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M.SC ZOOLOGY

ZOO501 - Developmental Biology

HANDOUTS TOPIC NO 1 TO 144

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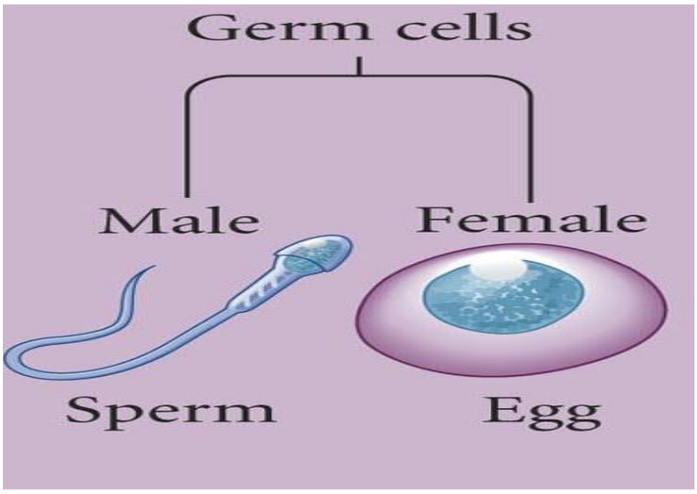
Topic.1 INTRODUCTION TO DEVELOPMENTAL BIOLOGY

All multicellular organisms arise by a slow process of progressive change called ***development.*** Development is a process by which a multicellular organism arises, initially from a single cell- the fertilized egg, or **zygote,** which divides mitotically to produce all the cells of the body.

The study of animal development has traditionally been called **embryology**, from that stage of an organism that exists between fertilization and birth.

Each day we replace more than a gram of skin cells (the older cells being sloughed off as we move), and our bone marrow sustains the development of millions of new red blood cells every minute of our lives.

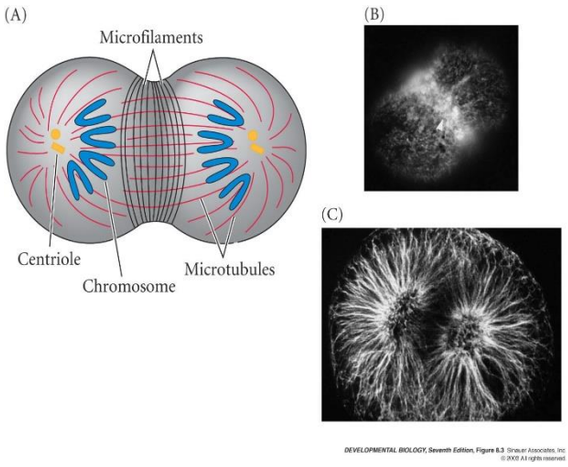


Fertilization

- How gametes fuse with each other

Cell Division

- How is cell division regulated?



