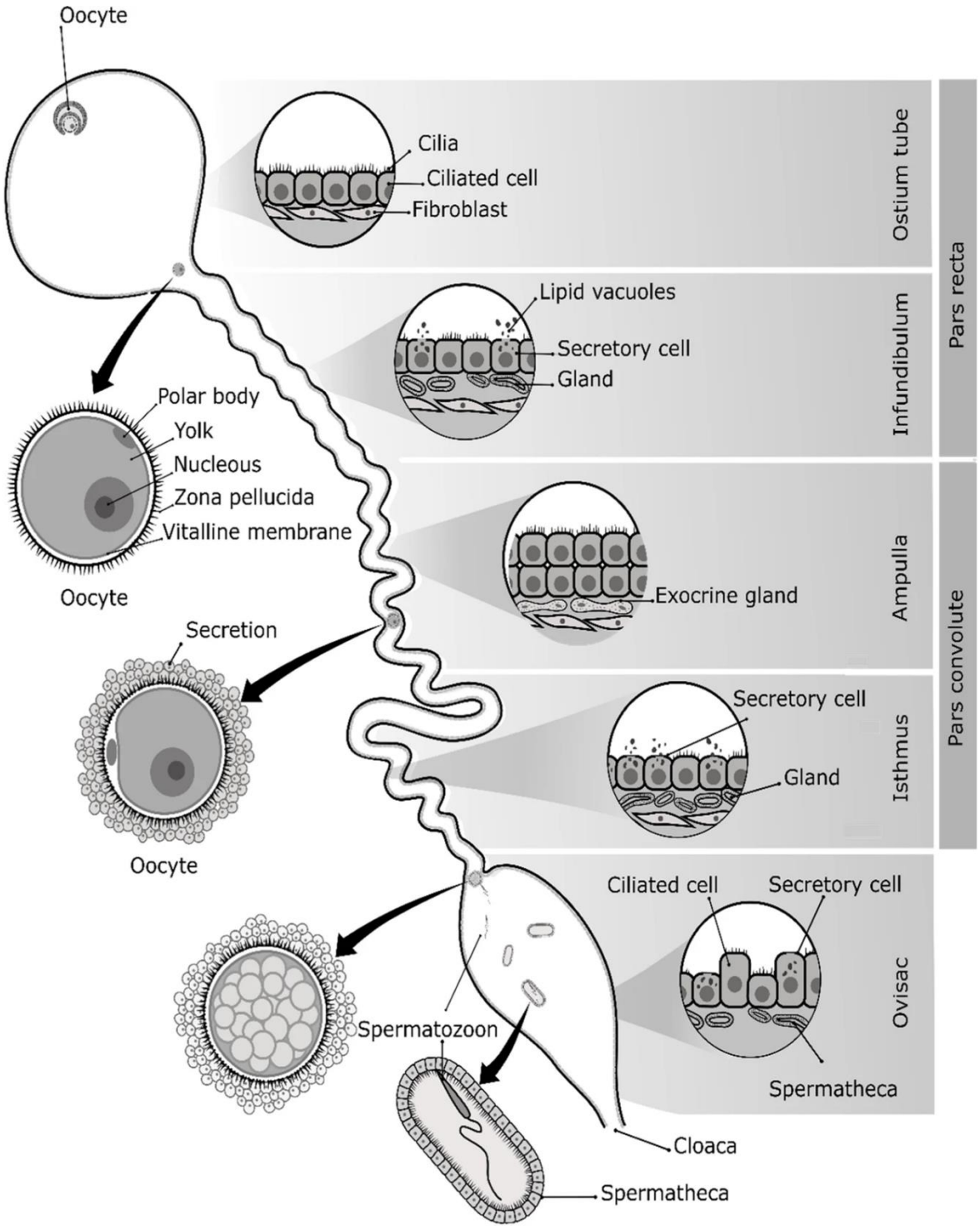

Figure 11: Oviductal regions of frogs..

The oviduct consists of three regions: the infundibulum, the ampulla, and the isthmus. These conform with the "pars convolute" and "pars recta". The "pars recta" nurtures and transports the oocytes to the ovisac. The ostium. The ostium tube is the transition from the "pars recta" into the infundibulum. Generally, the ostium tube is composed of numerous cilia and is aglandular. The infundibulum is formed of ciliated cells, glands, and secretory cells with lipid-secreting granules. The ampulla is a highly secretory portion of the "pars convoluta". The exocrine glands and epithelium secrete a jelly-like substance to surround and nurture the oocyte. The isthmus has mucosal folds, ciliated cells with short cilia, and ceramide-secreting cells. Finally, the ovisac stores the oocytes, and the spermatheca store sperm for fertilization(Graham & Morgan, 1966)

Stage I: Previtellogenic Oocytes

- ✓ Oocyte Size: 50–300 μm in Xenopus laevis, Rana lessonae, and other species.
- ✓ Characteristics: Transparent cells with granular cytoplasm, a large central nucleus, and Balbiani bodies (complexes of organelles) that are present only in early oogenesis.
- ✓ Follicle Structure: Oocytes are surrounded by a single layer of somatic pre-follicular cells. Primordial germ cells are large and yolk filled.
- ✓ Oogonia: Primary oogonia are small, mitotically active, surrounded by somatic cells, and become secondary oogonia.

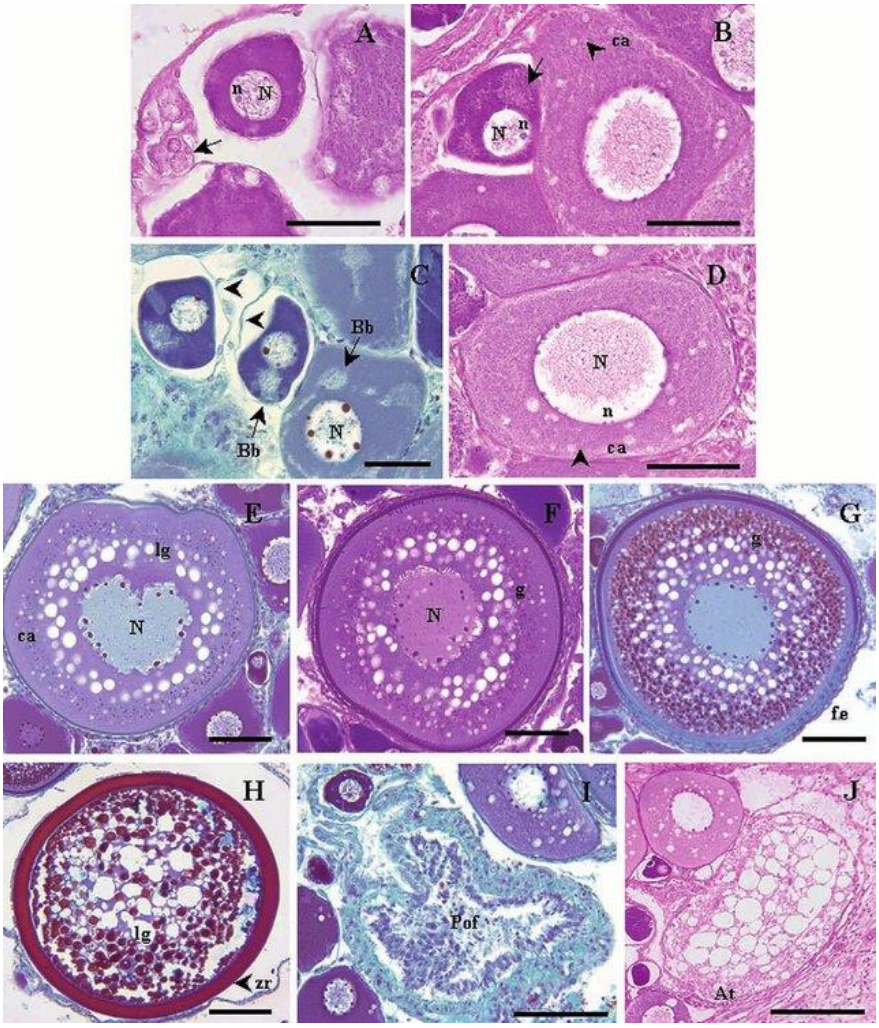

Figure 12:Stages of Oogenesis in O. minor: A Detailed Overview of Oocyte Development(Embryo Development and Ageing in Birds and Mammals - Search Results - EBSCO Discovery Service, n.d.)

Stage II: Early Vitellogenic Oocytes

- ✓ Oocyte Size: 300–450 µm in Xenopus laevis, 250–500 µm in other species.
- ✓ Characteristics: During the first meiotic prophase, oocytes accumulate yolk (in the form of vitellogenin) and start to grow. Oocytes become opaque and begin storing lipids and mucopolysaccharides.
- ✓ Follicle Structure: Oocytes are surrounded by follicle cells and an acellular vitelline envelope. Atretic (degenerating) follicles are absorbed.
- ✓ Pigmentation: Oocytes are not yet pigmented, with visible pigmentation absent due to low melanin synthesis.

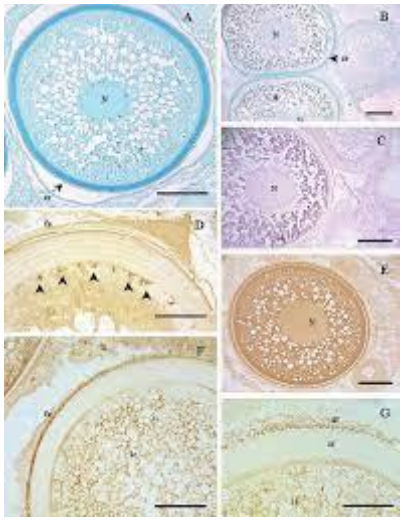

Figure 13:Photomicrography of Swordfish Ovarian Maturation Stages: Structural and Cytological Changes in Oogenesis(Méndez-Tepepa et al., 2023)

Stage III: Midvitellogenic Oocytes

- ✓ Oocyte Size: 400–600 µm in Xenopus laevis, 700–800 µm in Gastrotheca species.
- ✓ Characteristics: Oocytes undergo further vitellogenesis, accumulating more yolk proteins (up to 73% in some species). Melanin begins to accumulate, and the oocytes take on dark pigmentation.
- ✓ Follicle Structure: The follicles develop more blood vessels, and melanosomes give the oocytes a dark brown or black appearance.

- ✓ Pigmentation and Growth: Increased pigmentation and growth, with oocytes surrounded by two cellular layers.

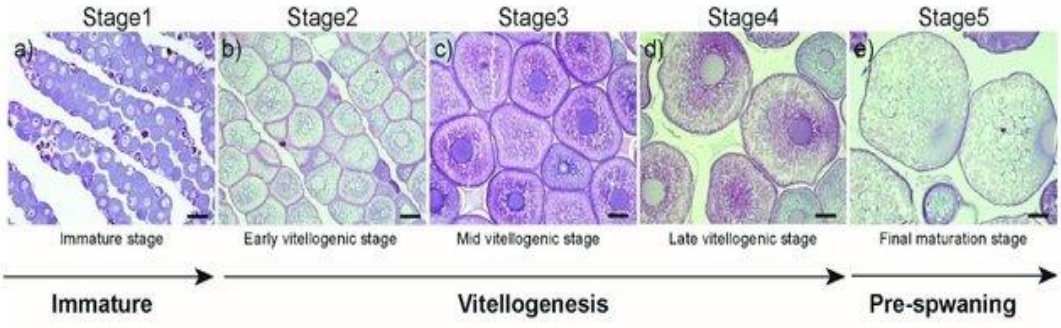

Figure 14:Histological Stages of Oocyte Development in Response to Salmon Pituitary Extract (SPE) Treatment(Méndez-Tepepa et al., 2023)

Stage IV: Late Vitellogenic Oocytes

- ✓ Oocyte Size: 600–1000 µm in Xenopus laevis, 800–900 µm in Gastrotheca species.
- ✓ Characteristics: Oocytes are filled with yolk and pigment granules, with a noticeable increase in blood vessels around the oocyte.
- ✓ Follicle Structure: Follicles consist of theca cells and granulosa cells. The theca cells are responsible for producing hormones like estradiol and progesterone.
- ✓ Growth: Oocytes protrude into the ovarian cavity, becoming increasingly large and pigmented.

Stage V: Vitellogenic Oocytes

- ✓ Oocyte Size: 1000–1200 µm in Xenopus laevis, 700–1000 µm in Rana ridibunda.
- ✓ Characteristics: Oocytes are uniformly pigmented and large, with well-developed microvilli on their surface.
- ✓ Follicle Structure: The follicle consists of thick cuboidal cells, with an increased number of blood vessels. Oocytes are surrounded by the zona pellucida, an extracellular glycoprotein layer.

- ✓ Animal-Vegetal Polarity: Polarity between animal and vegetal poles becomes well defined in some species.

Stage VI: Post vitellogenic Oocytes

- ✓ Oocyte Size: 1200–1300 µm in Xenopus laevis, up to 3000 µm in Gastrotheca species.
- ✓ Characteristics: Oocytes complete vitellogenesis and reach their full size. Pigmentation ranges from brown to pale yellow, depending on the species.
- ✓ Follicle Structure: Microvilli extend between the oocyte and follicle cells. A thick vitelline envelope forms around the oocyte.
- ✓ Hormonal Control: Maturation-promoting factors (MPF) trigger the breakdown of the nuclear membrane. Progesterone, produced by the follicle cells, plays a critical role in oocyte maturation leading to ovulation.

Figure 15:Oocyte Development Stages in Amphibians

9. Food and Feeding

Adult amphibians are carnivorous, feeding on a wide range of prey. Earthworms are the primary diet for burrowing caecilians, while anurans and salamanders eat insects, arthropods, and, in some cases, small vertebrates such as birds and mammals. Many salamanders and diurnal anurans actively forage, but others use a sit-and-wait approach. Caecilians locate underground prey using a specialized chemosensory tentacle and capture it with a strong bite. Aquatic salamanders, like lunged salamanders, suck prey into their mouths by expanding their oral cavities, while terrestrial salamanders extend sticky tongues to catch prey from a distance. Anurans, depending on their species, may also employ advanced feeding techniques, such as the "lingual flip," in which the tongue's surfaces twist and flip during extension to capture prey. In summary, amphibians showcase a rich variety of reproductive, developmental, and feeding strategies. Their life cycles, driven by complex hormonal and physiological processes, are adapted to aquatic, terrestrial, and even fossorial environments, reflecting their evolutionary success across diverse habitats.

iii Form and function

Amphibians possess several unique features that distinguish them from other vertebrates. Their skin is crucial for cutaneous respiration, allowing gas exchange and water balance, and it also contains poison glands for defence. They have evolved advanced vision, including colour perception, and a specialized hearing system that detects both airborne and seismic signals. The three amphibian orders salamanders, caecilians, and anurans have distinct structural adaptations. Salamanders are characterized by their elongated bodies and limbs, with less specialized locomotion. Caecilians are limbless, burrowing creatures with compact skulls and enhanced chemosensory abilities. Anurans are specialized for jumping, with powerful hind limbs and well-developed auditory and visual systems. These structural differences reflect each

group's unique evolutionary history and adaptations to their environments, ranging from terrestrial to aquatic and subterranean habitats.

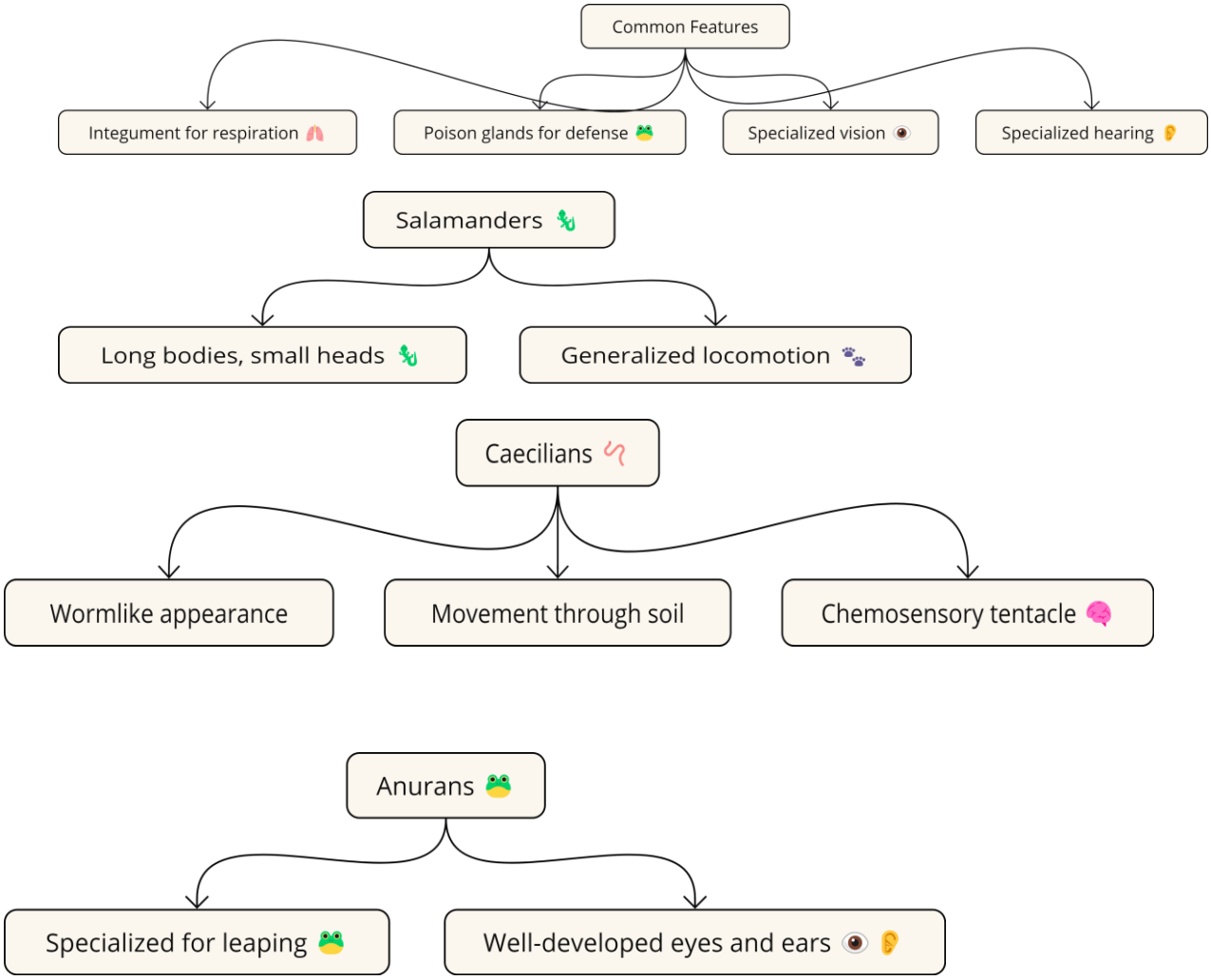

Figure 16:Comparative diagram of Amphibians: Salamanders, Caecilians, and Anurans

II.2.Development of Birds

Birds (class Aves) are a group of over 10,400 living species, distinguished primarily by their feathers, a unique characteristic among animals(Lack, 1947). They are warm-blooded vertebrates closely related to reptiles, more so than to mammals, and they share some traits with mammals, such as having a four-chambered heart. Birds have forelimbs modified into wings, similar to bats, and lay hard-shelled eggs(Embryo Development and Ageing in Birds and Mammals - Search Results - EBSCO Discovery Service, n.d.). They also possess keen vision, which is their primary sense for perceiving the environment, though their sense of smell is not well developed, and their auditory range is somewhat limited. Most birds are diurnal (active during the day), and more than 1,000 extinct species have been discovered through fossil records(Nord & Giroud, 2020).

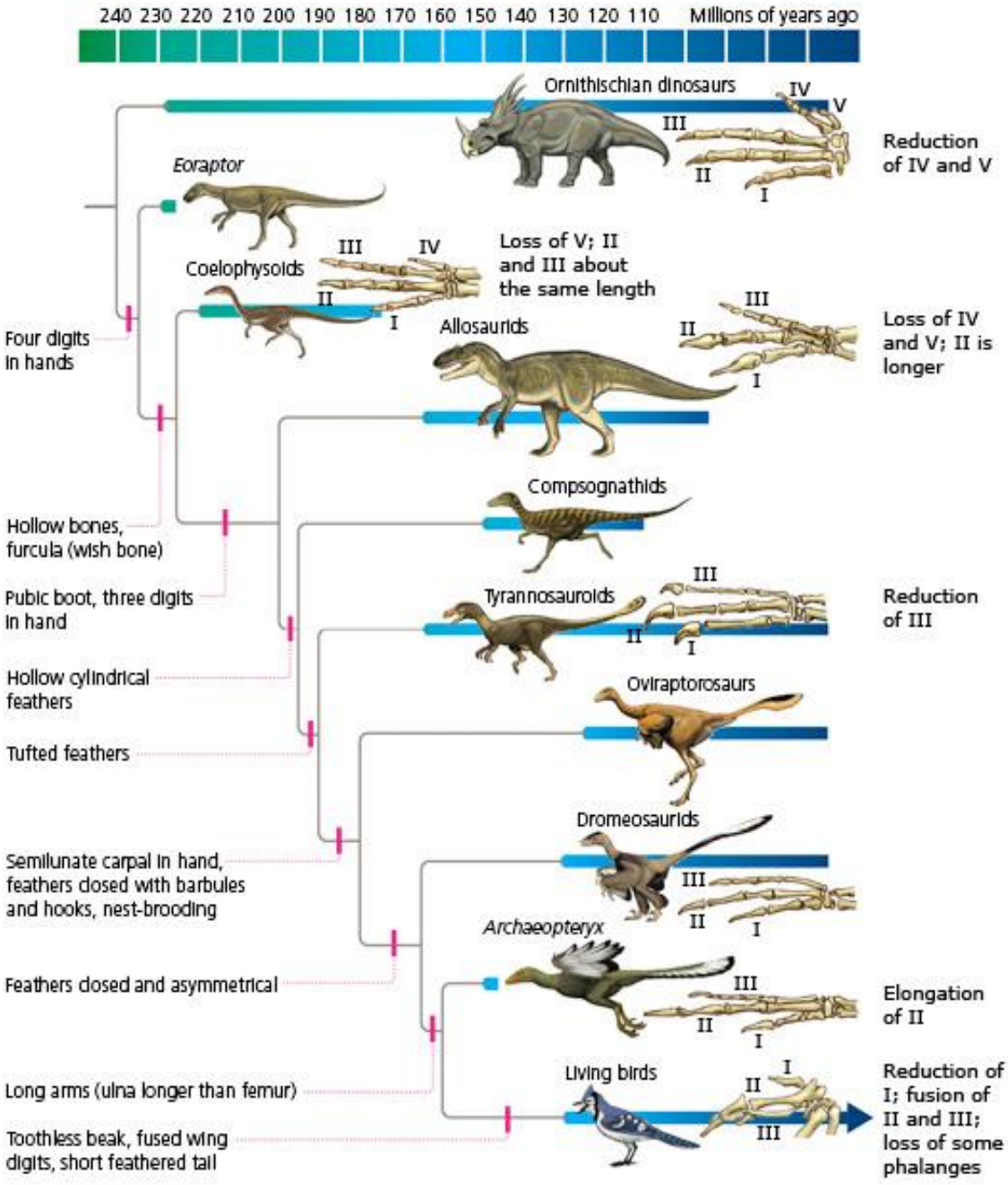

Figure 17:Bird phylogeny adapted from / branched downy feather and symmetrical contour feather from The Tangled Bank, used with permission of the author, Carl Zimmer, and publisher, Roberts & Company, Greenwood Village, Colorado(Gilbert SF. Developmental Biology., 2000)

Cultural Significance

Birds have long held both material and cultural importance. Prehistoric humans, as seen in the Lascaux Grotto in France, created depictions of birds, and birds have featured prominently in mythology and literature worldwide. Long before the advent of ornithology (the scientific study of birds), people's fascination with birds was reflected in conversation, stories, and art. For example, Ancient Egyptians included bird figures in their hieroglyphs and paintings, and the Bible mentions Noah's use of a raven and a dove during the Great Flood.