DEVELOPMENTAL BIOLOGY, Seventh Edition, Figure 8.3 Sinauer Associates, Inc
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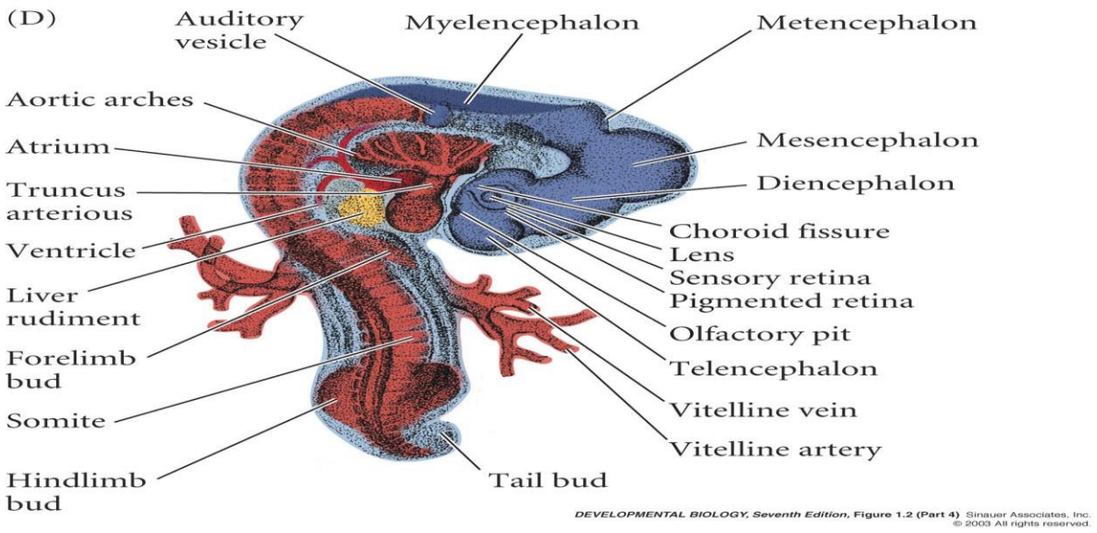
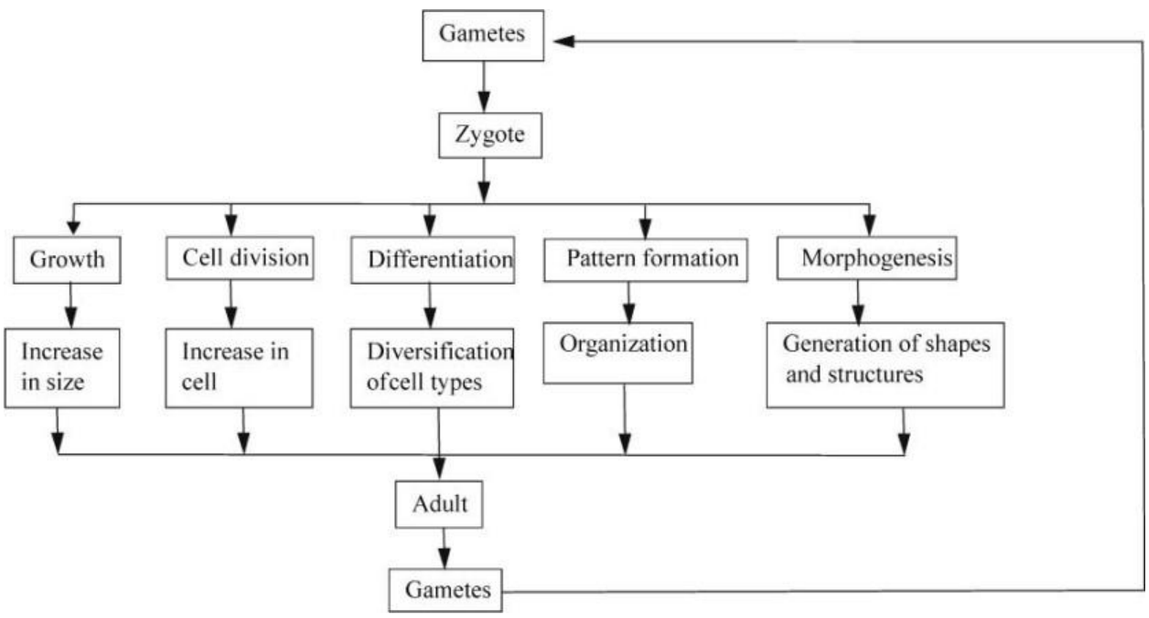
In addition, some animals can regenerate severed parts, and many species undergo metamorphosis (such as the transformation of a tadpole into a frog, or a caterpillar into a butterfly).

So **Developmental biology** is a discipline that studies embryonic and other developmental processes.

- **How do cells form ordered structures?**





Development accomplishes two major objectives:

- ✓ It generates cellular diversity and order within each generation.
- ✓ It ensures the continuity of life from one generation to the next.

Topic. 2 DEVELOPMENTAL HIERARCHY OF ANIMALS

The **developmental hierarchy** is the hierarchical series of decisions involving specific preexisting cell types.These developmental decisions are usually irreversible, that progressively and irreversibly restrict cell fate.

Animal development is the division of embryo into the three germ layers.

1. Ectoderm
2. Mesoderm
3. Endoderm

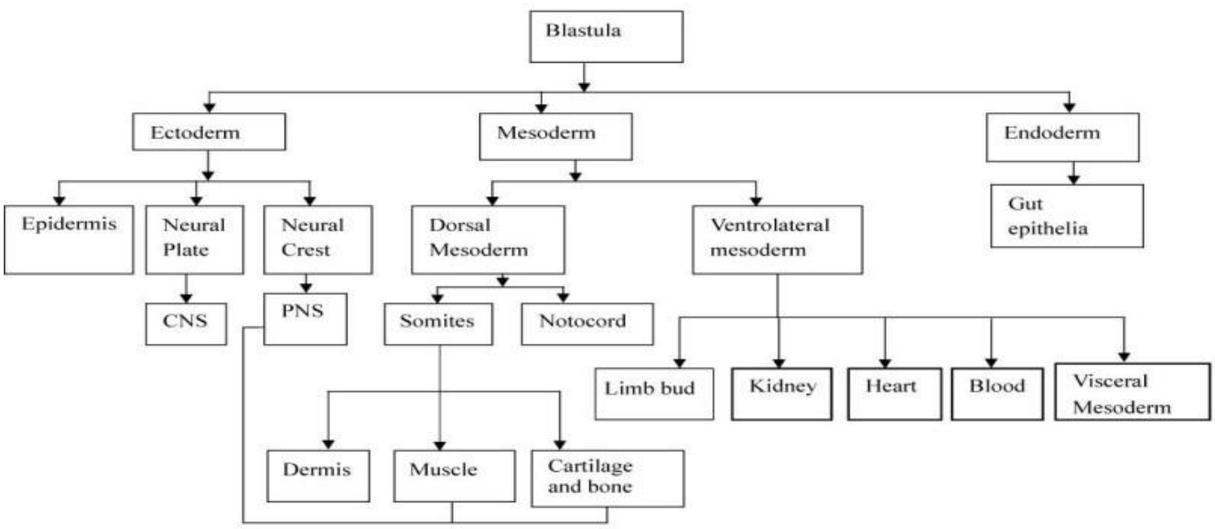
Potency and cell fate

1. Potency is the entire range of the cell types a particular cell can give rise to in all possible environments.

2. For example: A cell is pluripotent if it can give rise to all different blood cell lineages. There is a decrease in potency as cells become committed.

   Fate of a cell is all the different cell types, its descendants can become during normal development.

- Cell potency ≥ fate.
- Potency is intrinsic property.
- Cell fate is equal to potency plus environment.
- Cell fate becomes increasingly restricted until a cell is terminally differentiated.



Topic .3 ANATOMICAL APPROACHES TO DEVELOPMENTAL BIOLOGY

The basis of all research in developmental biology is the changing anatomy of the organism. What parts of the embryo form the heart? How do the tissues that form the bird wing relate to the tissues that form the fish fin or the human hand?

Following important parameters weave together to form the anatomical approaches to development.

- Comparative embryology
- Evolutionary embryology
- Teratology
- Mathematical modeling

- **Comparative embryology:**

The first strand is comparative embryology, the study of how anatomy changes during the development of different organisms. For instance, a comparative embryologist may study which tissues form the nervous system in the fly or in the frog.

- **Evolutionary embryology:**

The second strand, based on the first, is evolutionary embryology, the study of how changes in development may cause evolutionary changes and of how an organism's ancestry may constrain the types of changes that are possible.

- **Teratology:**

The third anatomical approach to developmental biology is **teratology**, the study of birth defects. These anatomical abnormalities may be caused by mutant genes or by substances in the environment that interfere with development. The study of abnormalities is often used to discover how normal development occurs.

➢ **Mathematical modeling:**

The fourth anatomical approach is mathematical modeling, which seeks to describe developmental phenomena in terms of equations. Certain patterns of growth and differentiation can be explained by interactions whose results are mathematically predictable.

Topic 04 .DEVELOPMENTAL COMMITMENT

Before a cell overtly differentiates, it can be said to be committed, that is, the cell has been instructed on its fate. An intrinsic program has been activated within the cell that causes it to follow a particular pathway of development.

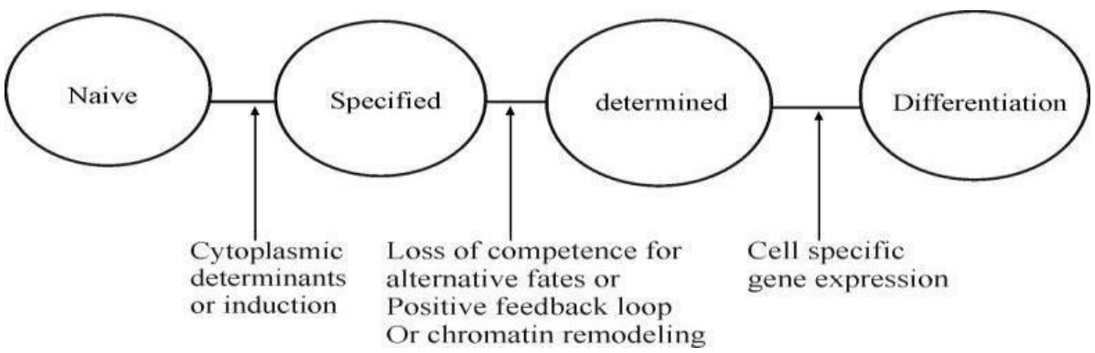
LEVELS OF DEVELOPMENTAL COMMITMENT

➢ As cell fate becomes restricted following each decision in the developmental hierarchy, cells become committed to a certain fate.