Birds have often been used symbolically in both language and art. Aesop's fables feature many bird characters, and the Physiologus and bestiaries of the Middle Ages used birds to convey moral lessons, though with limited understanding of the birds themselves. In some cultures, birds were believed to have supernatural abilities. For instance, Australian Aborigines believed the black-and-white flycatcher could overhear conversations and relay them to enemies, while Pacific Islanders viewed frigate birds as omens and symbols of the sun. The raven has long been a symbol of dark prophecy, immortalized in Edgar Allan Poe's poem "The Raven."

Birds have also served as symbols of power and prestige. Eagles, for instance, have represented strength in many cultures, from Native American rituals, where eagle feathers symbolized peace and were used in headdresses, to European heraldry. The resplendent quetzal, the national bird of Guatemala, was worshipped by the ancient Mayans and Aztecs. Other notable symbolic birds include the phoenix, representing resurrection, and the owl, often seen as a symbol of wisdom but also a harbinger of death in Native American traditions. In Christian iconography, birds have symbolized the transcendent soul, with a bird trapped in foliage representing the soul's entanglement in worldly materialism.(Gilbert SF. Developmental Biology., 2000; Nord & Giroud, 2020)

Modern Birdwatching and Environmentalism

In contemporary times, bird-watching has become a popular recreational activity, closely linked to the rise of environmentalism. This hobby evolved from the 19th-century practice of shooting and collecting birds, shifting toward identifying and observing birds with binoculars and scopes. The transition to non-lethal birdwatching began around 1900 with campaigns aimed at stopping the large-scale killing of birds for food and millinery (feathers for fashion). The activity gained momentum with the publication of field guides, improvements in photography and sound recording, and efforts to protect bird populations.

By the mid-20th century, bird-watching had evolved from simply keeping lists of observed species to more detailed studies of bird behavior, migration, and ecology. This shift was supported by the practice of bird banding (known as ringing in the UK), and organizations like the British Trust for Ornithology and the National Audubon Society helped coordinate both professional and amateur efforts to observe and study birds scientifically.

In summary, birds are remarkable creatures with a rich cultural history and significant scientific interest. From symbolic roles in mythology and art to modern conservation and recreational activities, birds continue to captivate human imagination and inspire efforts to protect their diverse populations.(Gilbert SF. Developmental Biology., 2000)

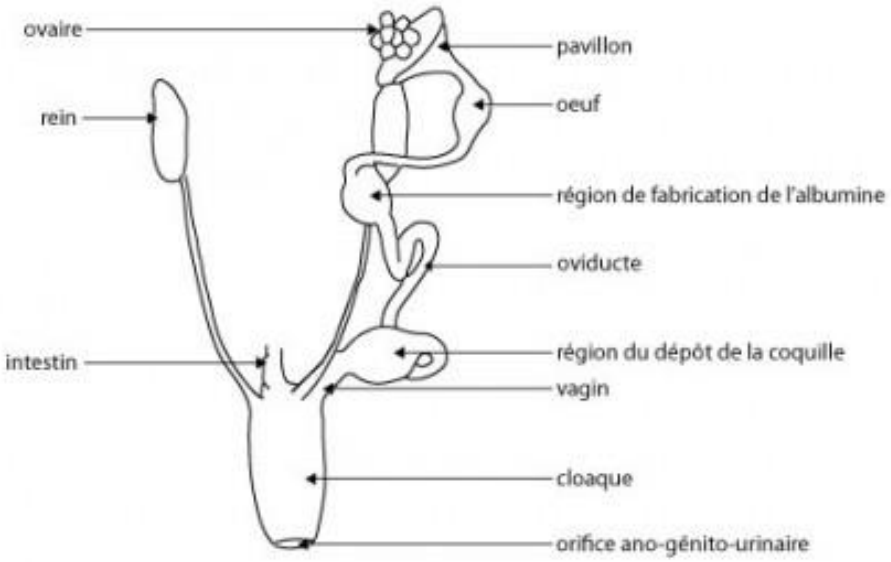

*Figure 18 from the diagram: ovary, pavilion, egg, region of albumin production, oviduct, region of shell deposition, vagina, cloaca, ano-genito-urinary orifice (*Reproduction in Birds | Vigie-Nature École*, n.d.)*

1. Egg Formation and Fertilization

Birds are oviparous, meaning they lay eggs. The development of a bird starts with the fertilization of an egg inside the female's reproductive tract. The fertilized egg consists of:

- Yolk: Provides nutrients for the growing embryo.
- Albumen (egg white): Provides additional nutrition and protection.
- Shell membranes and the shell: Protect the embryo and prevent water loss while allowing gas exchange.

The yolk, encased in protective membranes and surrounded by the albumen, is enclosed in a hard, calcium-rich shell before being laid by the female.(Gilbert SF. Developmental Biology., 2000)

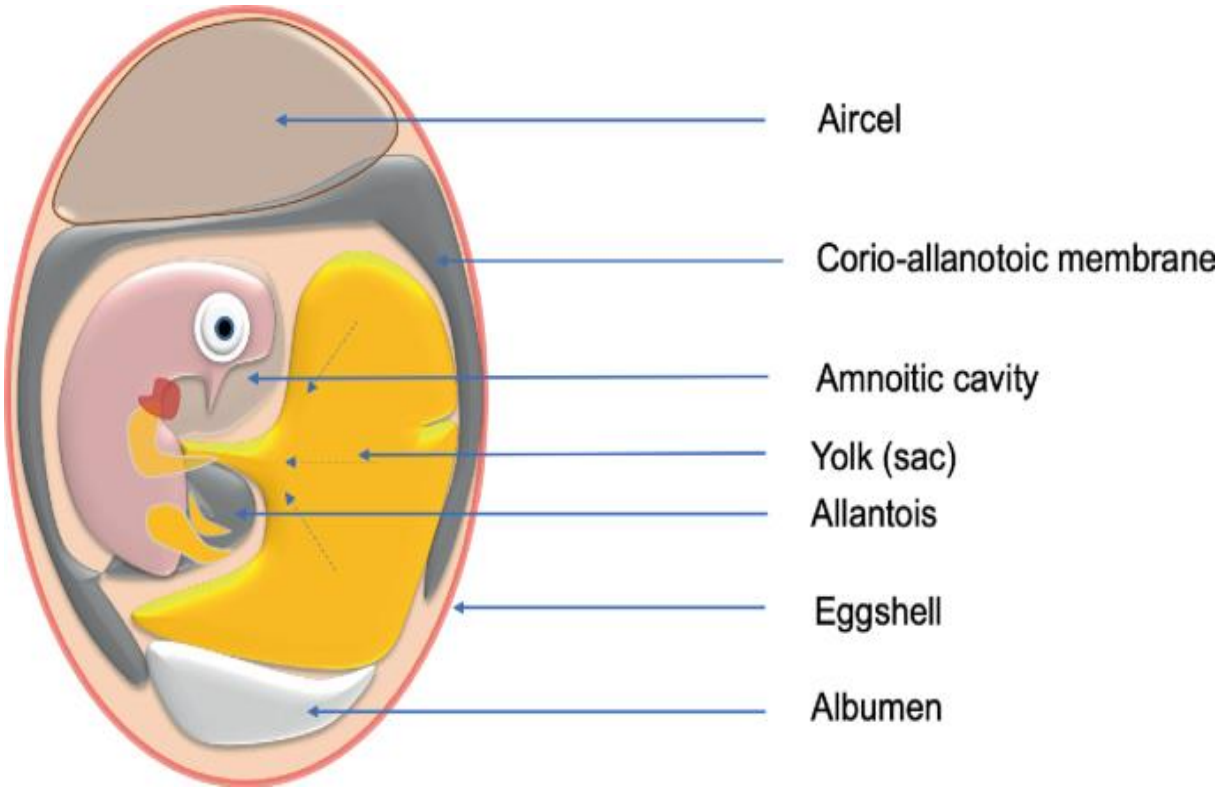

Figure 19 :Interior view of a chicken's egg

*(*Chicken Embryo Development - Candling Eggs during Incubation, *n.d.)*

2. Embryonic Development Inside the Egg

After the egg is laid, embryonic development begins. This process is influenced by the warmth provided by incubation (often by the parent birds sitting on the eggs). The key stages include:

- Cleavage: Shortly after fertilization, the embryo undergoes rapid cell division (cleavage), forming a blastoderm on the yolk's surface.
- Gastrulation: The blastoderm differentiates into three germ layers—ectoderm, mesoderm, and endodermthat will give rise to the different tissues and organs of the bird.
- Organogenesis: The major organs begin to form. The heart starts to beat early in development, and the nervous system, digestive system, and musculoskeletal system begin to take shape.(Gilbert SF. Developmental Biology., 2000; Toledo Fonseca et al., 2013)

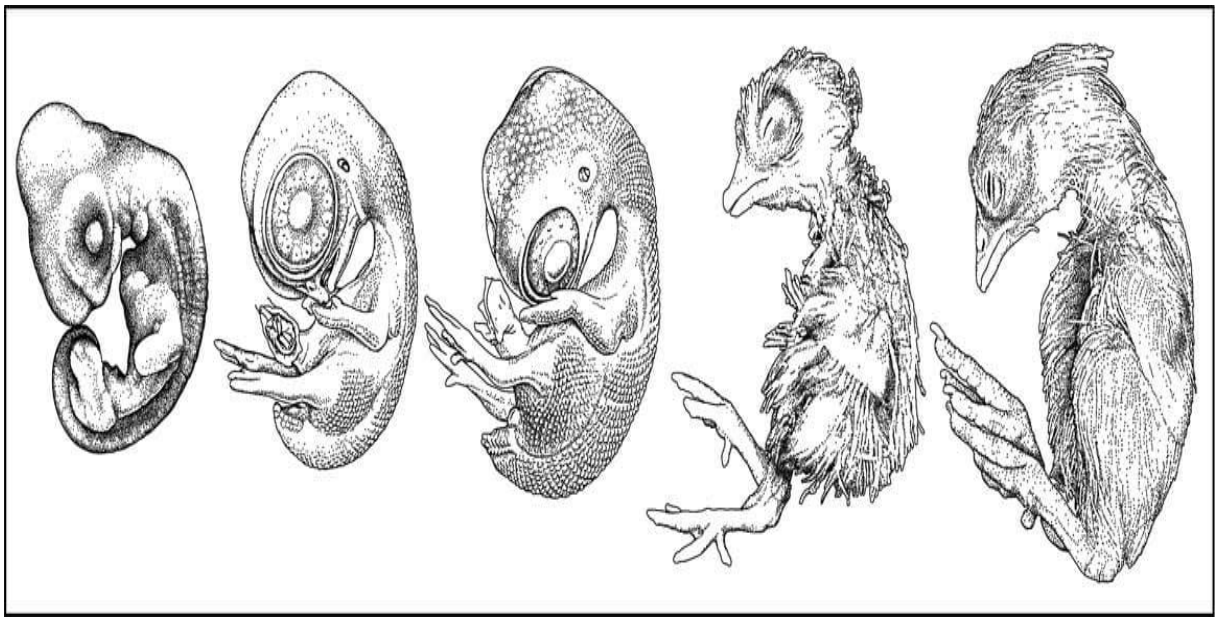

Figure 20:Embryonic Development Inside the Egg

*(*Raptor Resource Project: Bald Eagle and Bird of Prey Cams, *n.d.)*

During embryonic development, several specialized structures assist the growing bird:

- Amnion: A membrane that surrounds and protects the embryo in a fluid-filled cavity.
- Chorion: Assists in gas exchange between the embryo and the external environment.
- Allantois: Stores waste products and also helps with respiration.
- Yolk sac: Provides nutrients from the yolk to the embryo.

3. Hatching

- As the embryo matures, it fills most of the egg, consuming the yolk. When development is complete, the chick uses a specialized structure called the egg tooth (a small, temporary structure on the tip of its beak) to break through the eggshell in a process called pipping.
- Hatching can take several hours to a day, during which the chick breaks the shell and emerges. After hatching, the chick is initially weak and vulnerable but quickly begins to develop its physical strength.(Embryo Development and Ageing in Birds and Mammals - Search Results - EBSCO Discovery Service, n.d.)

Figure 21:This undated illustration shows a redition of a close-to-hatching oviraptorosaur dinosaur embryo, which is based on the new specimen 'Baby Yingliang' found in the Ganzhou, Jiangxi Province, southern China. (AFP)

4. Post-Hatching Development

The degree of development at hatching depends on whether the species is precocial or altricial:

- Precocial birds: Hatch with open eyes, covered in down feathers, and are capable of walking or swimming shortly after birth. These species, such as ducks and chickens, are more independent.
- Altricial birds: Hatch blind, featherless, and helpless. They rely on their parents for warmth, food, and protection. This category includes species like songbirds and raptors.(Gilbert SF. Developmental Biology., 2000)

5. Growth to Maturity

- After hatching, birds continue to grow and develop. This involves:
- Feather growth: Birds gradually replace their down feathers with adult feathers through a process called molting.
- Skeletal and muscular development: The bones and muscles, particularly those used for flight, strengthen as the bird matures.
- Flight: In most species, flight feathers develop during the juvenile stage, and the bird begins practicing flight.

Birds reach sexual maturity at varying ages, depending on the species. Once mature, they are capable of reproduction, completing the life cycle.

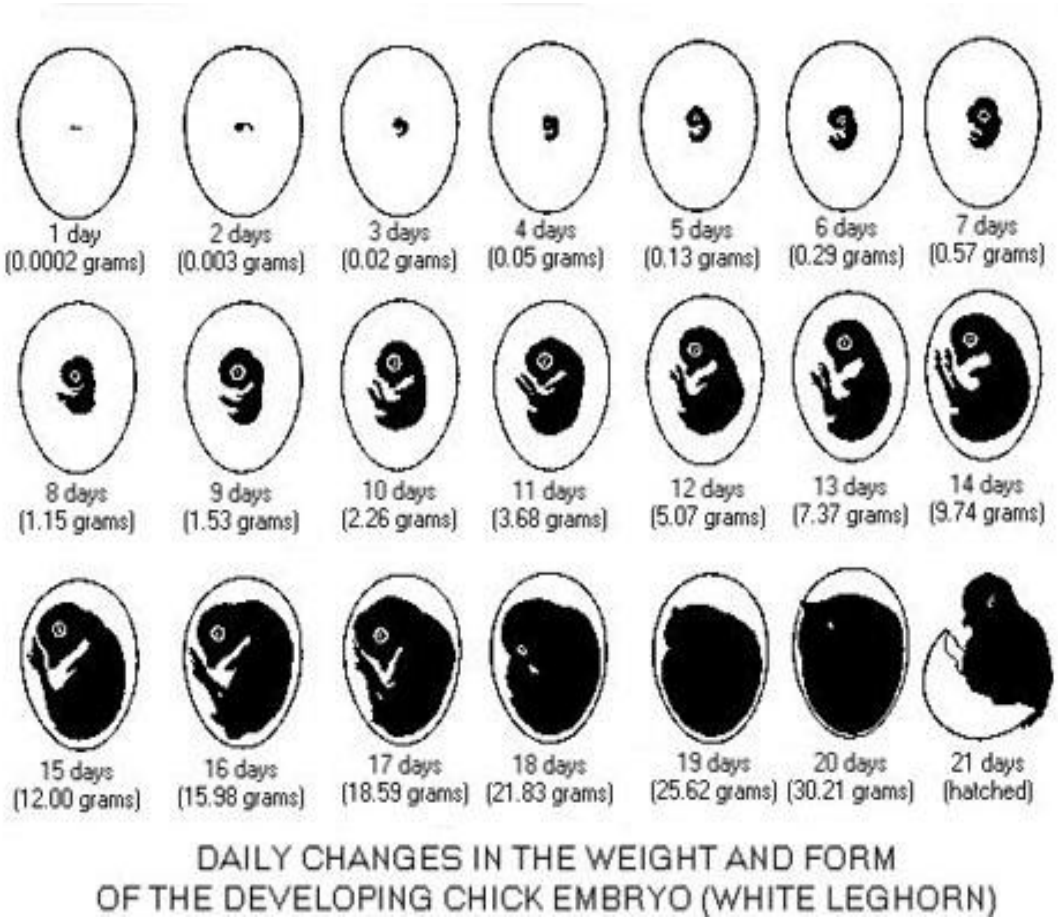

*Figure 22:transformation of the egg(*Stages in Chick Embryo Development | Mississippi State University Extension Service, *n.d.)*

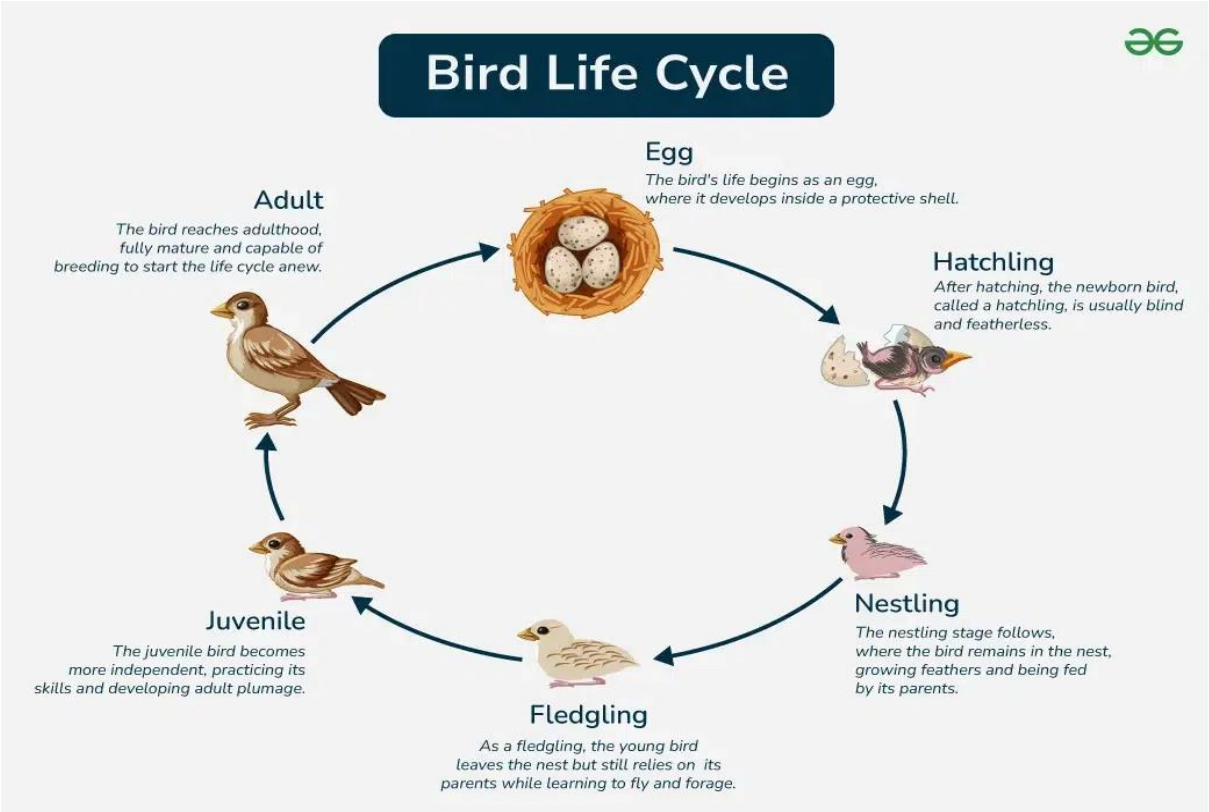

*Figure 23:Bird Life Cycle Diagram(*Bird Life Cycle - 7 Stages, Diagram, and Facts | GeeksforGeeks, *n.d.)*

Summary of Bird Development

- Egg formation and fertilization lead to the laying of an egg containing a developing embryo.
- The embryo undergoes gastrulation and organogenesis, with specialized membranes (amnion, chorion, allantois) supporting growth.
- Hatching occurs after a specific incubation period, with chicks using an egg tooth to break free.
- Post-hatching development varies by species: precocial birds are more developed, while altricial birds are helpless and dependent on parents.
- Birds grow to maturity through feather development, bone strengthening, and, for most species, learning to fly.

II.3.Development of Insects

Insects undergo different types of development depending on the species, with three main forms: ametabolous (no metamorphosis), hemimetabolous (incomplete metamorphosis), and holometabolous (complete metamorphosis).

Insects and mites belong to a large group of animals known as arthropods. Several characteristics differentiate insects from other arthropods. The body is segmented, and the segments are grouped into three sections: head, thorax and abdomen. The eyes, antennae and mouthparts are on the head, and the legs and wings are on the thorax(Costantini et al., 2018).

- **Ametabolous Development**

In ametabolous development, there is no metamorphosis. Insects in this category (such as silverfish) hatch from eggs as immature versions of the adult, differing only in size and reproductive maturity. These insects gradually increase in size through successive molts but do not change significantly in form. Examples: Silverfish, bristletails. In contrast, mites have no antennae, and adult forms usually have four pairs of legs. The head and thorax are smaller and fused in mites. The abdomen is relatively large and bulbous and is not distinctly segmented.(Mito et al., 2010; Nakajima & Ogura, 2022)

An insect’s skeleton is on the outside of its body and is called an exoskeleton. It serves as a support for muscles and internal organs as well as a covering. The body wall of an insect has three layers. The outer layer is cuticle containing chitin, proteins and often pigments. This is secreted by a cellular layer beneath it called

the epidermis. The inner layer is called the basement membrane.(Agrell & Lundquist, 1973)

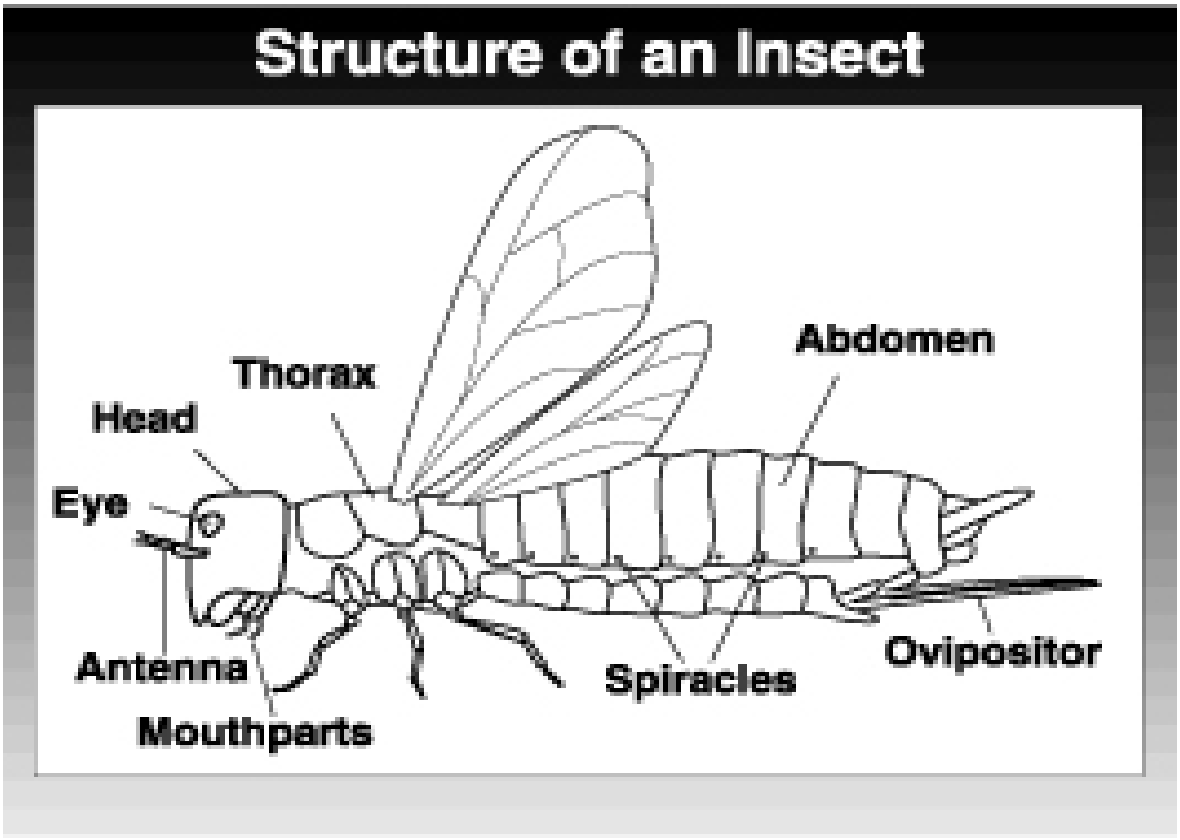

Figure 24:Insect Growth and Development(Costantini et al., 2018)

The type of mouthparts an insect has determines how it feeds and what sort of damage it does. There are two general types: chewing and sucking. Insects with sucking mouthparts suck liquid food through an elongated beak. Insects with chewing mouthparts tear off pieces of host tissue and ingest it. Growth progresses through successive stages. Females lay eggs, which hatch into an immature stage. After passing through a series of immature stages, the insect emerges as an adult. Adults mate and the cycle begins again. Most insects start as eggs, which vary in size, shape and color(Mito et al., 2010). They can be deposited singly or in compact masses but are usually protected in some way. They may be laid in the

ground or in or on plant tissue. Eggs left exposed are often protected from the elements and natural enemies by a coating. Eggs may be round, oval, barrel shaped, disk shaped or suspended on long stalks. The tough outer surface protects the embryo and prevents water loss.(Insect Collection

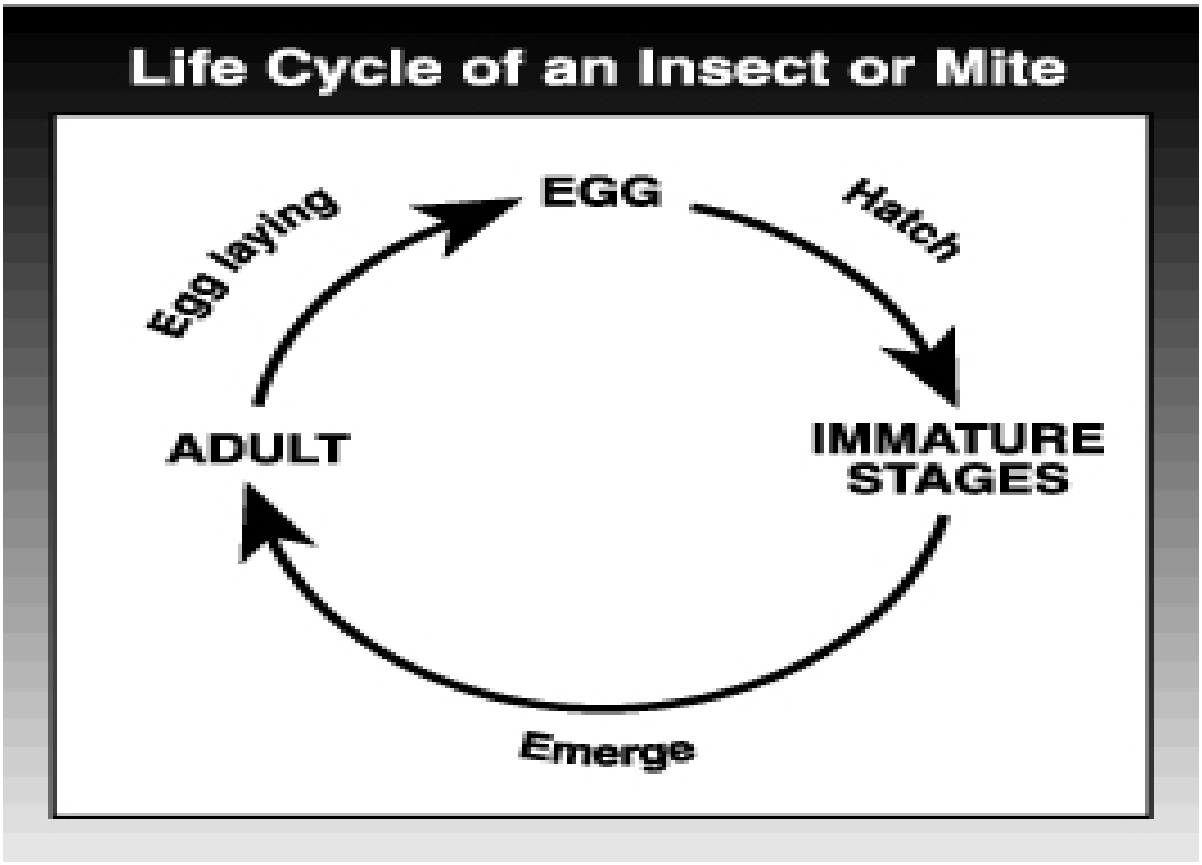

Figure 25: Life cycle of an insect(Costantini et al., 2018)

The first immature stage of the insect hatches from the egg. As the insect's rigid exoskeleton cannot expand much, it must be shed and replaced with a larger one as the insect grows. This process is called molting. The life stage between each molt is called an instar. Molting is governed by hormones. Cuticle secretion and the molt cycle are controlled by ecdysone, a steroid hormone. The hormone is secreted by a gland in the thorax, which is in turn controlled by a hormone from

the brain. Whenever the brain receives the appropriate stimulus, the insect will molt. A new cuticle forms under the old one, then the old exoskeleton splits and the insect wriggles its way out. Many insects eat their own discarded skin. The new cuticle is soft at first. The insect may swallow air to expand its own volume and stretch the new exoskeleton before in hardens, usually within about an hour Insects may undergo gradual metamorphosis, in which the transformation is subtle, or they can undergo a complete metamorphosis, in which each stage of the life cycle has a distinctly different appearance from the one before and the one after the current stage or they can experience something in between. Entomologists classify insects into three groups based on the type of metamorphosis they undergo: ametabolous, hemimetabolous, and holometabolous(*The Types and Stages of Insect Metamorphosis*, n.d.).

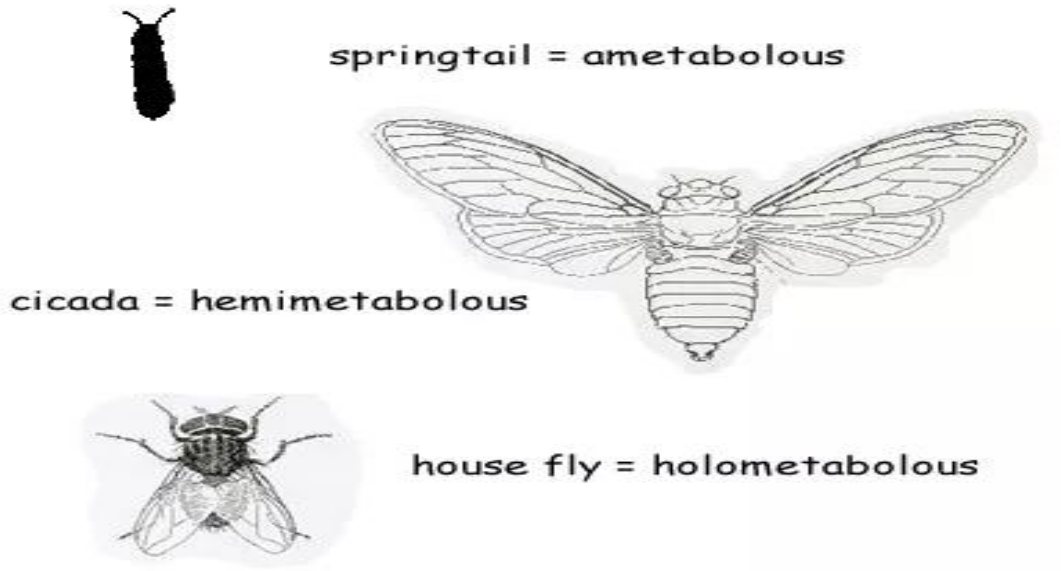

*Figure 26:e type of metamorphosis they undergo: ametabolous, hemimetabolous, and holometabolous.(*The Types and Stages of Insect Metamorphosis, *n.d.)*

- **Hemimetabolous Development (Incomplete Metamorphosis)**

Hemimetabolous insects undergo incomplete metamorphosis, where there is a gradual development from juvenile to adult. After hatching, the immature insect, called a nymph, resembles a smaller version of the adult but lacks fully developed wings and reproductive organs. Nymphs molt several times, and with each molt, they grow larger and develop more adult features. In the final molt, they become fully mature adults with functional wings (if the species has them).(Costantini et al., 2018; *The Types and Stages of Insect Metamorphosis*, n.d.)

Stages: Egg → Nymph → Adult

Examples: Grasshoppers, cockroaches, dragonflies.

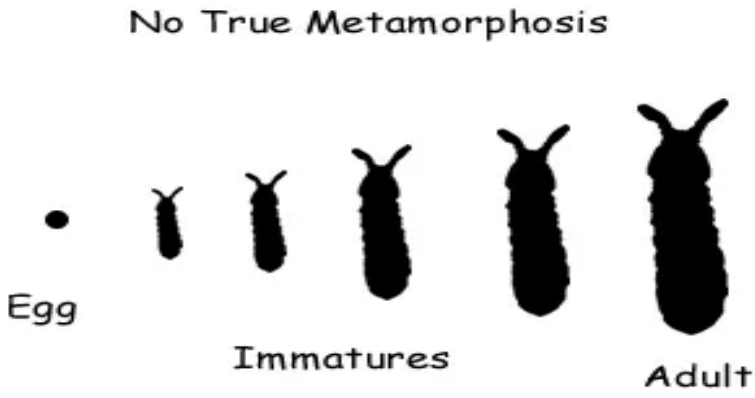

*Figure 27:Ametabolous: Little or No Metamorphosis(*The Types and Stages of Insect Metamorphosis, *n.d.)*

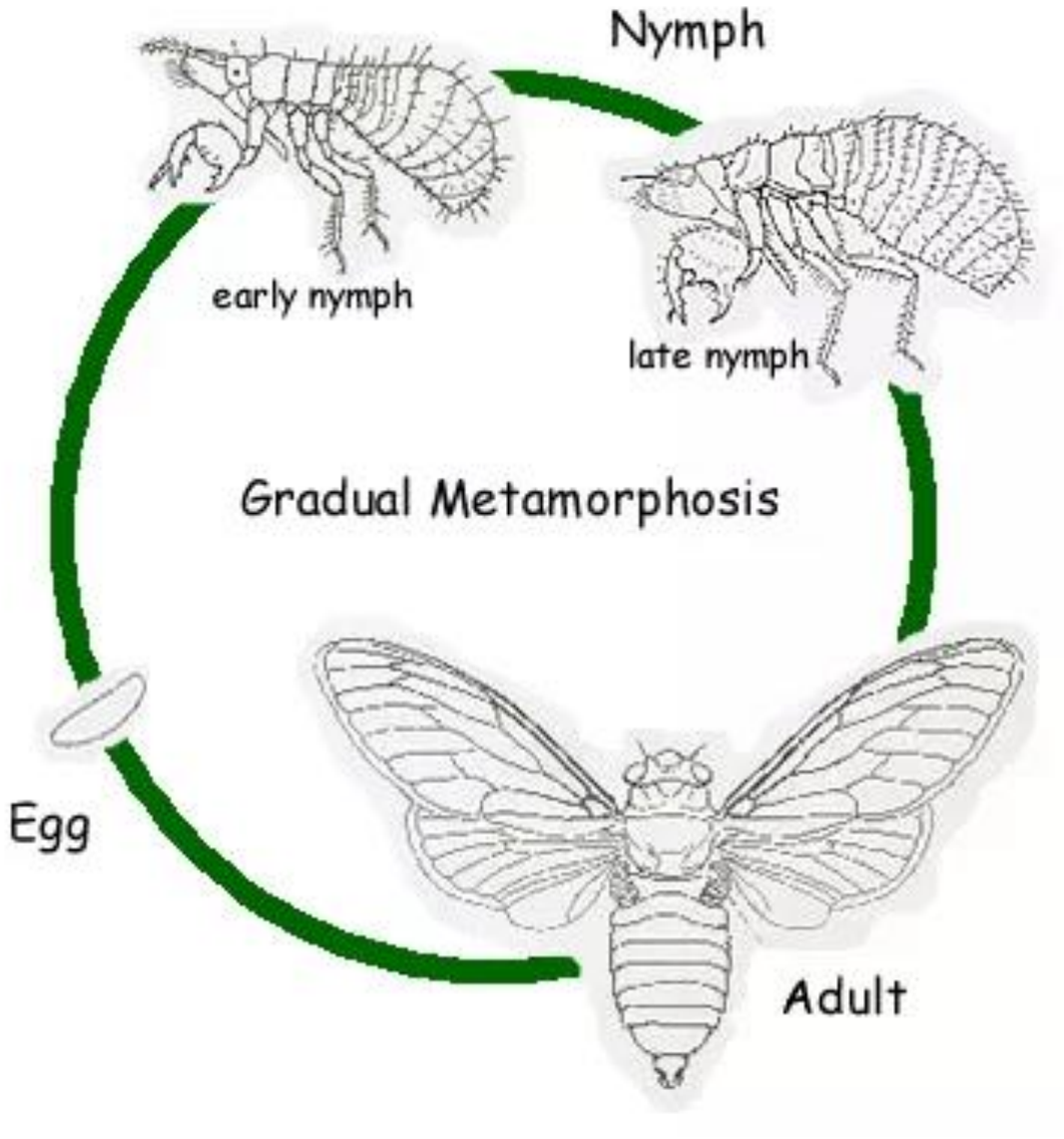

*Figure 28:Hemimetabolous: Simple or Gradual Metamorphosis.(*The Types and Stages of Insect Metamorphosis, *n.d.)*

- **Holometabolous Development (Complete Metamorphosis)**

Holometabolous insects undergo complete metamorphosis, which involves a dramatic transformation through distinct life stages. After hatching, the insect passes through the larval stage, where it often looks worm-like and has a completely different diet and lifestyle than the adult. The larva then enters the

pupal stage (cocoon or chrysalis), during which it undergoes significant reorganization and development into the adult form. Finally, the insect emerges as an adult with wings and reproductive capabilities.(Costantini et al., 2018; *Insects | MAYERiment Gardens*, n.d.)

Stages: Egg → Larva → Pupa → Adult

Examples: Butterflies, beetles, bees, ants.

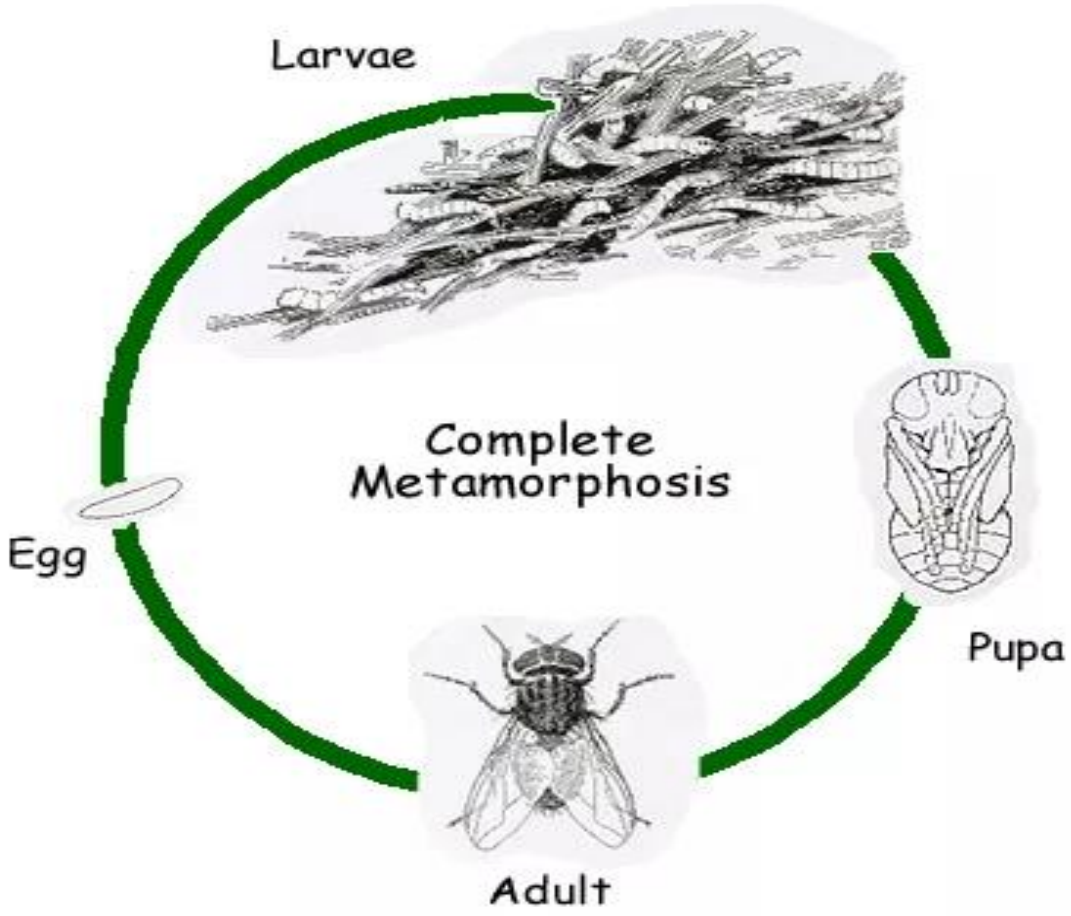

*Figure 29:Holometabolous: Complete Metamorphosis(*The Types and Stages of Insect Metamorphosis, *n.d.)*

The growth and moulting in insects are controlled by hormones. The neurosecretory cells in the brain, under certain stimuli, secrete a hormone prothoracicotropic hormone (PTTH), which activates the prothoracic glands. As a result of this activation, the growth and differentiation hormone (GDH) or ecdysone is secreted. This hormone induces the insects to moult. The corpora alata produce the juvenile hormone QH) or neotenin. High concentration of JH results in larval-pupal moult, lower concentration results in larval-pupal moult and absence of JH results in pupal-adult moult. The two other hormones which also play a role in development are the eclosion and tanning hormones. The eclosion hormone released by the brain controls the process of eclosion in insects while the tanning hormone or burscion regulates the process of tanning and sclerotization of the new cuticle.(*Growth and Development in Insects | Zoology*, n.d.)

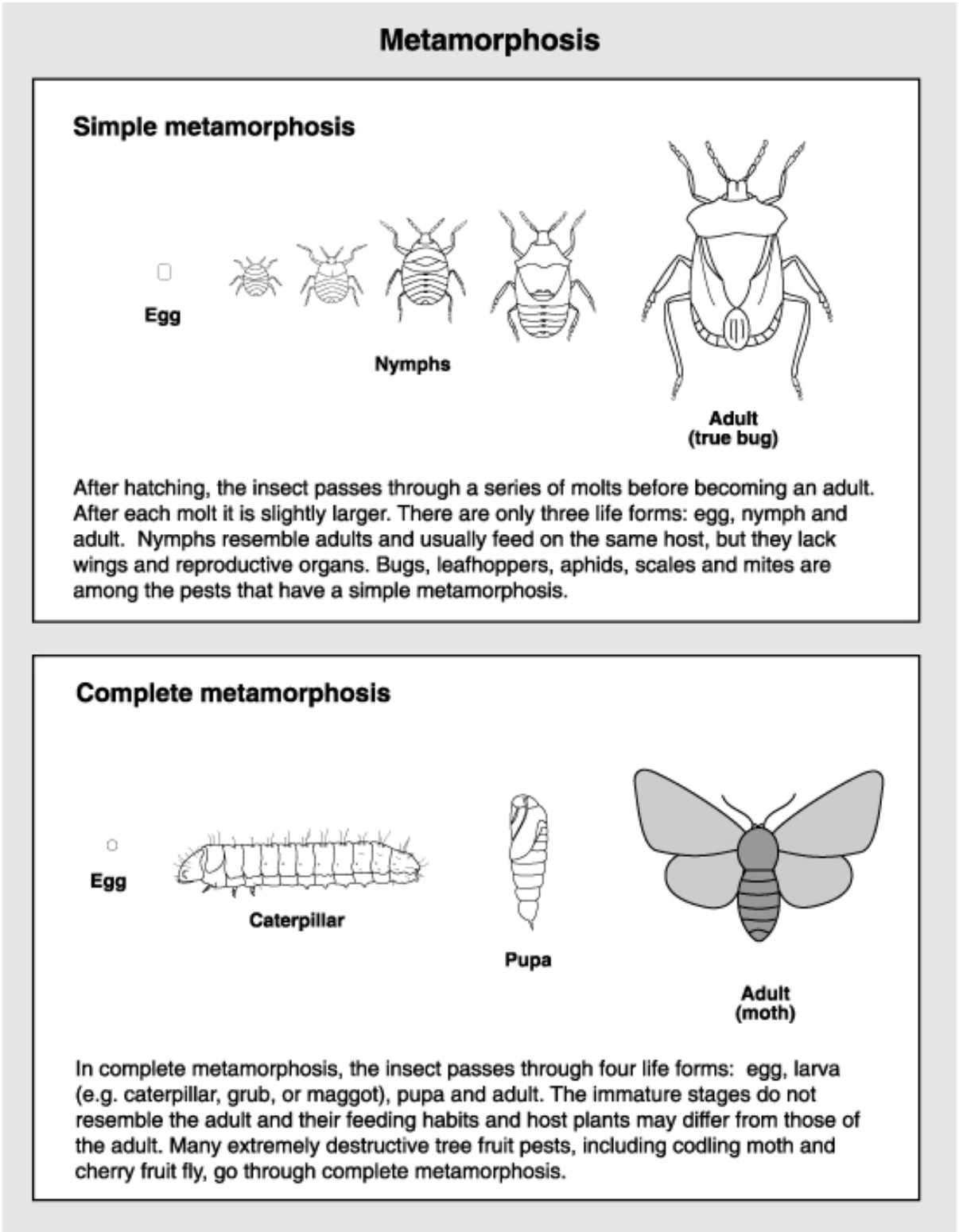

Figure 30:Metamorphosis(Costantini et al., 2018)

Key Features of Insect Development

- Molting: All insects grow by molting their exoskeletons, a process called ecdysis. Each molt allows for growth and the development of adult features.
- Metamorphosis: Insects with incomplete metamorphosis gradually change form, while those with complete metamorphosis undergo a radical transformation between life stages.
- Environmental Influence: Temperature, food availability, and other environmental factors often influence the duration and success of development in insects.

In summary, insect development ranges from gradual changes (as in ametabolous and hemimetabolous insects) to dramatic metamorphoses (in holometabolous insects), with each stage playing a vital role in the insect's lifecycle and ecological adaptation.

III. ELEMENTS NECESSARY FOR DEVELOPMENT

The development of organisms, whether animals, plants, or other life forms, requires several critical elements and factors. These include genetic, environmental, nutritional, and physiological components that contribute to successful growth and differentiation from a single cell to a fully formed organism.

i. Genetic Information

Development begins with genetic material the DNA present in every cell. Genes provide the blueprint for the organism's growth, determining the structure, function, and regulatory processes throughout development. Key aspects include:

- ✓ Genes: Sequences of DNA that encode proteins necessary for cellular function and differentiation.
- ✓ Regulatory genes: Control the timing and extent of gene expression, ensuring that development proceeds in an organized manner.
- ✓ Mutations: Changes in DNA that can alter development, leading to potential abnormalities or evolutionary adaptations.

ii. Environmental Factors

External environmental conditions significantly influence development, particularly for organisms that develop outside the mother's body (such as birds or plants). Important environmental factors include:

- ✓ Temperature: Many organisms require specific temperature ranges for successful embryonic development (e.g., bird eggs needing proper incubation).
- ✓ Moisture: Water is essential for cellular processes, and in many species (e.g., amphibians), development is tightly linked to aquatic environments.
- ✓ Light: Some species, particularly plants, rely on light for the development of certain structures (e.g., leaves) and physiological processes (e.g., photosynthesis).

iii. Nutritional Resources

Adequate nutrition is critical for development, as nutrients provide the building blocks and energy needed for growth. Nutritional resources can come from:

- ✓ Yolk: In animals like birds, the yolk of the egg provides essential nutrients to the developing embryo.
- ✓ Placental transfer: In mammals, nutrients are delivered to the developing fetus through the placenta.
- ✓ Photosynthesis: In plants, nutrients are derived from photosynthesis and nutrient absorption from the soil.

iv. Hormonal and Chemical Signals

Chemical signals regulate many stages of development, ensuring that different parts of the organism grow and differentiate at the correct times and in the right locations. Key elements include:

- ✓ Hormones: These signaling molecules, such as thyroid hormones in amphibians or auxins in plants, guide development, including metamorphosis, growth, and organ formation.
- ✓ Growth factors: Proteins that promote cell division and differentiation, helping tissues and organs form properly.
- ✓ Cell signaling: Communication between cells that coordinates their behavior during development, ensuring organized growth.

v. Cellular Differentiation and Organogenesis

As development progresses, cells begin to specialize into different types through cell differentiation. Critical components include:

- ✓ Stem cells: Undifferentiated cells that have the potential to become specialized cells (e.g., muscle, nerve, or blood cells).

- ✓ Tissue interactions: Different tissue layers interact (e.g., during gastrulation in animals) to form specific organs and systems.

vi. Oxygen and Respiration

Oxygen is crucial for energy production in cells. In embryos that develop within eggs, such as birds, gas exchange occurs through specialized membranes (e.g., the chorioallantois) to ensure the growing embryo receives sufficient oxygen.

vii. Protective Mechanisms

Many organisms have protective structures or mechanisms to safeguard the developing embryo:

- ✓ Egg shells: In birds and reptiles, hard shells protect the embryo from physical damage and desiccation.
- ✓ Amniotic sac: In mammals and birds, this fluid-filled sac cushions the embryo and prevents dehydration

III.2 Heterogeneity of the distribution of reserves

A. Oocyte and Early Embryonic Stages

During oocyte development, the distribution of nutrients (such as yolk in eggs) and organelles (mitochondria, ribosomes) can be uneven. This is known as cytoplasmic heterogeneity. This unevenness can influence the initial polarity of the embryo, where certain regions become more specialized for future developmental roles (e.g., establishing anterior-posterior or dorsal-ventral axes).

B. Localized Determinants

In many species, specific proteins or mRNA molecules are unevenly distributed within the egg or early embryo. These molecules act as morphogenetic determinants, influencing the fate of cells based on their location. For example, maternal effect genes in Drosophila deposit mRNA in certain areas of the egg to guide early patterning.

C. Developmental Gradients

Morphogen gradients, which are concentrations of signaling molecules, are established through differential distribution. Cells exposed to varying levels of these morphogens activate different gene expression programs, leading to cell differentiation.

A classic example of this is the Bicoid gradient in Drosophila embryos, where the heterogeneity in distribution creates a developmental blueprint.

D. Metabolic and Energy Reserves

The allocation of metabolic reserves (such as lipids, proteins, and carbohydrates) is also heterogeneous within embryos. This influences how regions of the embryo develop by providing differential access to energy needed for cell growth and division.

For species with yolky eggs (such as birds and reptiles), the yolk is a significant reserve unevenly distributed, and it supports embryonic development.

E. Implications for Developmental Anomalies

Uneven or abnormal distribution of developmental reserves can lead to developmental anomalies. If certain areas of an embryo lack the necessary nutrients, factors, or signaling molecules, it could result in malformations or failure to develop certain structures.

In summary, the heterogeneity of the distribution of reserves is a critical concept in embryology as it influences how the embryo grows, differentiates, and establishes its basic body plan. Understanding this heterogeneity helps explain why certain cells or regions take on specific developmental roles based on their access to and interaction with these unevenly distributed resources.

III.3 Vitreousness of the envelopes which protect the gamete

The vitreousness of the envelopes which protect the gamete refers to the glass-like, transparent, or brittle quality of the outer layers or membranes that surround and protect the gametes (sperm and egg cells). In a biological context, this term may be used metaphorically to describe the physical properties of these protective layers. In various organisms, gametes are often enclosed in specific protective structures, which vary in composition and properties. These layers can serve different functions depending on the species and the type of reproduction.

1. Zona Pellucida (in Mammals)

- Composition: The zona pellucida is a transparent glycoprotein layer surrounding the mammalian oocyte. Its vitreous (glass-like) transparency makes it an ideal structure for observing fertilization and early developmental processes.
- Function: This layer protects the oocyte and mediates species-specific sperm recognition, preventing polyspermy (fertilization by multiple sperm cells). The zona undergoes changes after fertilization, becoming less penetrable to other sperm.
- Structure: The zona is a porous yet flexible shell, giving it a certain fragility or vitreous quality. Its transparency allows visibility during in vitro fertilization (IVF) procedures.

2. Vitelline Envelope (in Non-Mammalian Species)

- Composition: In species such as amphibians, fish, and insects, the vitelline envelope is the outer protective layer of the egg. It is primarily made of glycoproteins and is thinner and more delicate than hard shells seen in birds or reptiles.
- Vitreous Nature: The vitelline envelope is often transparent or translucent, allowing researchers to study gamete interaction without interference. This envelope acts as a barrier to external pathogens and regulates sperm entry during fertilization.
- Post-Fertilization Changes: After fertilization, this envelope can undergo structural changes, hardening to form a protective layer to prevent additional sperm from entering the egg.

3. Perivitelline Space

- Location: Between the zona pellucida and the oocyte membrane in mammals, this space is filled with fluid that further protects the egg.
- Transparency: This area, combined with the zona pellucida, contributes to the overall vitreousness of the egg's outer layers, ensuring the clarity necessary for observation and manipulation in reproductive technologies.
- Function: It supports the fertilized egg during early cleavage stages and provides a cushioning environment for the developing zygote.

4. Sperm Plasma Membrane

- Structure and Function: The plasma membrane of sperm is not typically described as vitreous but can be considered somewhat transparent and fragile. It serves as the outer boundary of the sperm, containing receptors crucial for interacting with the egg's zona pellucida.
- Fusion with Egg: The membrane undergoes significant changes when the sperm approaches the egg, preparing for fusion. This delicate interaction highlights the fragility or glass-like nature of these structures at the cellular level.

5. Harder External Envelopes (Birds and Reptiles)

- In species like birds and reptiles, the eggs are encased in harder, non-transparent outer shells. These layers protect the developing embryo but are not described as vitreous. However, just beneath the hard shell, there is often a transparent or thin membrane layer that plays a role similar to the zona pellucida in mammals.
- The inner membranes beneath the hard shells can still be described as more delicate or semi-transparent, which are critical during the early stages of embryonic development.

6. Protective Mechanisms and Evolutionary Significance

- The vitreousness (or transparency and delicate nature) of these envelopes often plays a role in evolution, as it balances protection with permeability to allow the right conditions for fertilization.
- Transparency: The transparent nature allows easier penetration by sperm while keeping external threats like pathogens or harsh environmental conditions out.
- Fragility and Rigidity: In many species, once fertilization occurs, these envelopes may harden, reducing the risk of additional sperm penetrating the egg, thereby preventing polyspermy. This change highlights the balance between the initial fragility (which aids fertilization) and post-fertilization hardening (which aids protection).

7. Fertilization Process and Morphogenesis

- The initial vitreous-like structure of these membranes allows not only the fusion of gametes but also clear observation of the early cell division processes that lead to the formation of the zygote and eventual embryo.
- For species undergoing external fertilization (such as many fish or amphibians), the vitelline envelope serves as the first line of defense after gametes are released into the external environment. The transparency of this layer facilitates fertilization in water, where eggs and sperm meet externally.

8. Manipulation in Assisted Reproductive Technologies (ART)

- In assisted reproductive technologies such as IVF, the transparency or vitreousness of these protective envelopes (especially the zona pellucida) is critical for monitoring and assisting fertilization. Embryologists often use this quality to assess the health and viability of the gametes or embryos.
- Techniques like intracytoplasmic sperm injection (ICSI) directly manipulate the zona pellucida, where its delicate and semi-transparent nature is advantageous for precise handling.

Conclusion

The vitreousness of the protective envelopes around gametes highlights the delicate balance between protection, permeability, and transparency. These features ensure that gametes are shielded from external threats while maintaining the ability to undergo successful fertilization. Understanding the nature of these structures provides insight into their role in embryonic development and reproductive technologies.

IV. Fertilization

Fertilization is the fundamental biological process through which the genetic material of the male sperm and the female egg combine to form a zygote, the first cell of a new organism. This event marks the beginning of embryogenesis and involves both the structural modification of the egg and the biochemical activation that prepares it for subsequent cell division and development. Following sperm-egg fusion, a cascade of molecular and cellular events ensures the zygote's readiness for the early stages of embryonic growth.

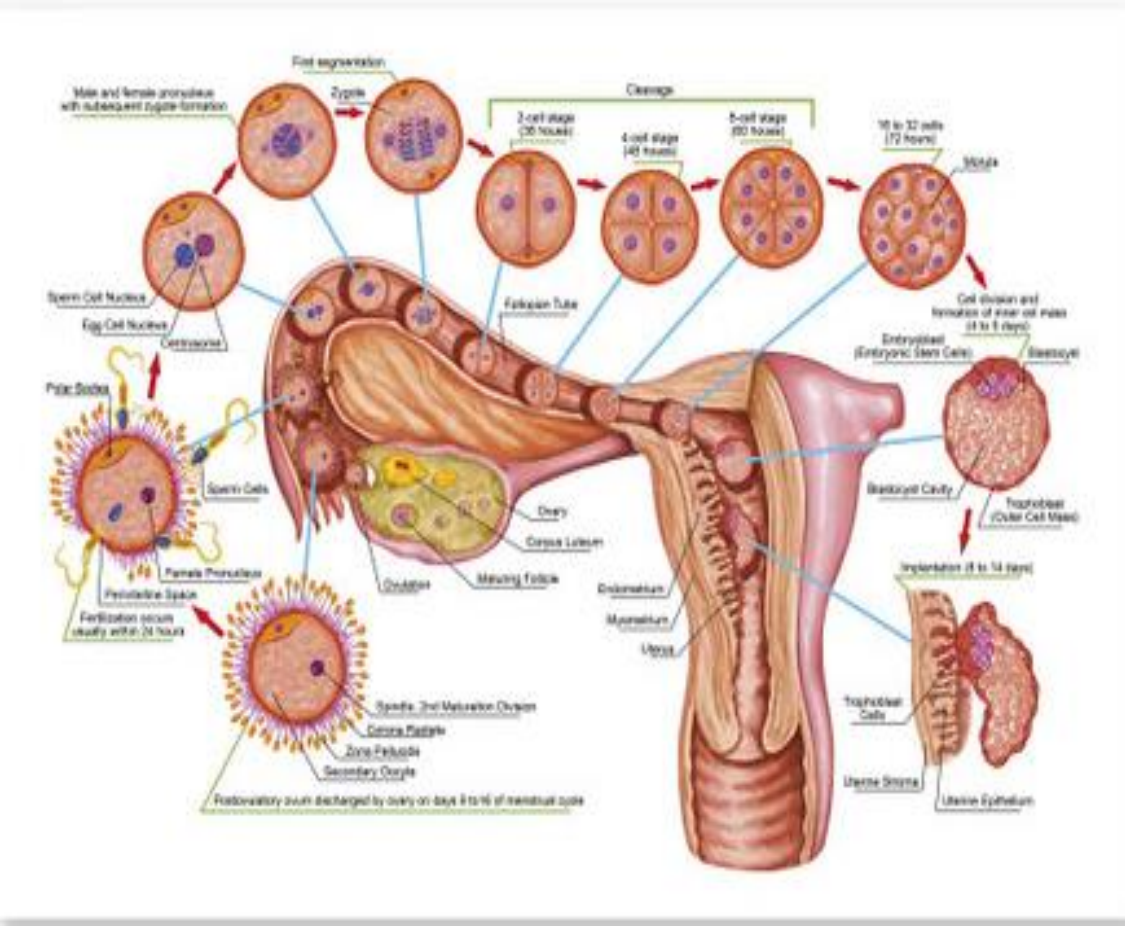

Figure 31:Fertilization Process(Fertilization - Egg Activation, Sperm Fusion, Zygote | Britannica, *n.d.)*

VII.1.Modification of the structure of the egg after fertilization

Upon fertilization, the structure of the egg undergoes several critical modifications that are vital to ensure proper development and prevent polyspermy (the fertilization of an egg by more than one sperm). These structural changes occur rapidly after the sperm penetrates the egg's outer layers.

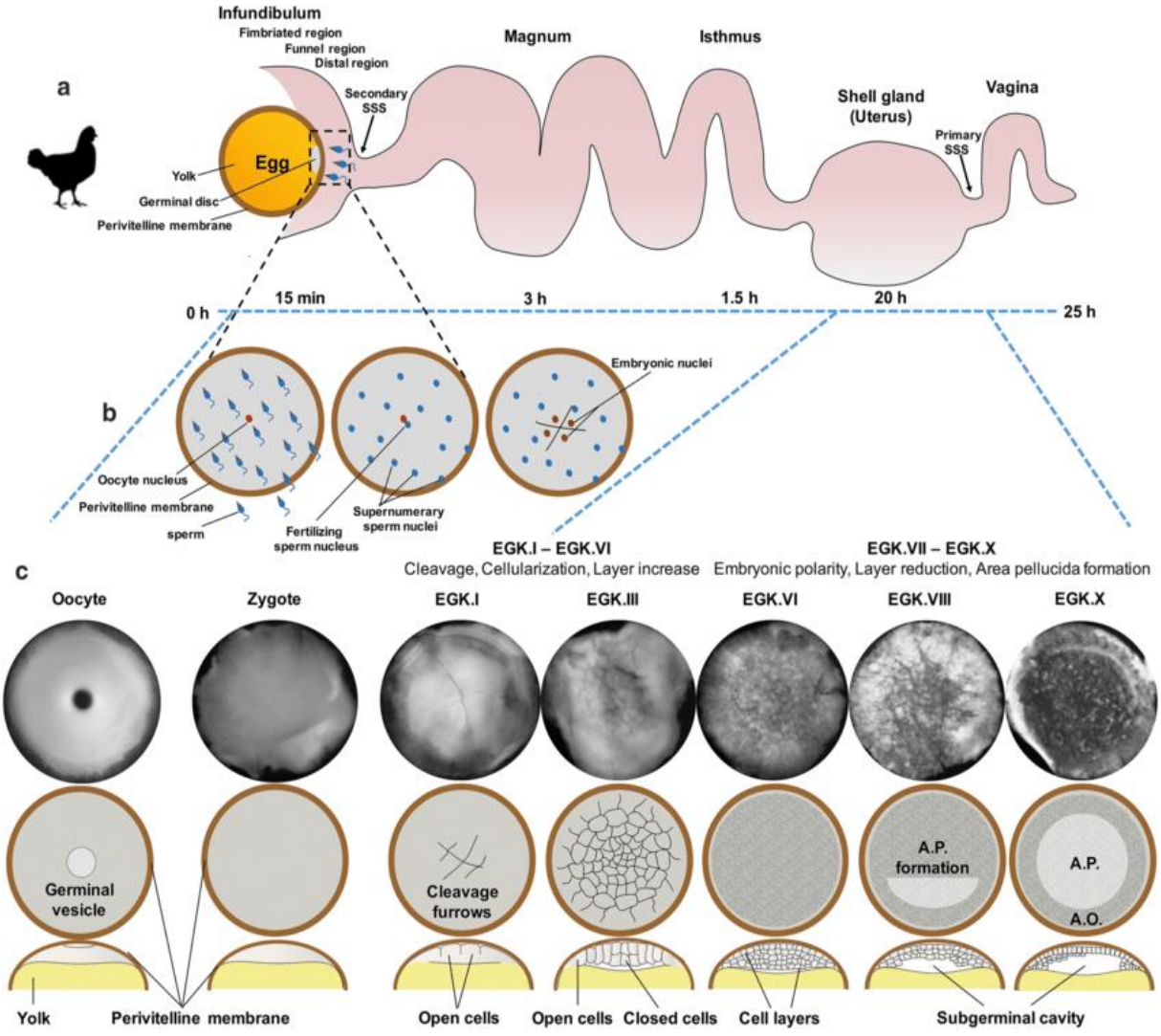

Figure 32:Schematic Diagram of Oviduct Structure, Fertilization, and Embryonic Development in Chickens(Marlow, 2010)

1. **Cortical Reaction**

- One of the earliest and most significant structural changes is the **cortical reaction**. Immediately after the sperm enters the egg, cortical granules, which are small secretory vesicles located just beneath the plasma membrane of the egg, fuse with the plasma membrane and release their contents into the perivitelline space (between the egg membrane and the zona pellucida or vitelline envelope).
- The exocytosis of these granules modifies the egg's outer layer, specifically altering the structure of the zona pellucida or vitelline envelope. In mammals, this reaction leads to the modification of **ZP2 and ZP3** glycoproteins within the zona pellucida, hardening the layer and creating a physical and chemical barrier to additional sperm entry.
- The cortical reaction also leads to the degradation of sperm-binding receptors, thus ensuring **polyspermy block**, a necessary step to prevent the fusion of more than one sperm, which could result in chromosomal imbalance and developmental failure.

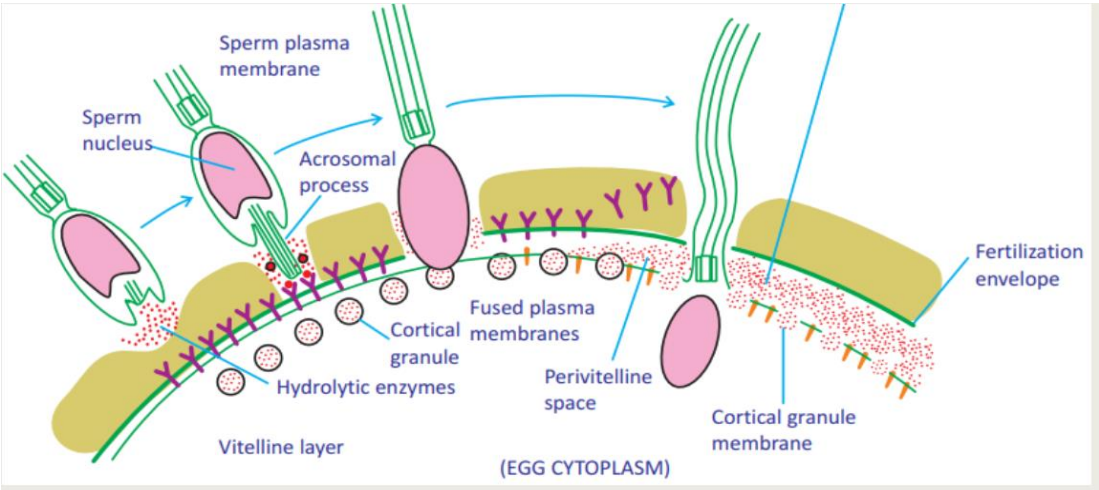

*Figure 33:Cortical Granule Reaction(*Cortical Granule Reaction (Sea Urchin) - Rethink Biology Notes, *n.d.)*

2. **Zona Reaction**:
    - The **zona reaction** is closely related to the cortical reaction and represents the biochemical changes in the zona pellucida that contribute to the block to polyspermy. This involves the **cross-linking of glycoproteins** in the zona pellucida, transforming it into a tough, impermeable barrier.
    - In species with an external vitelline envelope, similar modifications occur, where the envelope becomes impenetrable to prevent further sperm from entering.
    - This transformation is critical in maintaining the integrity of the fertilization process and ensuring that only one set of paternal chromosomes enters the egg.
3. **Membrane Depolarization**:
    - The initial **depolarization of the egg membrane** is a fast block to polyspermy that occurs within seconds of sperm fusion. Upon sperm entry, ion channels in the egg's plasma membrane open, leading to a rapid influx of sodium ions, which changes the electrical charge across the membrane.
    - This change in membrane potential temporarily prevents additional sperm from binding to the egg membrane, providing an immediate block to polyspermy before the slower but more permanent cortical and zona reactions take place.
4. **Pronuclear Fusion**
    - After the successful entry of the sperm, the male and female pronuclei, which contain the genetic material from the sperm and egg, respectively, migrate toward each other. This process is facilitated by the microtubule network within the egg's cytoplasm.

- **Karyogamy**, or the fusion of the male and female pronuclei, results in the formation of a single, diploid nucleus, marking the creation of a **zygote**. This fusion event is essential for

combining the haploid sets of chromosomes from both parents into a complete diploid genome, initiating the zygote's genetic program for development.

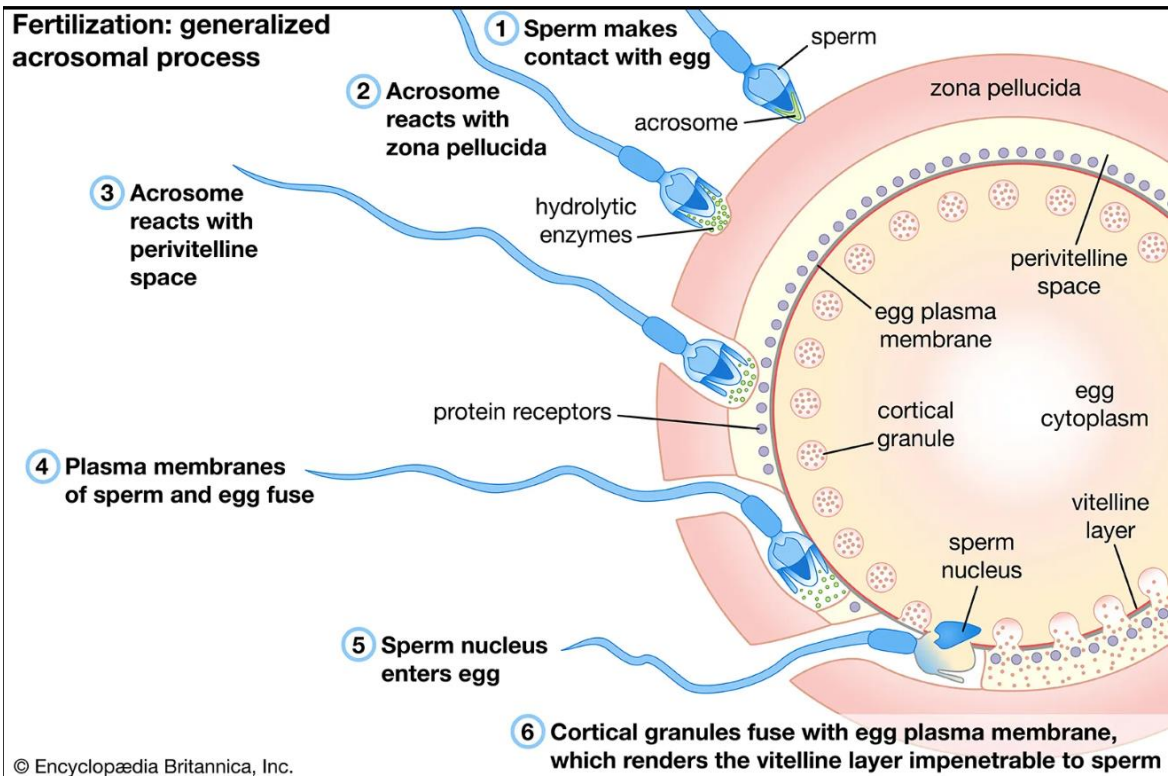

*Figure 34:Steps of the Acrosomal Reaction During Fertilization: Sperm-Egg Interaction and Membrane Fusion(*Fertilization - Egg Activation, Sperm Fusion, Zygote | Britannica, *n.d.)*

VII.2. Activation of the egg

Egg activation is the sequence of biochemical and cellular events that follow sperm entry, triggering the egg's transition from a dormant state into one capable of initiating embryonic development. This activation is crucial as it signals the egg to resume meiosis, initiate metabolic processes, and enter the cell cycle.

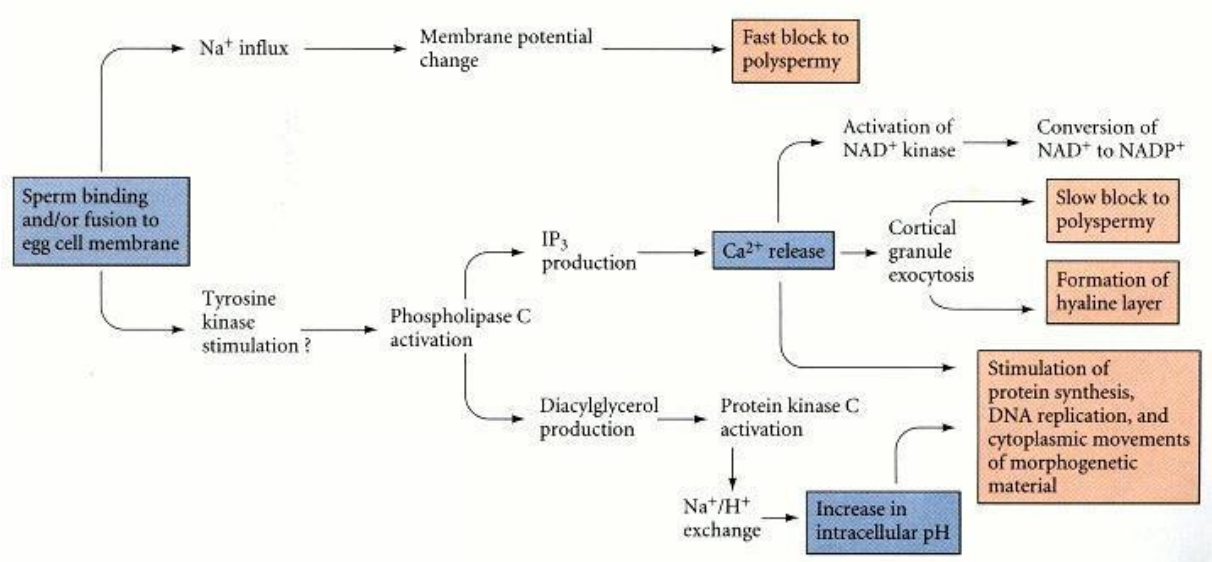

Figure 35:Model of possible pathway of egg activation in the sea urchin (After Epel 1980; L. A. Jaffe, personal communication.)(Gilbert, 2000b)

A. Calcium Wave and Egg Activation:

- ✓ One of the most critical events in egg activation is the release of calcium ions from intracellular stores, which creates a **calcium wave** that sweeps across the egg. This rise in calcium concentration is triggered by the fertilizing sperm and activates various enzymes and pathways within the egg.
- ✓ The calcium wave initiates key processes, such as the **completion of meiosis II**, which allows the egg to complete its maturation and expel the second polar body. The egg was previously arrested in **metaphase II** and required this calcium signal to proceed through the final stages of meiotic division.

- ✓ Calcium also activates other intracellular pathways, leading to the **decondensation of the male pronucleus**, the reorganization of the cytoplasm, and the activation of maternal mRNA and proteins stored in the egg.

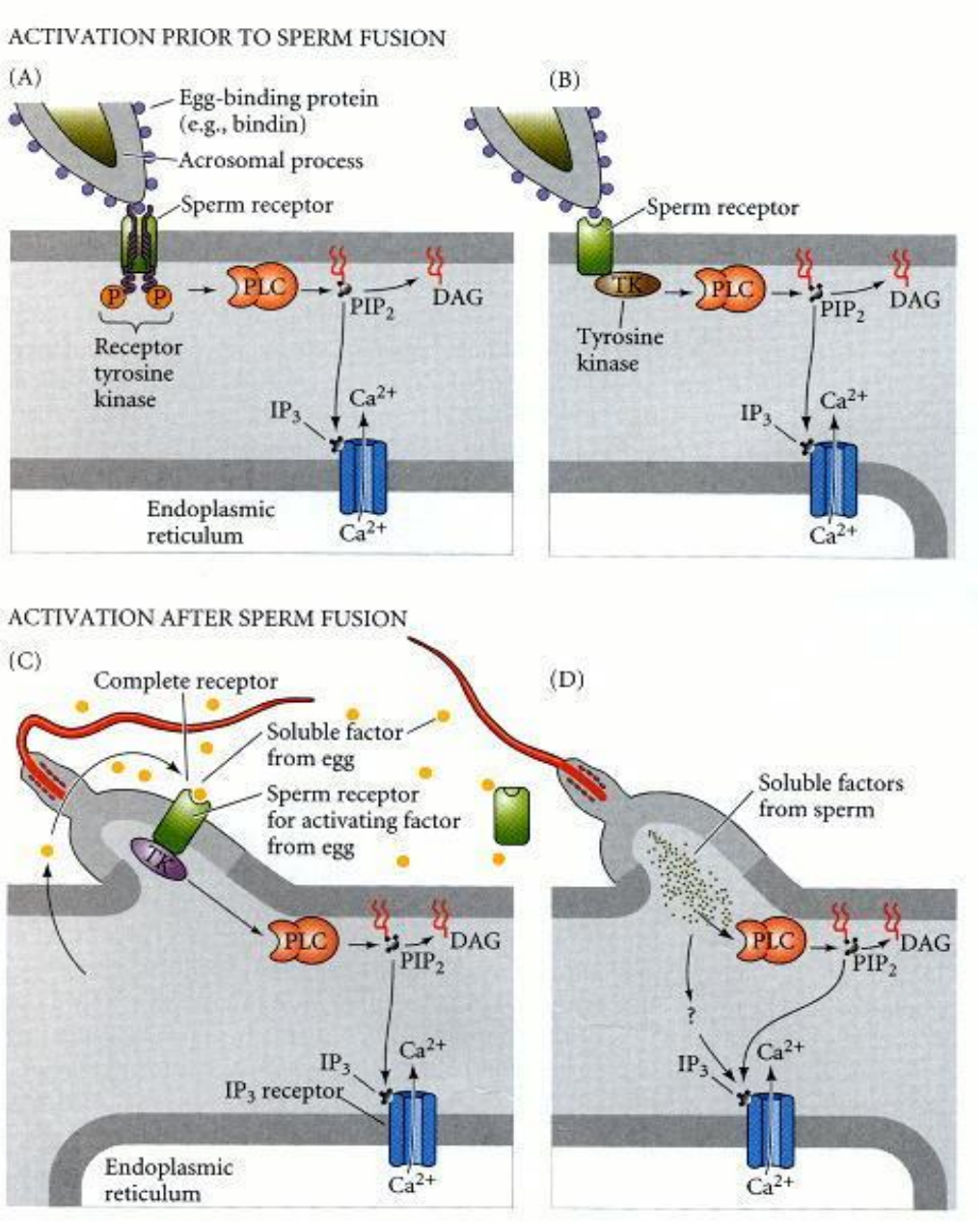

*Figure 36:Possible mechanisms of egg activation. (A) The bindin receptor in the egg plasma membrane has tyrosine kinase activity. The tyrosine kinase activates PLC. (B) The bindin receptor activates a cytoplasmic tyrosine kinase. (C) An activated tyrosine kinase(*Fertilization - Egg Activation, Sperm Fusion, Zygote | Britannica, *n.d.)*

B. Resumption of the Cell Cycle

- ✓ Before fertilization, the egg is typically arrested in a state of meiotic arrest (metaphase II in most species), and fertilization is the signal for the egg to complete meiosis. Once calcium levels rise, the egg resumes the cell cycle, completing the second meiotic division.
- ✓ Following this, the newly formed zygote enters the **mitotic cell cycle**, initiating the series of cell divisions known as **cleavage**. Cleavage is characterized by rapid cell divisions without an increase in overall embryo size, leading to the formation of smaller cells called **blastomeres**. This marks the beginning of embryonic development.

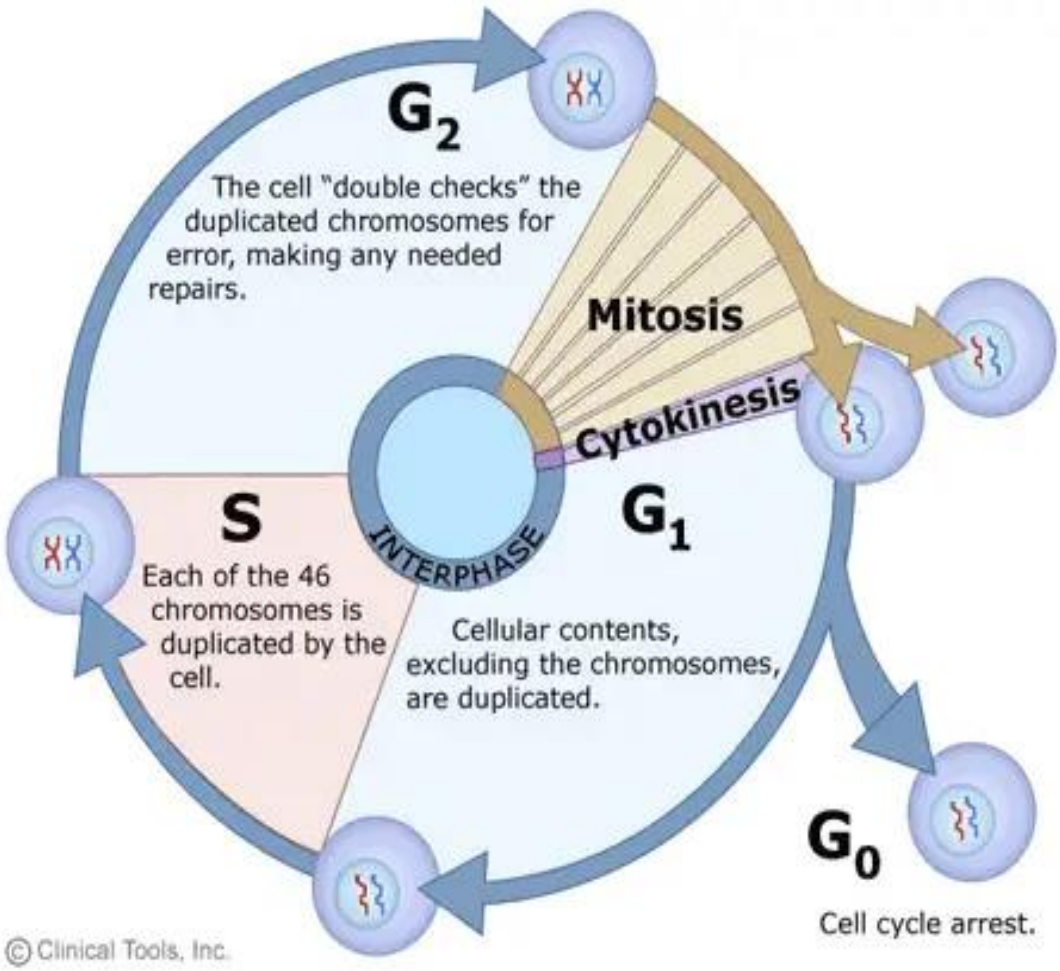

Figure 37:The basic cell cycle steps where telophase forms the last leg of the mitosis phase (M-phase) | Image Credit: University of Leicester(Bower et al., 2017)

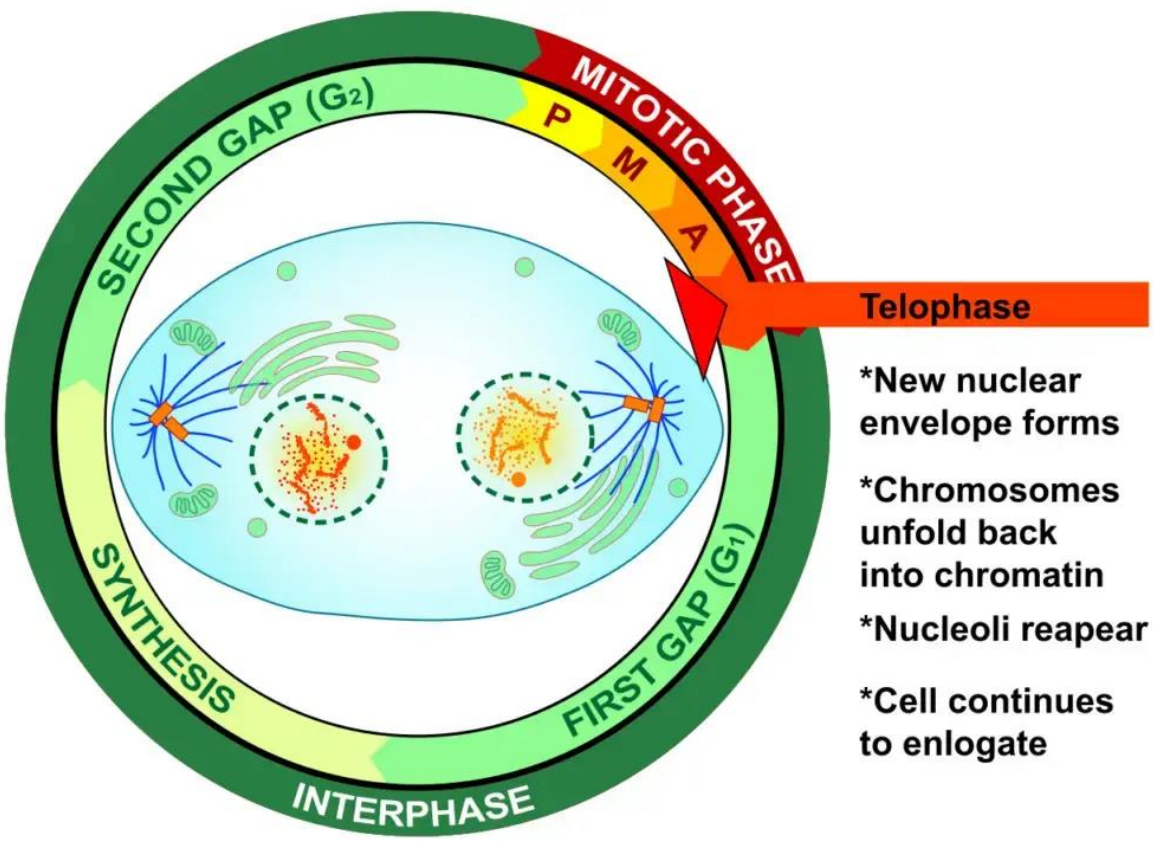

Figure 38:Telophase: The Final Stage of Mitosis(Bower et al., 2017)

C. Metabolic Activation:

- ✓ Fertilization also triggers a significant increase in the egg's metabolic activity. Prior to fertilization, the egg is largely dormant, with most of its mRNA and proteins stored in an inactive form. The calcium wave leads to the activation of these stored mRNA molecules, initiating protein synthesis.
- ✓ The increase in protein synthesis is critical for the early stages of development, as the embryo relies heavily on maternally derived mRNA and proteins during the early divisions before its own genome is fully activated in a process called **zygotic genome activation (ZGA)**.(Bower et al., 2017)

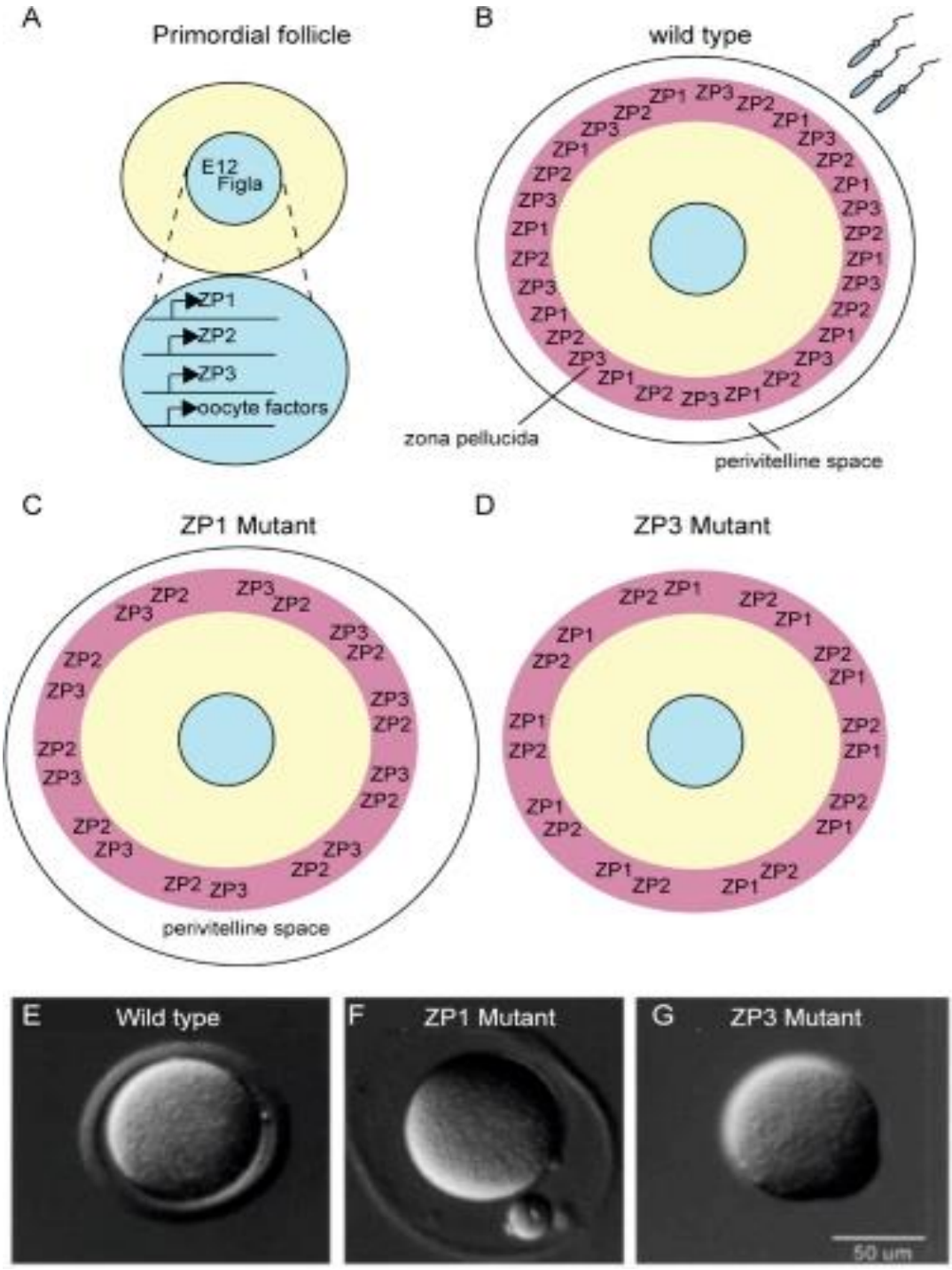

*Figure 39:(A) Figla promotes expression of the three-mammalian zona pellucida proteins. (B–D) Each uniquely contribute to the formation, function, and integrity of the zona as can be seen (F, G) in the mouse mutant phenotypes. (E–G) Images of unfixed ovulated egg(*Cortical Granule Reaction (Sea Urchin) - Rethink Biology Notes, *n.d.)*

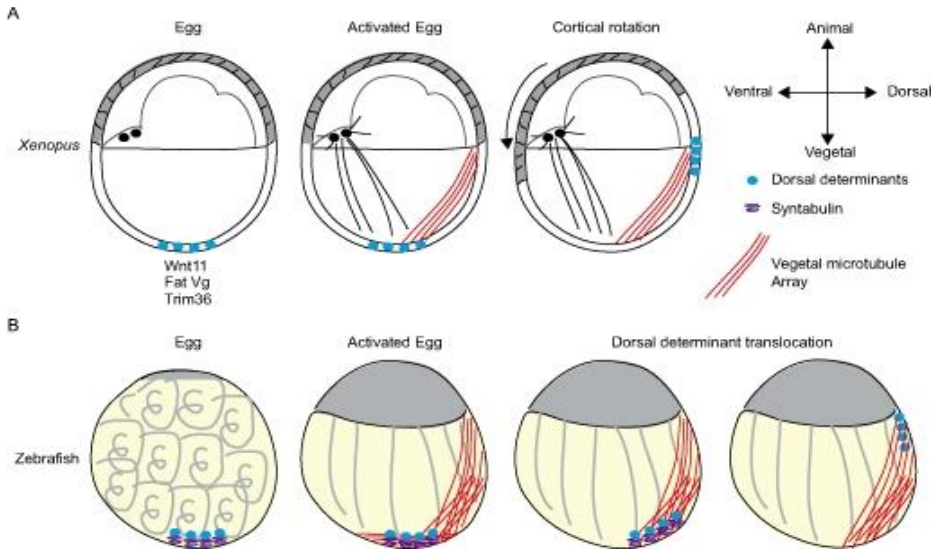

Figure 40:Mechanisms of Cytoplasmic and Dorsal Determinant Movement in Xenopus and Zebrafish Oocytes(Marlow, 2010)

D. DNA Replication and Initiation of Cleavage:

- ✓ With the fusion of the male and female pronuclei, the newly formed zygote begins **DNA replication** in preparation for the first mitotic division. Cleavage divisions occur rapidly, with cells dividing to increase the number of cells (blastomeres) but without increasing the overall size of the embryo.
- ✓ The cleavage stage eventually leads to the formation of a blastula, a multicellular structure that represents one of the early stages of embryogenesis.

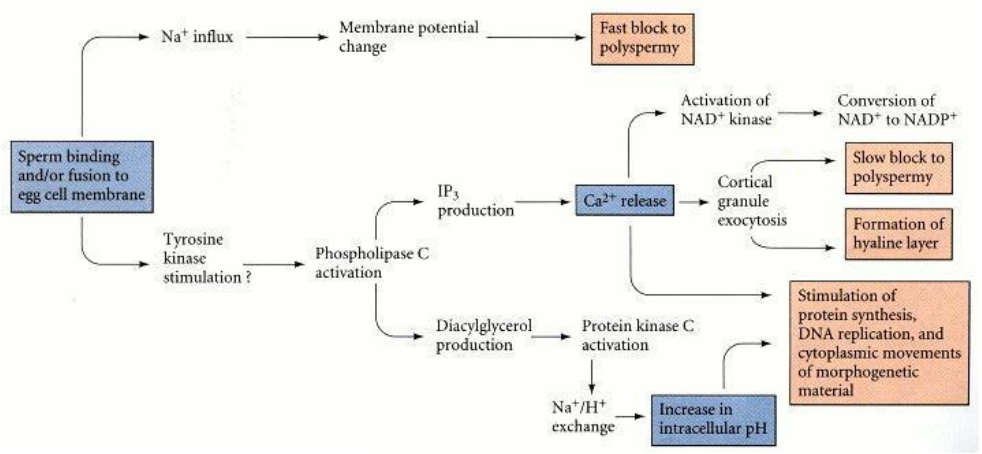

*Figure 41:Mechanism of DNA replication(*The Activation of Egg Metabolism - Developmental Biology - NCBI Bookshelf, *n.d.)*

Conclusion

Fertilization marks the beginning of a complex and highly regulated process that ensures the proper development of the zygote. The modifications to the egg structure, such as the cortical and zona reactions, protect the egg from polyspermy and maintain the integrity of the fertilization process. Concurrently, egg activation triggered by a calcium wave initiates crucial events, including the completion of meiosis, resumption of the cell cycle, and metabolic activation. These coordinated changes prepare the zygote for cleavage and the subsequent stages of embryonic development, setting the foundation for the formation of a fully developed organism.

V. SEGMENTATION

Segmentation is a critical process in embryonic development, where a single-celled fertilized egg (zygote) undergoes a series of cell divisions, eventually forming a multicellular organism with specialized tissues and body structures. The transition from a single cell to a multicellular organism involves intricate coordination between molecular signals, cellular interactions, and regulatory mechanisms. Below is a review covering key aspects of segmentation in embryology, focusing on the transformation of the egg into a multicellular structure, the molecules involved, cellular interactions and affinities, and the regulation of segmentation.

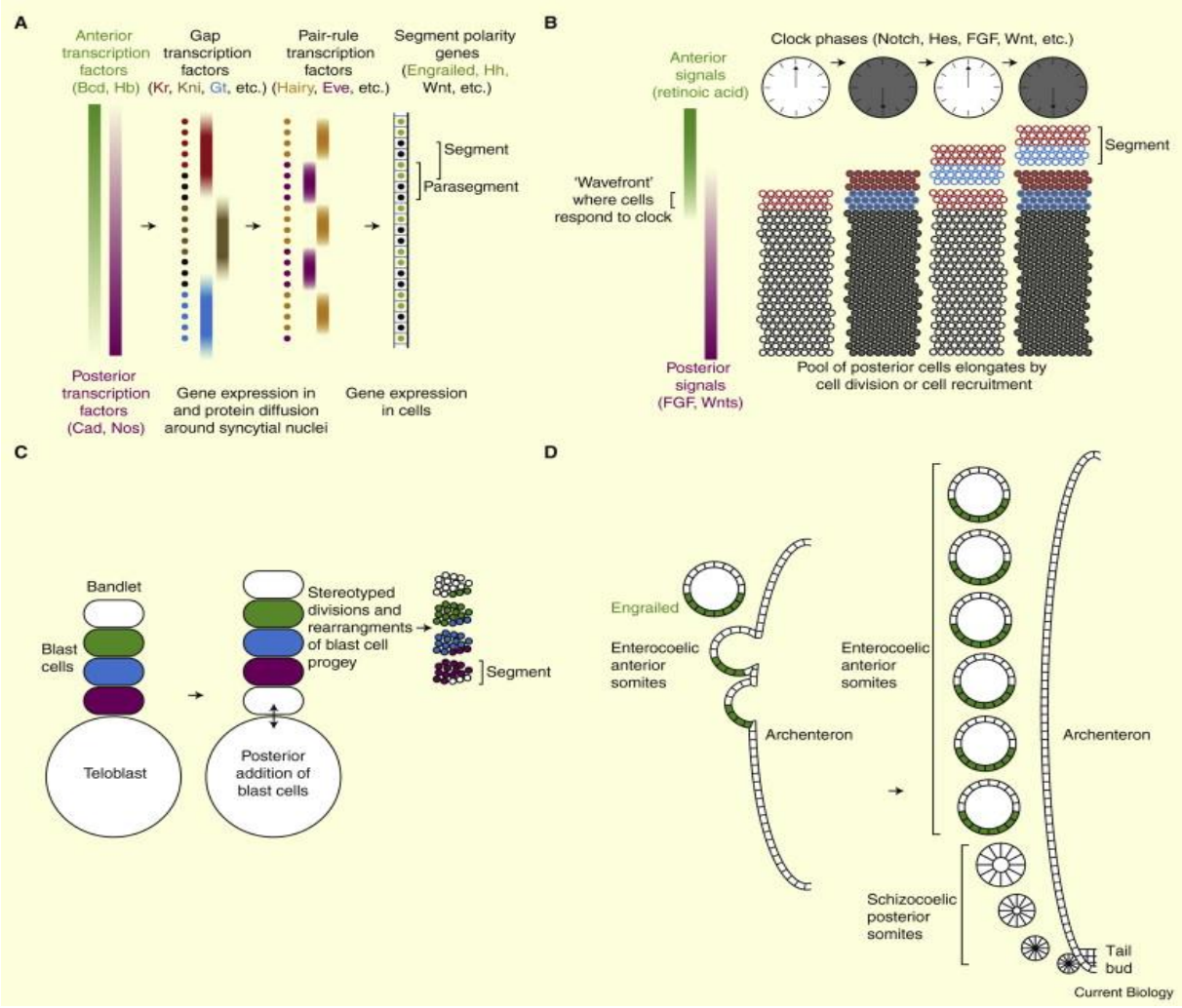

Figure 42:Different mechanisms of segmentation.(Blair, 2008)

V.1.Transformation of the egg into a multicellular structure

The transformation of the egg (zygote) into a multicellular structure begins immediately after fertilization and is a crucial step in embryogenesis. This process is marked by cleavage, a rapid series of mitotic divisions that increase the number of cells without increasing the overall size of the embryo. The resulting cells are called blastomeres.

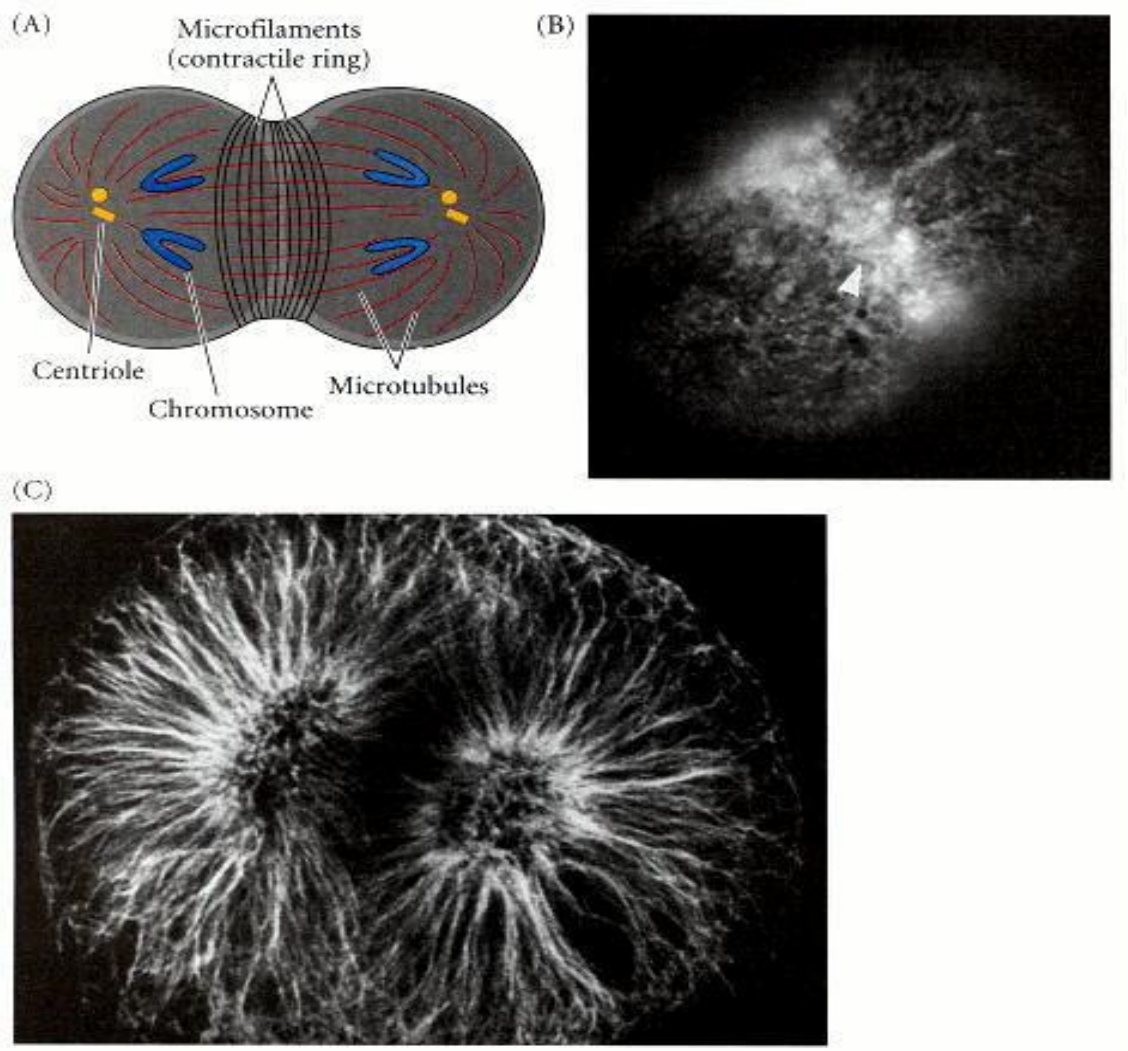

Figure 43:Role of microtubules and microfilaments in cell division. (A) Diagram of first-cleavage telophase. The chromosomes are being drawn to the centrioles by microtubules while the cytoplasm is pinched in by the contraction of microfilaments. (B) Localization(Gilbert, 2000a)

- **Cleavage Patterns**

Different species exhibit different cleavage patterns depending on the distribution of yolk in the egg. For example, holoblastic cleavage (complete division) occurs in eggs with little yolk (e.g., mammals), while meroblastic cleavage (incomplete division) is seen in yolk-rich eggs (e.g., birds and reptiles).

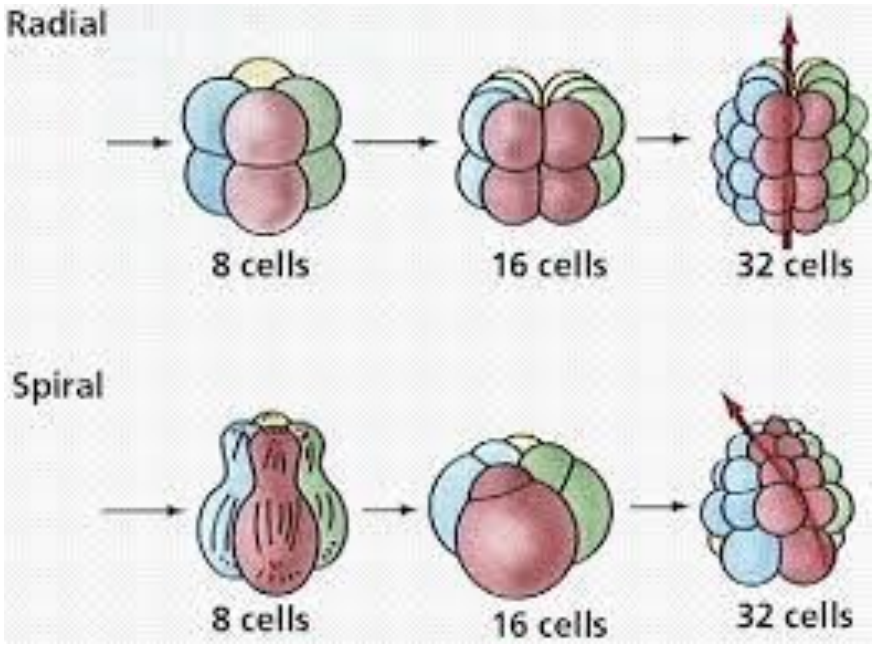

*Figure 44:different cleavage pattern(*43.5B: Cleavage, the Blastula Stage, and Gastrulation - Biology LibreTexts, *n.d.)s*

- **Blastulation**

As cleavage progresses, the blastomeres continue to divide and form a hollow ball of cells called the blastula. Inside the blastula is a fluid-filled cavity called the blastocoel. This stage marks the beginning of cellular differentiation, where certain cells start to become specialized for future roles(*43.5B: Cleavage, the Blastula Stage, and Gastrulation - Biology LibreTexts*, n.d.).

- **Gastrulation**

Following blastulation, the process of gastrulation occurs, where the blastula reorganizes into a multilayered structure known as the gastrula. This is the stage where the three primary

germ layers ectoderm, mesoderm, and endoderm are established, setting the foundation for the development of various tissues and organs.

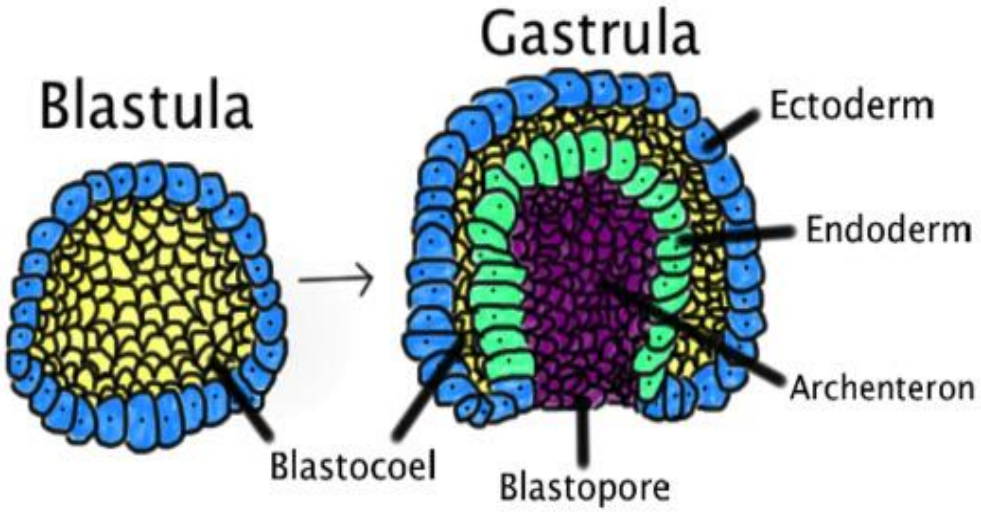

Figure 45:Comparison of blastula to gastrula.(Muhr et al., 2023)

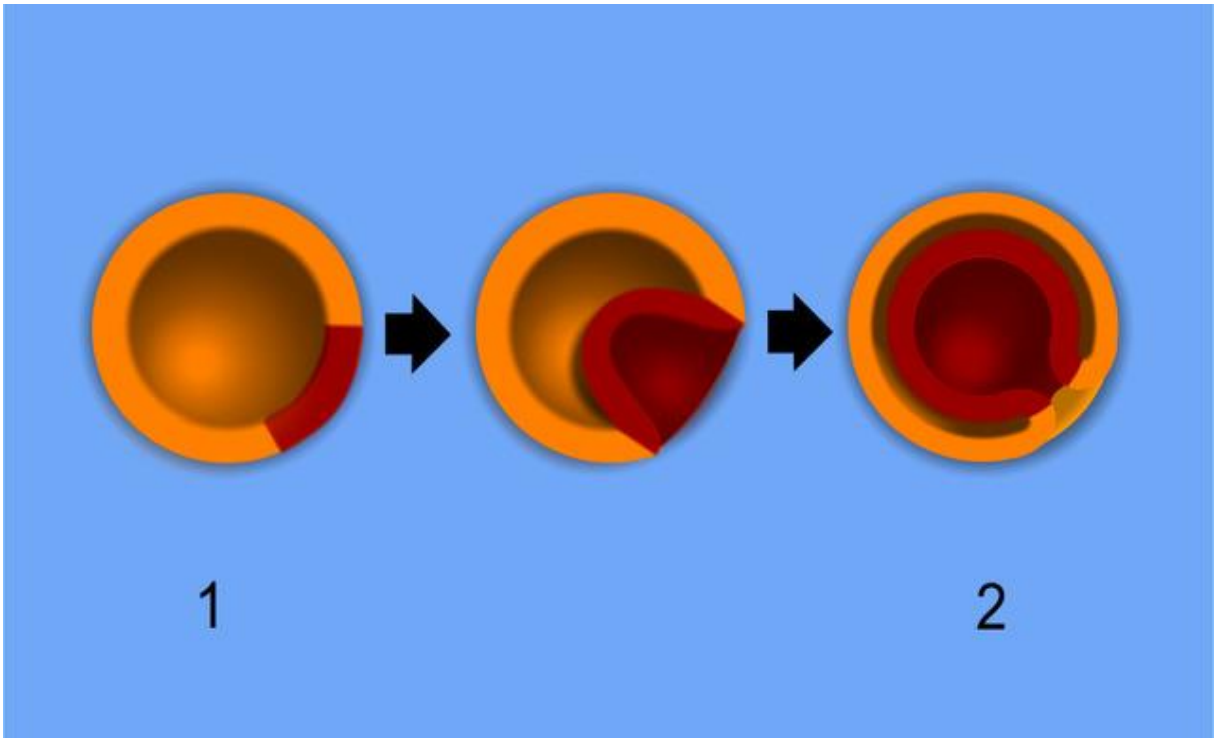

Figure 46:Diagram of gastrulation of a blastula.(Muhr et al., 2023)

Molecular signaling plays a pivotal role in guiding segmentation and the differentiation of cells into specialized tissues. Key molecules involved in segmentation include morphogens, transcription factors, and signaling pathways.

- **Morphogens**

Morphogens are signaling molecules that form concentration gradients and influence the fate of cells based on their position within the embryo. One well-known morphogen is Bicoid in Drosophila embryos, which establishes the anterior-posterior axis by creating a gradient from the anterior (head) to the posterior (tail).

- **Segmentation Genes**

In model organisms like Drosophila, segmentation is controlled by a hierarchy of segmentation genes:

- ✓ Gap genes (e.g., Hunchback) define broad regions of the embryo.
- ✓ Pair-rule genes (e.g., Even-skipped) further refine these regions into segments.
- ✓ Segment polarity genes (e.g., Hedgehog) define the boundaries of individual segments.

The first direct evidence that DNA is rearranged during B cell development came in the 1970s from experiments in which molecular biologists compared DNA from early mouse embryos, which do not make antibodies, with the DNA of a mouse B cell tumor, which makes a single species of antibody molecule. The specific variable (V)-region and constant (C)-region coding sequences that the tumor cells used were present on the same DNA restriction fragment in the tumor cells but on two different restriction fragments in the embryos. This showed that the DNA sequences encoding an antibody molecule are rearranged at some stage in B cell development (Figure47-48)(Alberts et al., 2002; *The Generation of Antibody Diversity - Molecular Biology of the Cell - NCBI Bookshelf*, n.d.).

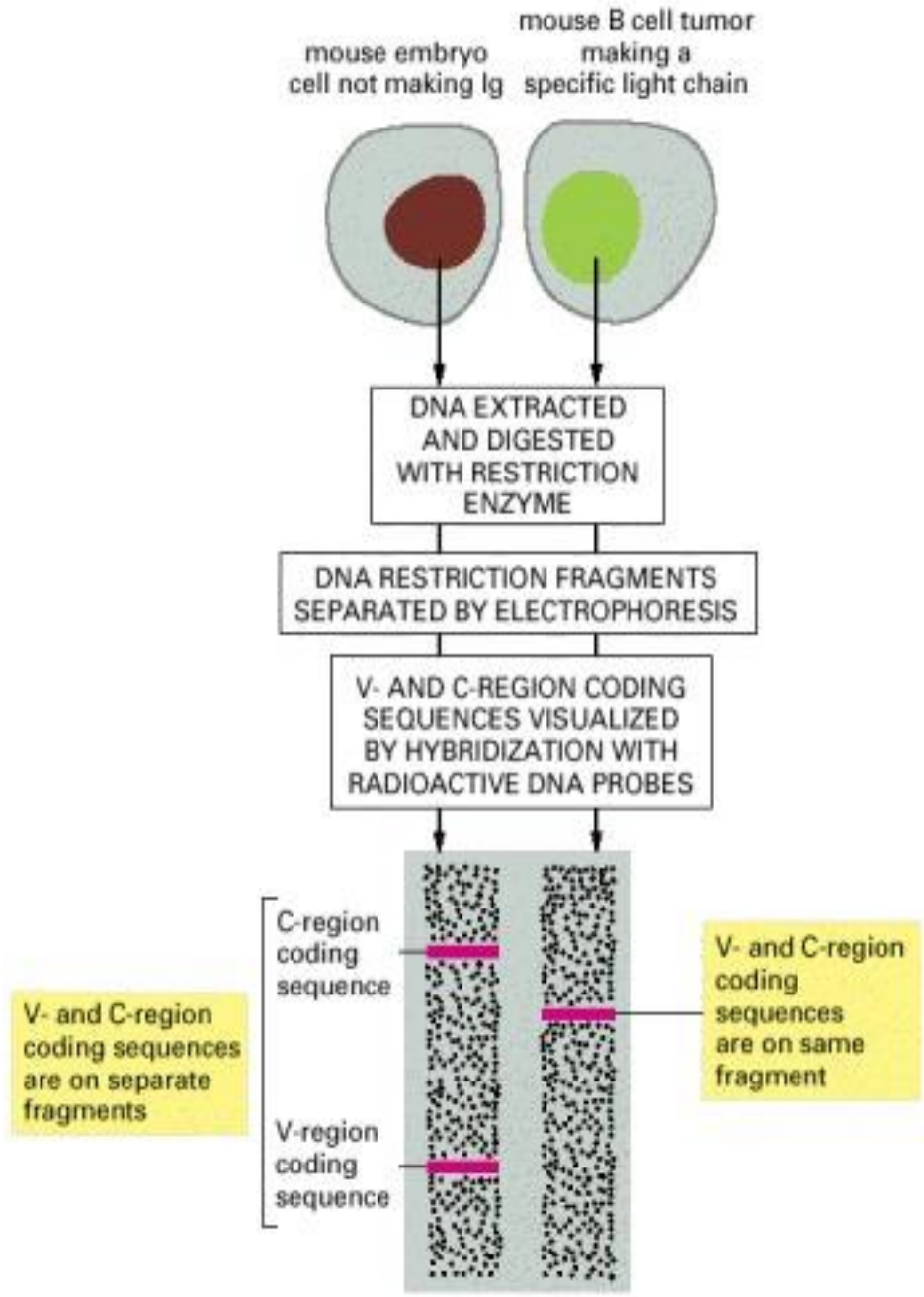

*Figure 47:Drawing of an experiment that directly demonstrates that DNA is rearranged during B cell development.(*The Generation of Antibody Diversity - Molecular Biology of the Cell - NCBI Bookshelf, *n.d.)*

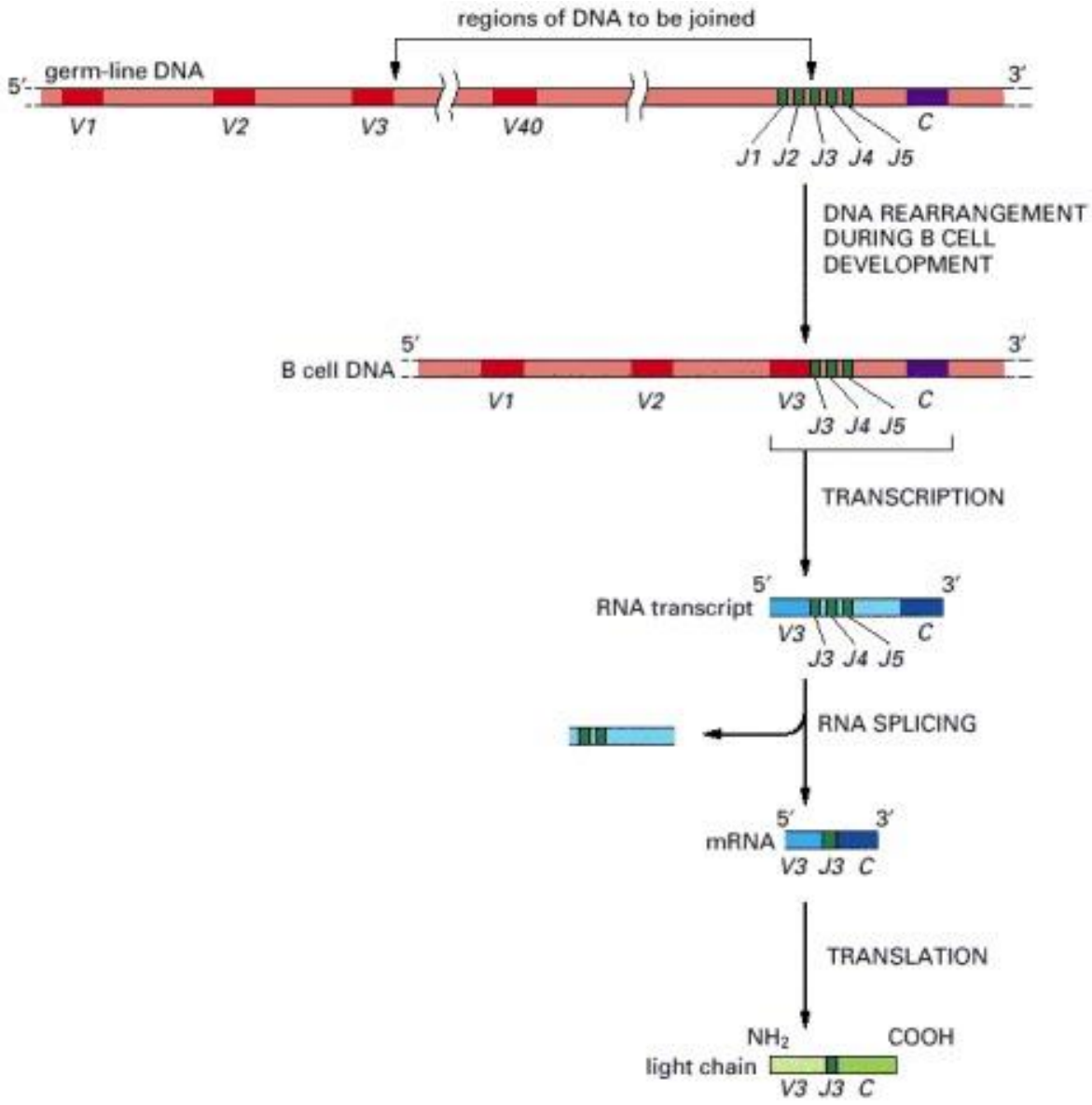

Figure 48 :The V-J joining process involved in making a human κ light chain(Alberts et al., 2002)

In the "germ-line" DNA (where the antibody genes are not being expressed and are therefore not rearranged), the cluster of five J gene segments is separated from the C-region exon by a short intron and from the 40 V gene segments by thousands of nucleotide pairs. During the development of a B cell, the randomly chosen V gene segment (V3 in this case) is moved to lie precisely next to one of the J gene segments (J3 in this case). The "extra" J gene segments (J4 and J5) and the intron sequence are transcribed (along with the joined V3 and J3 gene segments and the C-region exon) and then removed by RNA splicing to generate mRNA molecules in which the V3, J3, and C sequences are contiguous. These mRNAs are then

translated into κ light chains. A J gene segment encodes the C-terminal 15 or so amino acids of the V region, and the V-J segment junction coincides with the third hypervariable region of the light chain, which is the most variable part of the V region(Alberts et al., 2002).

- **Wnt and Hedgehog Signaling Pathways**

Wnt and Hedgehog signaling pathways are essential in regulating cell division, cell differentiation, and segment polarity. These pathways work by controlling the expression of target genes that determine the fate and position of cells in the embryo.

- **Notch Signaling**

The Notch pathway plays a crucial role in mediating cell-cell communication, ensuring that neighboring cells adopt different fates during segmentation. This is especially important in the development of tissues like the nervous system. The Hh signaling pathway is an important cascade for cellular growth and differentiation during the embryonic development. The pathway was first identified in Drosophila fruit flies, and has been shown to be highly conserved in vertebrates and invertebrates. Kim *et al(Kim et al., 2010)* investigated differentiation-associated signal interactions between the Wnt and Hh signaling pathways in gastric cancer cells, and indicated that the expression levels of Shh signaling components were increased, whereas those of the Wnt signaling pathway were decreased. Further research indicated that ectopic expression of GLI1 increased the level of SFRP1 transcript, and that increased expression of GLI1 decreased nuclear β-catenin staining. By contrast, inhibition of GLI1 reduced SFRP1 expression. Thus, the Shh and Wnt pathways are differentially involved according to the differentiation of gastric cancer cells (Figure. 3)(Borday et al., 2012)

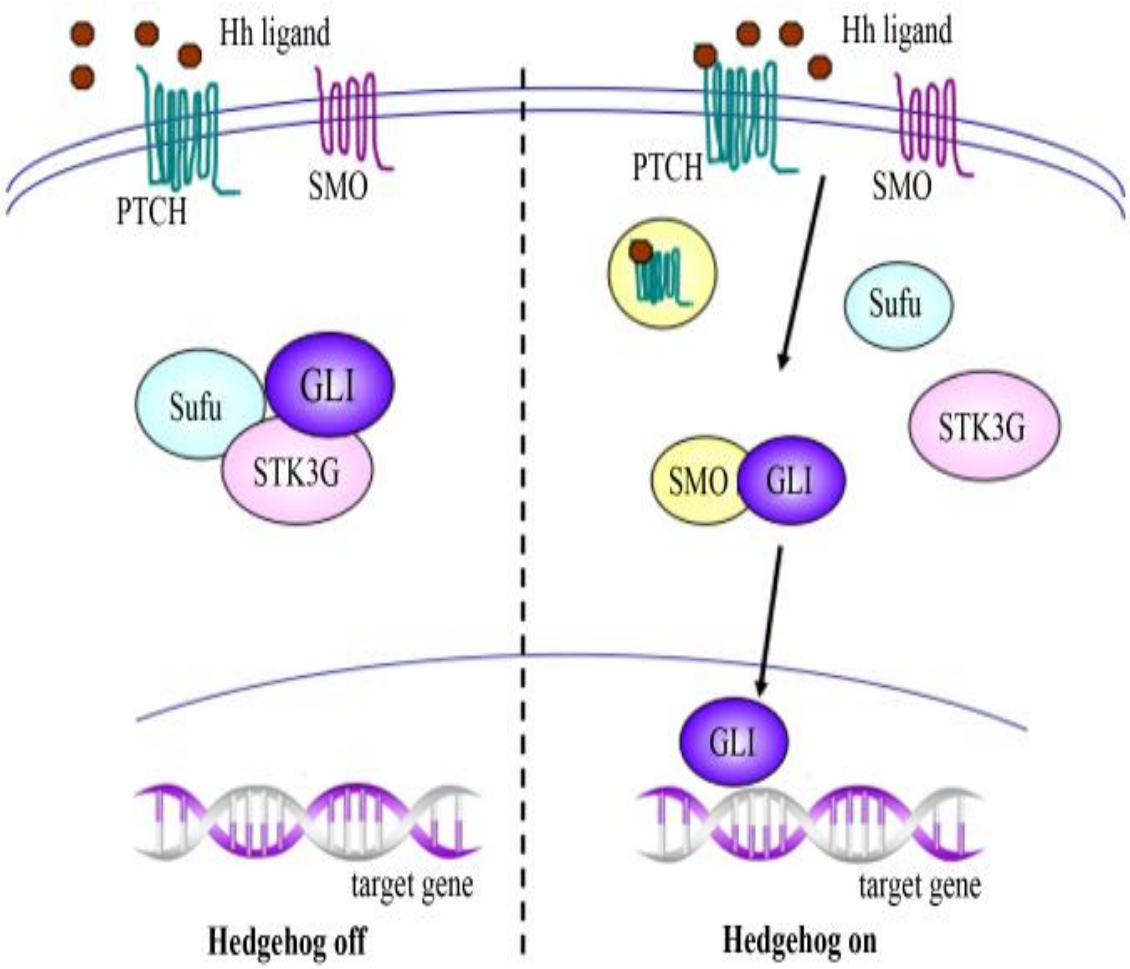

Figure 49:Activation of the Hh signaling pathway results in the activation of SMO and migration of GLI into nucleus. Hh, Hedgehog; SMO, Smoothened; GLI, glioma-associated oncogene homolog; PTCH, Patched; Sufu, Suppressor of fused; STK3, serine/threonine kinase 3(Borday et al., 2012)

Crosstalk between the Hh and Wnt pathways forms regulatory loops. The Hh signaling pathway negative regulates Wnt activity via SFRP1, and Wnt/β-catenin pathway feedback regulates Hh activity via GLI3 transcriptional regulation. Hh, Hedgehog; SFRP1, secreted frizzled-related protein 1; GLI, glioma-associated oncogene homolog; PTCH, Patched; SMO, Smoothened; Sufu, Suppressor of fused; STK3, serine/threonine kinase 3; LRP, lipoprotein receptor-related protein; GSK-3β, glycogen synthase kinase 3β; APC, adenomatous polyposis coli; HCNR2/3, highly conserved non-coding DNA region 2/3; TCF, T cell factor

V.2.Cellular interactions and affinities

As segmentation progresses, cells must interact with each other and their environment to ensure proper tissue and organ formation. These interactions are governed by cell adhesion molecules and cell signaling.

- **Cell Adhesion Molecules (CAMs)**

Proteins such as cadherins and integrins are vital for maintaining the structural integrity of tissues. Cadherins, for instance, are involved in cell-cell adhesion, ensuring that cells remain bound to their neighbors during development. The differential expression of cadherins helps organize cells into specific layers or regions.

- **Differential Cell Affinity**

Early embryonic cells demonstrate differential affinities for one another based on their gene expression patterns. Cells with similar affinities tend to group together, leading to the segregation of cells into distinct germ layers or tissues.

- **Cell Communication**

Cells communicate through direct contact or via signaling molecules to coordinate their activities during segmentation. These interactions ensure that cells divide at the correct times, migrate to their appropriate locations, and differentiate into the correct cell types.

- **Inductive Interactions:**

Inductive signaling occurs when one group of cells influences the fate of another group. For example, the Spemann-Mangold organizer in amphibians releases signals that induce neighboring cells to form specific structures, like the nervous system.

V.3.Regulation of segmentation

Segmentation is tightly regulated by a network of genes and signaling pathways to ensure that cells divide and differentiate at the right times and in the correct locations.

- **Gene Regulatory Networks**

Gene regulatory networks (GRNs) involve interactions between transcription factors, enhancers, and repressors that control the expression of segmentation genes. In Drosophila, for instance, the precise timing and location of gene expression are controlled by a cascade of transcription factors that include the gap, pair-rule, and segment polarity genes.

- **Positional Information and Patterning**

The concept of positional information is key in regulating segmentation. Cells in different positions within the embryo receive signals that instruct them on how to behave. For example, the French flag model describes how a morphogen gradient can provide cells with positional information, allowing them to adopt distinct fates depending on their distance from the morphogen source.

- **Temporal Regulation**

Segmentation is also regulated temporally, meaning that the timing of cell division and differentiation is critical. The segmentation clock in vertebrates, for example, governs the rhythmic and sequential formation of somites (segments in the developing body). This clock is regulated by oscillations in the expression of specific genes like Notch, Wnt, and FGF.

- **Feedback Mechanisms**

Positive and negative feedback loops help regulate the precision of segmentation. For example, once a segment is formed, inhibitory signals may prevent further division in that region while promoting segmentation elsewhere.

VI. GASTRULATION

Gastrulation is a pivotal stage in embryonic development, marking the transition from a simple blastula to a complex structure with three distinct germ layers: the ectoderm, mesoderm, and endoderm. These layers give rise to all tissues and organs in the body. Gastrulation involves highly coordinated movements of cells and is regulated by a variety of molecular signals and cellular interactions. Below is a detailed review of the key processes in gastrulation, including the positioning of the three primordial tissues, primary and secondary inductions, and egg activation.

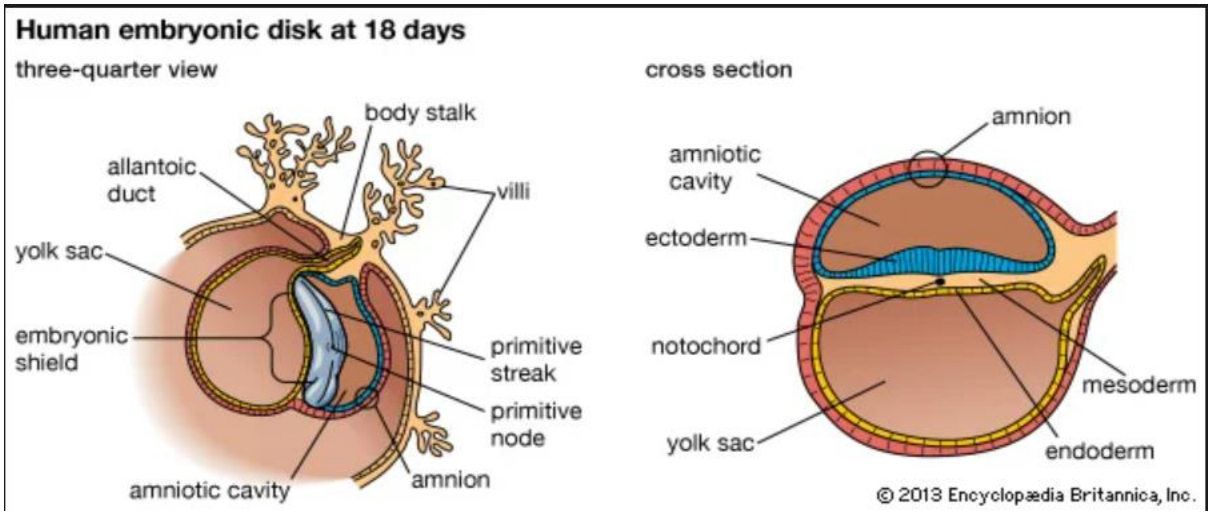

Figure 50:Development of the human embryo at 18 days, at disk or shield stage, shown in (left) three-quarter view and (right) cross section.(Borday et al., 2012)

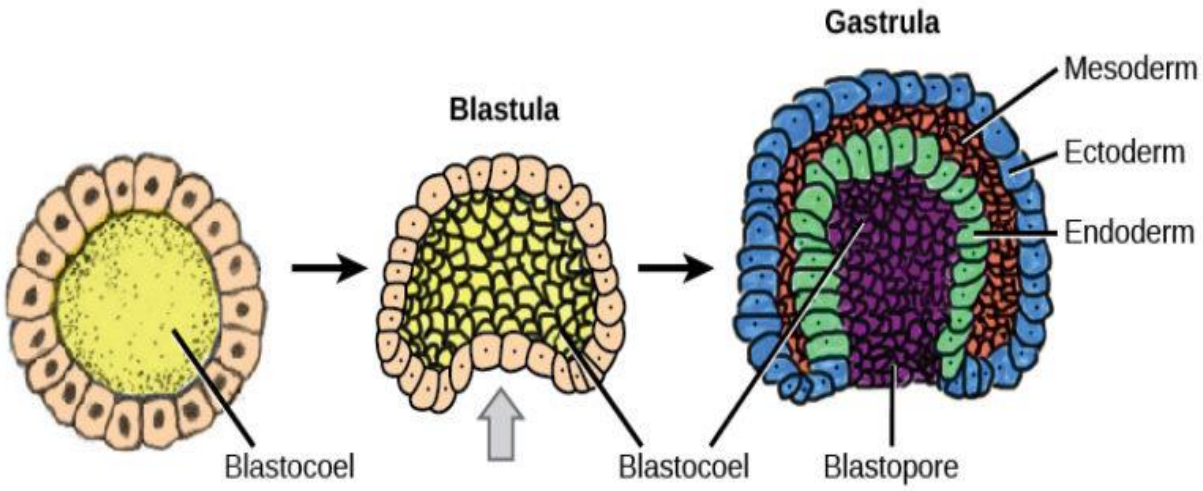

Figure 51: Gastrulation is the process wherein the cells in the blastula rearrange themselves to form the germ layers. (credit: modification of work by Abigail Pyne)(Molnar & Gair, 2015)

VI.1. Positioning of the Three Primordial Tissues (Ectoderm, Mesoderm, Endoderm)

The primary purpose of gastrulation is to reorganize the simple blastula into a structure containing the three germ layers, each of which has a distinct role in forming the body's tissues and organs

1. **Ectoderm**:
    - The outermost layer, the ectoderm, gives rise to structures such as the skin, nervous system, and sensory organs. During gastrulation, cells that remain on the surface of the embryo generally form the ectoderm.
2. **Mesoderm**:
    - The middle layer, the mesoderm, forms muscles, bones, the circulatory system, and other connective tissues. Mesodermal cells migrate between the ectoderm and endoderm layers during gastrulation. In some species, the **primitive streak** or **Hensen's node** helps guide mesodermal cells into the correct positions.
3. **Endoderm**:
    - The innermost layer, the endoderm, forms the lining of the gut, respiratory tract, and associated organs like the liver and pancreas. During gastrulation, some cells move inward to form this layer through processes like **invagination** and **involution**.

Key Movements During Gastrulation:

- **Invagination**: Cells fold inward to create a cavity, such as the **archenteron**, which becomes the future digestive tract.
- **Involution**: Sheets of cells roll over an edge and spread into the embryo's interior, creating new layers.
- **Epiboly**: The thinning and spreading of cell layers, such as ectodermal cells covering the embryo.

- **Convergent Extension**: Cells elongate and narrow as they intercalate, helping to shape the embryo.

VI.2. Primary and Secondary Inductions

During gastrulation, **induction** is the process by which certain groups of cells (inducing tissues) signal neighboring cells (responding tissues) to adopt specific fates, guiding the formation of tissues and organs. There are two types of induction: **primary induction**, which sets up the basic body plan, and **secondary induction**, which refines and diversifies tissue formation.

1. **Primary Induction**
   - The classic example of primary induction is the interaction between the **Spemann-Mangold organizer** (in amphibians) and surrounding cells. The organizer secretes signals that instruct neighboring cells to form the **neural ectoderm**, establishing the foundation for the central nervous system.
   - The **dorsal lip of the blastopore** in amphibians and Hensen's node in birds and mammals play similar roles in inducing the formation of the notochord and neural tube, which are central to early body plan development
2. **Secondary Induction**:
   - After the basic body plan is established, secondary induction refines the development of organs and specialized tissues. For example, interactions between the mesoderm and ectoderm induce the formation of structures like the limbs, kidneys, and eyes.
   - **Reciprocal Induction**: Sometimes, tissues that are induced will send feedback signals to further influence the development of the inducing tissue, creating a feedback loop that fine-tunes development.
3. **Molecular Signals in Induction**
   - **Bone Morphogenetic Proteins (BMPs)** and **Noggin**: BMPs promote ectodermal cells to become epidermis, while inhibitors like **Noggin** and **Chordin**, secreted by

the organizer, block BMP signaling in the dorsal ectoderm to allow neural tissue to form.

- **Fibroblast Growth Factors (FGFs)**: These are critical in mesodermal and endodermal induction, helping cells to adopt specific tissue identities.
- **Wnt and Hedgehog Pathways**: These are also key in orchestrating both primary and secondary induction, directing cells to differentiate into specific types based on their position within the embryo.

VI.3. Control of Transcription by Cytoplasmic Factors

During the early stages of embryonic development, the regulation of transcription is controlled by **cytoplasmic factors** that are inherited maternally. These factors play a critical role in the early activation of the embryonic genome and in the spatial patterning of the embryo.

1. **Maternal mRNAs and Proteins**:

   - **Maternal effect genes** encode mRNAs and proteins that are deposited in the egg during oogenesis. These cytoplasmic factors govern the initial stages of development before the zygotic genome is activated. Examples include **Bicoid** and **Nanos** in *Drosophila*, which establish the anterior-posterior axis through localized mRNA distribution and translational control.

2. **Cytoplasmic Localization**:

   - The localization of cytoplasmic factors within the egg is crucial for establishing the embryo's polarity. For example, in many vertebrates, factors like **Vg1** and **Wnt11** are localized in the vegetal pole of the egg and are involved in specifying the dorsal axis. **β-catenin**, activated by the **Wnt signaling pathway**, accumulates in the dorsal side of the embryo and plays a role in the activation of the organizer genes.

3. **Zygotic Genome Activation (ZGA)**:

- As gastrulation proceeds, the embryonic genome becomes fully activated in a process called **zygotic genome activation (ZGA)**. Transcription factors like **Smad**, which are activated by **TGF-β signaling**, and **β-catenin**, regulated by **Wnt signaling**, initiate the expression of genes necessary for gastrulation and further development. The timing of ZGA varies among species, occurring at the **mid-blastula transition** in organisms like *Xenopus* and *Drosophila*.

These cytoplasmic factors control gene expression during the critical early stages of development, ensuring that the embryonic genome is properly activated and that cell fates are determined according to the body plan.

VI.4. Molecules Involved in Cellular Migration

Cell migration is a hallmark of gastrulation, allowing cells to move to their appropriate positions in the embryo to form the three germ layers. This movement is directed by various **extracellular matrix (ECM) proteins**, **adhesion molecules**, and **chemotactic signals** that coordinate the migration of cells.

1. **Fibronectin and Integrins**:
   - **Fibronectin** is an ECM glycoprotein that provides a scaffold for migrating cells. Cells express **integrins**, which are transmembrane receptors that bind to fibronectin and other ECM components, facilitating cellular adhesion and migration. This interaction is crucial for mesodermal cells as they move inward during involution and convergent extension.
2. **Cadherins**:
   - **Cadherins** are cell-cell adhesion molecules that mediate the adherence of cells within tissues. During gastrulation, **E-cadherin** helps maintain the integrity of the epithelial layer, while changes in cadherin expression allow cells to dissociate and migrate. For example, a reduction in **E-cadherin** expression facilitates the **epithelial-to-mesenchymal transition (EMT)**, a process by which epithelial cells lose their polarity and adhesion properties and become migratory mesenchymal cells.
3. **Chemotactic Signals**:
   - **Wnt**, **FGF**, and **TGF-β** pathways provide chemotactic cues that direct the migration of cells toward specific regions of the embryo. These signaling molecules create gradients that cells follow during gastrulation, ensuring the proper positioning of the mesoderm and endoderm layers.

4. **Cytoskeletal Regulation**:
   - The cytoskeleton, particularly **actin filaments**, plays a crucial role in driving the movements required for cell migration. **Rho GTPases**, such as **Rho**, **Rac**, and **Cdc42**, regulate cytoskeletal dynamics, allowing cells to form **lamellipodia** and **filopodia** for migration.

These molecules and pathways are essential for coordinating the cellular migrations that shape the embryo during gastrulation, ensuring that cells reach their correct destinations to form the body's tissues.

VI.5. Morphogenetic Movements

Morphogenetic movements are the coordinated movements of cells and tissues that give the embryo its final shape. These movements are integral to the proper formation of the germ layers and the establishment of the body axes during gastrulation.

1. **Invagination**
   - **Invagination** is a process by which a sheet of cells bends inward to form a pocket or cavity. This movement is seen in organisms like sea urchins and amphibians, where the **archenteron** (primitive gut) forms through the invagination of cells at the vegetal pole.
2. **Involution**
   - In **involution**, a layer of cells rolls inward over an edge, such as the **blastopore**, creating a new internal layer. This movement is critical for mesodermal and endodermal formation in amphibians and other vertebrates.

3. **Epiboly**

   - **Epiboly** is the thinning and spreading of the ectoderm over the embryo's surface, which occurs in species such as fish and amphibians. During epiboly, the outer cell layer extends to enclose the internal cell layers, helping to shape the embryo.

4. **Convergent Extension**

   - **Convergent extension** involves the intercalation of cells, whereby cells move toward the midline of the embryo and elongate, contributing to the narrowing and lengthening of tissues. This movement is crucial for the formation of structures like the **notochord** and the neural plate.

5. **Delamination**

   - **Delamination** occurs when a single cell layer splits into two distinct layers. This movement is seen in birds and mammals during the formation of the **hypoblast** and **epiblast**.

Morphogenetic movements drive the large-scale reorganization of cells during gastrulation, establishing the foundational structure of the embryo and enabling the correct formation of tissues and organs.

Conclusion

Gastrulation is a highly coordinated process that establishes the three primary germ layers and defines the body axes, positioning cells for subsequent organogenesis. The regulation of cellular migration, transcription by cytoplasmic factors, and the execution of morphogenetic movements are all crucial for successful gastrulation. Inductive interactions and the precise control of signaling pathways ensure that cells differentiate appropriately, laying the groundwork for the development of the organism's complex body structures. Understanding these processes provides insight into the fundamental mechanisms of embryogenesis and the molecular basis of development.

VII. NEURULATION

Neurulation is a key developmental process that establishes the nervous system in vertebrates. It involves the transformation of a portion of the ectoderm into the neural tube, which will later develop into the central nervous system (CNS). This process also involves the formation of **neural crest cells**, which contribute to the peripheral nervous system, including the autonomic ganglia.

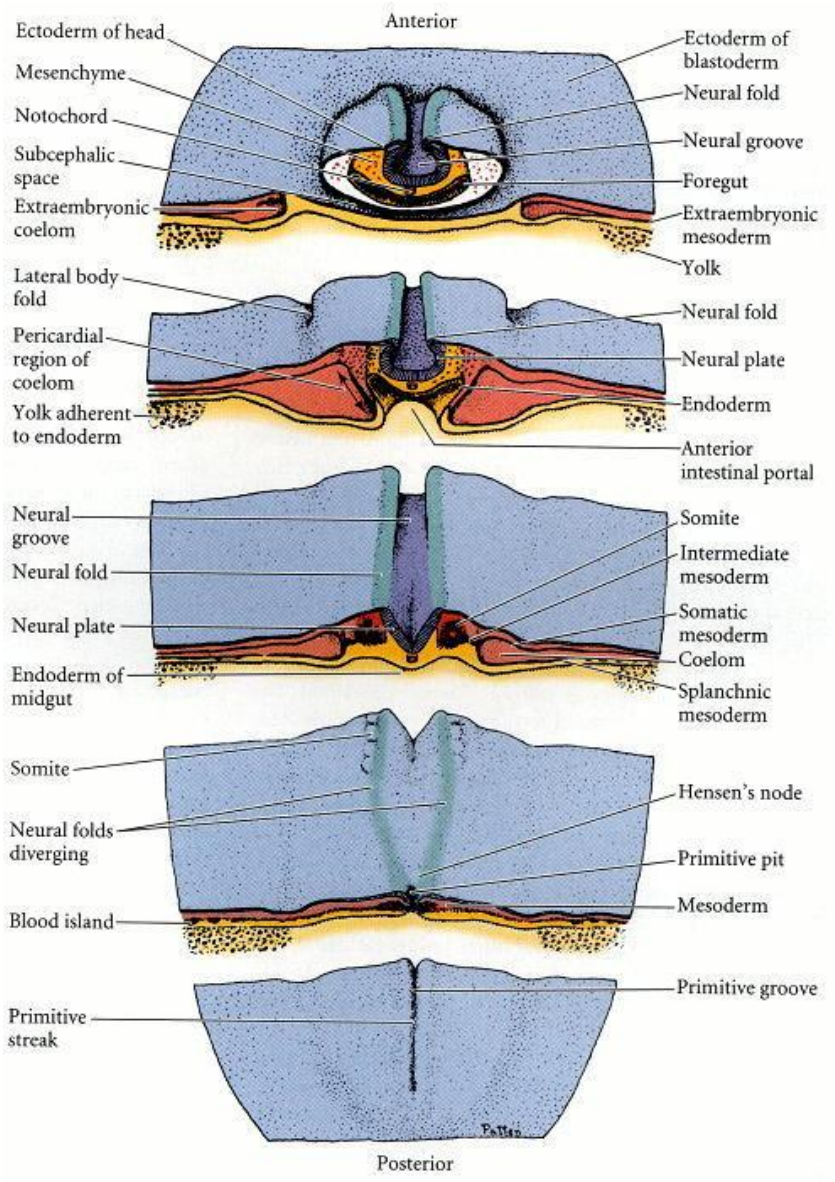

Figure 52:Stereogram of a 24-hour chick embryo. Cephalic portions are finishing neurulation while the caudal portions are still undergoing gastrulation(Borday et al., 2012).

1. Establishment of the Neural Tube and Autonomic Ganglia

1. **Neural Tube Formation**:

   - **Neurulation** begins when the **neural plate** forms from the ectoderm in the dorsal side of the embryo, guided by signals from the underlying notochord and mesoderm.
   - The neural plate cells undergo morphological changes, causing them to invaginate and form a **neural groove**. The lateral edges of the groove elevate, creating **neural folds**.
   - The neural folds move towards each other and fuse along the midline, forming the **neural tube**, which gives rise to the brain and spinal cord.
   - Neural tube closure is a sequential process, starting in the middle of the embryo and proceeding bidirectionally. Improper closure of the neural tube can lead to conditions such as **spina bifida** (if the posterior end fails to close) or **anencephaly** (if the anterior end fails to close).
   - The neural tube's anterior end enlarges to form the **brain vesicles**, while the rest becomes the **spinal cord**.

2. **Neural Crest Cells and Autonomic Ganglia**:

   - As the neural tube forms, a unique population of cells called **neural crest cells** emerges from the edges of the neural plate. These cells detach and migrate throughout the embryo, contributing to a wide range of structures.
   - Neural crest cells give rise to the **autonomic ganglia**, which are clusters of neurons that regulate involuntary functions like heartbeat, digestion, and

respiratory rate. These ganglia are part of the **autonomic nervous system** (ANS) and are subdivided into the **sympathetic** and **parasympathetic** ganglia.

- Neural crest cells also contribute to other structures, such as sensory ganglia, melanocytes, and components of the facial skeleton.

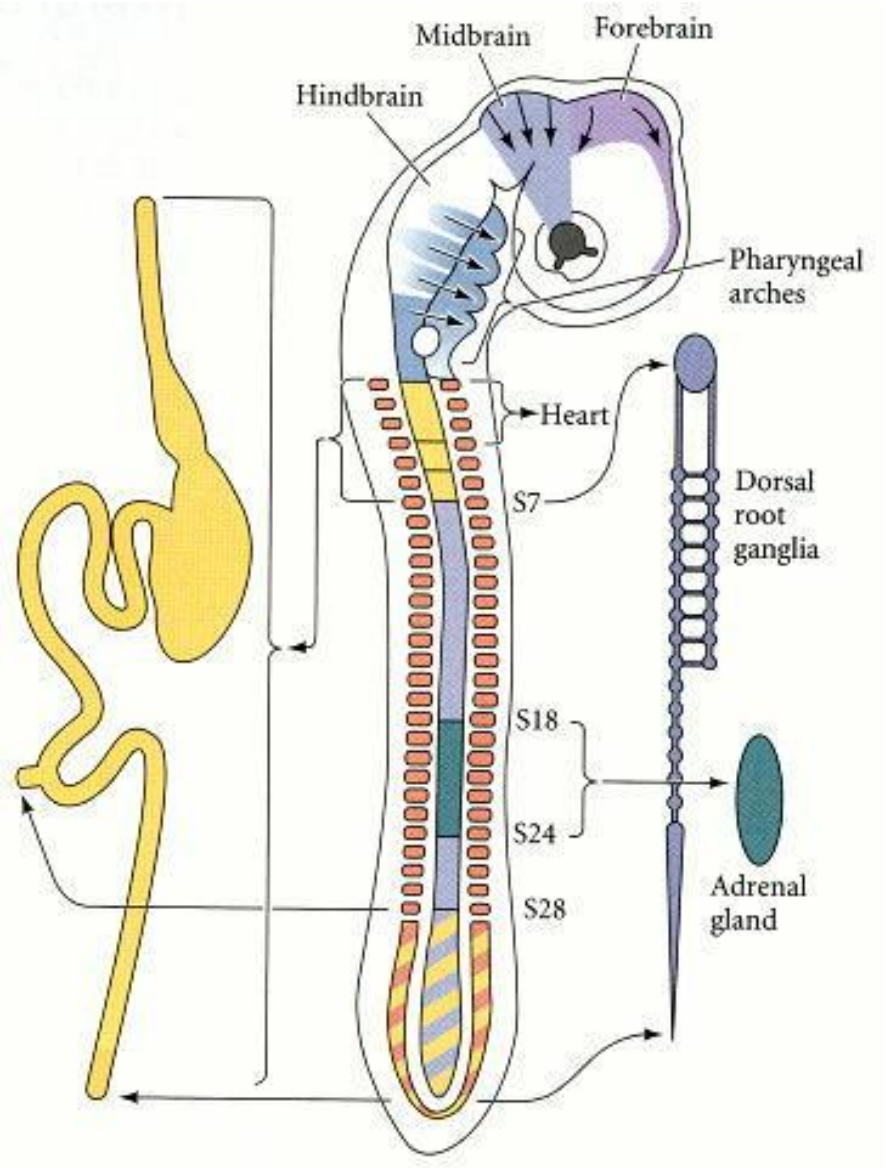

Figure 53:Regions of the neural crest. The cranial neural crest migrates into the branchial arches and the face to form the bones and cartilage of the face and neck(*Molnar & Gair, 2015*)

VIII. ORGANOGENESIS

Organogenesis is the stage of embryonic development where the three germ layers (ectoderm, mesoderm, and endoderm) differentiate into specific organs and tissues. It occurs after gastrulation and neurulation, as the basic body plan is laid out.

The first organ system to develop during organogenesis is the cardiovascular system. The heart has established its 4 chambers by 4 weeks of development, whereas week 6 involves cardiac outflow separation and descent of the heart (and lungs) into the thorax. The separation divides the truncus arteriosus into the ascending aorta and pulmonary artery; this occurs via spiraling of the aorticopulmonary septum. Anatomically, the aorta and pulmonary appear to wrap around each other superior to the heart. That appearance is the result of embryologic spiraling. The aorticopulmonary septum may also be called the spiral or conotruncal septum(Schittny, 2017).

Lung development occurs from the embryonic period through the fetal period until birth. In particular, lung growth begins in early embryonic development when right and left lung buds are formed from an initial outpouching, the respiratory diverticulum. The buds enlarge and branch to form the respiratory tree. The appearance of the visceral and parietal pleura takes place during weeks 5 through 7. Both types of pleura arise from mesoderm. The visceral pleura covers the developing bronchial tree, and the parietal pleura covers the internal chest wall. Pleuroperitoneal membranes form and fuse with the diaphragm, which separates the pleural and peritoneal body cavities. Closure of the pleuroperitoneal canal by these membranes takes place by approximately week 7(Giancotti et al., 2019; Schittny, 2017). The neural tube closes around week 4 and is the early derivative of the brain and spinal cord. During weeks 5 through 8, the CNS develops its vesicles, embryologic precursors to different brain structures. The forebrain, midbrain, and hindbrain all develop from vesicles. These three structures are the prosencephalon, mesencephalon, and rhombencephalon, respectively. The prosencephalon later

develops into the diencephalon and telencephalon. The diencephalon gives rise to the thalami, hypothalamus, optic cups, and neurohypophysis, while the telencephalon grows to surround the diencephalon, midbrain, and hindbrain. The mesencephalon forms the aqueduct of Sylvius, superior and inferior colliculi, and tegmentum. The rhombencephalon gives rise to the fourth ventricle and the metencephalon, a structure that eventually develops into the pons and cerebellum.(Fotos et al., 2011)

1. **Ectoderm Derivatives**:

The **ectoderm** gives rise to the nervous system, skin, and sensory organs.

- The **neural tube** (from the ectoderm) forms the brain and spinal cord.
- The outer ectoderm forms the **epidermis**, hair, nails, and parts of the eye (e.g., lens).
- **Neural crest cells**, derived from ectoderm, contribute to the peripheral nervous system and many craniofacial structures.

2. **Mesoderm Derivatives**:

The **mesoderm** gives rise to the skeletal system, muscles, cardiovascular system, and more.

- ✓ The **somites**, segmented blocks of mesoderm, give rise to the vertebrae, ribs, and muscles.
- ✓ The **intermediate mesoderm** forms the kidneys and gonads.
- ✓ The **lateral plate mesoderm** forms the heart, blood vessels, and components of the limbs.

3. **Endoderm Derivatives**:

The **endoderm** forms the lining of the digestive and respiratory systems and associated organs.

- ✓ The **gut tube** forms the stomach, intestines, and other digestive organs.
- ✓ The endoderm also forms the **lungs**, liver, pancreas, and thyroid.

IX. MORPHOGENESIS OF EMBRYONIC

Morphogenesis is the biological process that causes an organism to develop its shape. In vertebrates, the development of limbs and other appendages involves a complex interplay of molecular signals that regulate growth, patterning, and differentiation.

1. **Limb Bud Formation**:

   - ✓ Limb development begins with the formation of **limb buds**, which are small protrusions on the lateral sides of the embryo.
   - ✓ These limb buds consist of **mesodermal cells** derived from the lateral plate mesoderm and are covered by ectoderm.
   - ✓ The mesodermal cells within the limb bud differentiate into skeletal elements, muscles, and connective tissues, while the ectoderm forms the skin.

2. **Apical Ectodermal Ridge (AER)**:

   - ✓ The **Apical Ectodermal Ridge (AER)** is a specialized thickened region of ectoderm at the tip of the limb bud. It plays a crucial role in promoting the outgrowth of the limb along the **proximal-distal axis** (shoulder to fingertip).
   - ✓ The AER secretes signaling molecules, such as **Fibroblast Growth Factors (FGFs)**, which promote cell proliferation and prevent differentiation until the proper structures have been formed.

3. **Zone of Polarizing Activity (ZPA)**:

   - ✓ The **Zone of Polarizing Activity (ZPA)** is a region of mesoderm located on the posterior side of the limb bud. It secretes **Sonic Hedgehog (Shh)**, which provides positional information to the developing limb.

- ✓ The gradient of Shh helps establish the **anterior-posterior axis** (thumb to pinky) and ensures the correct number and identity of digits.

4. **Proximal-Distal and Dorsal-Ventral Patterning**:

- ✓ **Proximal-distal patterning** is regulated by interactions between the AER and mesoderm. FGFs from the AER promote the distal development of the limb (e.g., fingers).
- ✓ **Dorsal-ventral patterning** (back to palm in limbs) is controlled by signals from the ectoderm, such as **Wnt7a**, which influences the dorsal side of the limb, and **BMPs**, which influence the ventral side.

X. GENETIC CONTROL OF DEVELOPMENT

The genetic regulation of development is orchestrated by a complex network of genes that control when and where cells divide, migrate, and differentiate. In model organisms like *Drosophila* (fruit flies) and vertebrates, a well-understood set of genes regulates the body plan and organ formation.

X.1. Expression of the Development Plan in *Drosophila*

1. **Segmentation Genes**

The development of *Drosophila* is governed by a hierarchy of segmentation genes, including:

- **Maternal Effect Genes**: Establish the anterior-posterior axis. For example, **Bicoid** mRNA is deposited in the egg and forms a gradient that influences the anterior structures.
- **Gap Genes**: Define broad regions of the embryo. Mutations in gap genes, like **Kruppel**, can result in the loss of large segments of the body.
- **Pair-Rule Genes**: Further refine segmentation by establishing alternating bands of gene expression (e.g., **Even-skipped** and **Fushi Tarazu**).
- **Segment Polarity Genes**: Define the anterior-posterior polarity within each segment, such as **Engrailed** and **Hedgehog**.

2. **Homeotic Genes**

- **Homeotic (Hox) Genes** control the identity of segments by regulating which body parts will form in each segment. In *Drosophila*, mutations in these genes

can lead to transformations, such as legs growing in place of antennae (as seen in **Antennapedia** mutants).

X.2. Regulatory Genes in Vertebrate Development

1. **Hox Genes in Vertebrates**:
    - **Hox genes** are also critical in vertebrate development and are expressed in specific patterns along the anterior-posterior axis. These genes ensure that each body region develops its appropriate structures (e.g., vertebrae in the spinal column).
    - Hox gene expression is spatially and temporally regulated, with **Hox clusters** controlling body patterning in mammals, birds, and other vertebrates.
2. **Transcription Factors**:
    - Regulatory genes such as **Pax6** are crucial for the development of organs like the eyes, while **Sox2** is important for neural development.
    - **FGF**, **Wnt**, and **Hedgehog** pathways are highly conserved in vertebrates and regulate processes such as limb development and organogenesis.

XI. PLACENTA

The **placenta** is a temporary organ that forms during pregnancy in mammals, providing essential nutrients and oxygen to the developing fetus while removing waste products. It also functions as an endocrine organ, producing hormones that regulate pregnancy and fetal development. The placenta is a vital organ with multiple functions, such as endocrine, immune, and physiological. The placenta is formed gradually during the first three months of pregnancy, while, after the fourth month, it grows parallel to the development of the uterus. Once completed, it resembles a spongy disc 20 cm in diameter and 3 cm thick. It is a temporary organ, whose genetic characteristics are identical to those of the developing child. The placenta interacts with the environment in which it is present and vice versa. Proper development of the placenta is essential for a successful pregnancy. There are several layers of tissue that make up this delicate organ that need to develop normally for proper function during gestation. Without proper function, there can be devastating consequences to the pregnancy(Herrick & Bordoni, 2023).

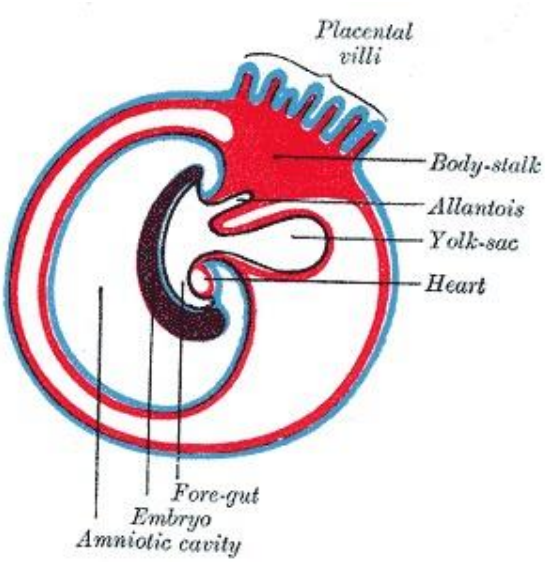

Figure 54:Development of the Fetal membrane and the Placenta, Diagram showing the expansion of amnion and delimitation of the umbilicus, Heart, Fore-gut, Embryo, Amniotic cavity, Placental villi Henry Vandyke Carter, Public Domain, via Wikimedia Commons(Fotos et al., 2011)

1. **Formation**:

The placenta forms from the **trophoblast** cells of the blastocyst and the maternal uterine tissue. **Chorionic villi** extend into the uterine wall, facilitating the exchange of nutrients and gases between the mother and fetus. The placenta is a fetal organ made up of its parenchyma, chorion, amnion, and umbilical cord. The fetal structures form from the zygote and therefore separate the fetus from the endometrium. The fetal tissues form from the chorionic sac - which includes the amnion, chorion, yolk sac, and allantois. These tissues get delivered after birth. The maternal part comes from the endometrium and is called the decidua. There are three parts to the decidua - the decidua basalis (deep at the implantation site), the decidua capsularis (covers the implantation site), and the decidua parietalis (everything else)(Burton & Jauniaux, 2015)

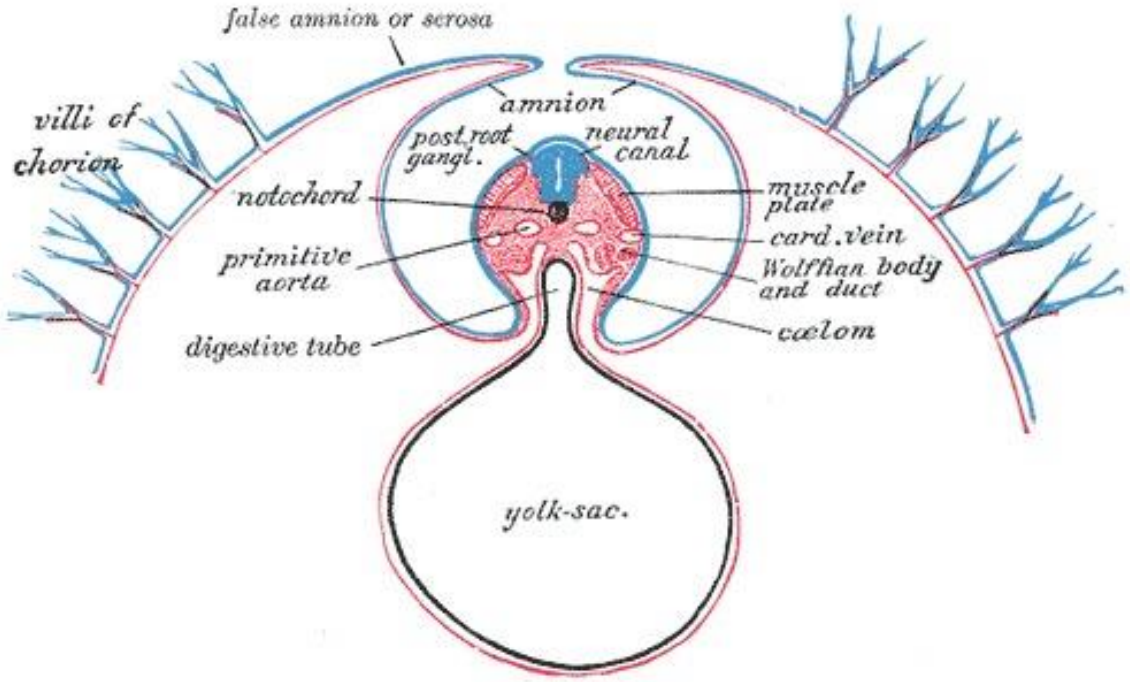

Figure 55:Development of the Fetal membrane and the Placenta, Diagram of a transverse section, showing the mode of formation of the amnion in the chick. The amniotic folds have nearly united in the middle line, Ectoderm is blue is the mesoderm; red is the entoder(Ando, 2010)

2. **Fonctions**

The placenta is the means of communication between mom and fetus. The placental membrane is where the exchange of substances happens between mother and fetus. This exchange is essential for the transfer of gases, electrolytes, hormones, maternal antibodies, fetal waste, and nutrition such as water, amino acids, glucose, vitamins, and free fatty acids. Fetal waste includes urea, uric acid, and bilirubin. Alpha-fetoprotein and other proteins also get exchanged. These transfers are beneficial to the fetus, but many harmful substances can pass through the placental membrane such as certain drugs, live vaccines, carbon monoxide, anti-Rh antibodies, and several infectious agents ("ToRCHes" infections)(Kaplan, 2013; Neu et al., 2015).

- **Nutrient and Gas Exchange**: The placenta allows for the diffusion of oxygen, carbon dioxide, nutrients, and waste between maternal and fetal blood without direct mixing of the two blood supplies.
- **Hormonal Regulation**: The placenta secretes hormones such as **human chorionic gonadotropin (hCG)**, **progesterone**, and **estrogen**, which are essential for maintaining pregnancy.
- **Immune Protection**: The placenta acts as a selective barrier, protecting the fetus from pathogens while allowing the passage of maternal antibodies to confer immunity to the fetus.

3. **Placental Disorders**

- Disorders like **placenta previa** (where the placenta covers the cervix) and **placental abruption** (premature separation from the uterine wall) can pose risks to both the mother and fetus during pregnancy.

CONCLUSION

The study of developmental biology, as outlined in this manual, provides a comprehensive understanding of the processes guiding the formation and differentiation of various life forms, from amphibians and birds to insects. These organisms, while diverse, follow distinct yet interconnected stages of development, illustrating fundamental biological principles that span across species.

Through a closer look at amphibians, birds, and insects, we examined how the stages of development reflect each organism's adaptation to its environment. Each species exhibits unique developmental features that are tightly controlled by genetic, molecular, and environmental factors. From the distribution of reserves essential for early growth to the protective envelopes of gametes, these foundational elements play pivotal roles in ensuring the survival and successful development of the embryo.

Fertilization marks a crucial turning point, initiating significant structural changes in the egg, followed by its activation. This leads to segmentation, the transformation of a single cell into a multicellular organism, which is meticulously regulated by molecular and cellular interactions. The precision of gastrulation and neurulation stages ensures the proper organization and positioning of primary tissues and the formation of the neural structures necessary for life.

Subsequent processes, such as organogenesis and morphogenesis, complete the organism’s structure, creating complex appendages and internal systems necessary for independent life. The genetic control of development serves as an underlying framework for these stages, where regulatory genes govern the expression of development plans and specify the organism’s final form. This is evident in models such as Drosophila and vertebrate systems, which highlight the universality and specificity of genetic regulatory mechanisms across species.

In conclusion, this manual highlights the interconnected stages of embryogenesis and development, where each phase builds upon the previous one in a complex dance directed by genetic, cellular, and environmental cues. This intricate orchestration underlies not only individual development but also evolutionary adaptations that enable life to thrive in diverse forms.

Final Words from Dr. Fatima Benchikh

As we conclude this practical manual of Embryology, I am reminded of the incredible journey we undertake each time we delve into the development of life. This manual, crafted with care, represents not just a guide through embryology but an invitation to engage with the profound mechanisms that govern the earliest and most essential phases of life across species.

Our study underscores that embryology is more than just cellular divisions, genetic instructions, and molecular interactions; it is a testament to nature's extraordinary precision and adaptability. From the first stages of fertilization to the intricate steps of organogenesis, each process reflects a finely tuned choreography that has evolved over millennia, providing invaluable insights into the interconnectedness of life forms.

I hope this manual serves as a source of knowledge, inspiration, and curiosity for future scholars, researchers, and practitioners. May it empower you to explore further and uncover new dimensions of developmental biology, pushing the boundaries of what we know and contributing to the ever-growing field of embryological science.

Thank you for embarking on this journey with me. May this manual be a foundation for your own discoveries and a steppingstone towards greater scientific exploration.

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