➢ An uncommitted cell can be described as naive, meaning that it has received no instructions directing it along a particular developmental pathway.

➢ The fate of a cell is said to be determined if it cannot be changed, regardless of cell's environment. Commitment at this stage becomes irreversible.

➢ Fate of a cell is said to be specified if the cell is directed to follow a certain developmental pathway and does so when placed in isolation, which should provide a neutral environment. Commitment at this stage is reversible as it may be specified if placed in different environment.

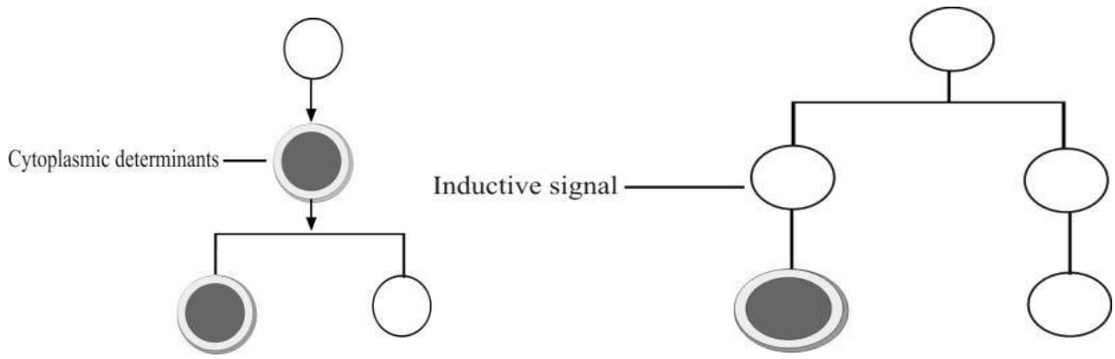


TOPIC 05.MECHANISMS OF DEVELOPMENTAL COMMITMENT

There appear to be two major strategies for establishing commitment and hence initiating the series of events that result in cell differentiation.

- **The inheritance of cytoplasmic determinants:** Cytoplasmic determinants are the molecules in cytoplasm that can help to determine cell fate. The asymmetric distribution of cytoplasmic determinants indicates that the mechanism of differentiation is entirely intrinsic.

  - For example, if a mother cell contains cytoplasmic determinants that is localized to one pole as the cell under goes division, that determinant will be inherited by only one of the daughters.



- **The perception of external inductive signals:** The process where one cell or group of cells changes developmental fate of another is termed induction. It is extrinsic process that depends on the position of a cell in the embryo.
- Two identical cells can follow alternative fates if one is exposed to an external signal (often secreted by a different cell).

Topic 06. MODES OF COMMITMENT

Three basic modes of commitment are,

- ❖ Autonomous specification
- ❖ Conditional specification
- ❖ Syncytial specification

AUTONOMOUS SPECIFICATION:

Specification by differential acquisition of certain cytoplasmic molecules present in the egg.

- ➢ Characteristic of most invertebrates.
- ➢ Cell type specification precedes any large-scale embryonic cell migration.
- ➢ Autonomous specification gives rise to a pattern of development referred to as **mosaic development**, in which the embryo appears to be constructed like a tile mosaic of independent self-differentiating parts.
- ➢ Invariant cleavages produce the same lineages in each embryo of the species. Blastomere fates are generally invariant.
- ➢ Produces "mosaic" ("determinative") development: cells cannot change fate if a blastomere is lost.

CONDITIONAL SPECIFICATION:

- ➢ Characteristic of all vertebrates and few invertebrates. o Specification by interactions between cells. Relative positions are important. o Variable cleavages produce no invariant fate assignments to cells. o Massive cell rearrangements and migrations precede or accompany specification.
- ➢ Capacity for "regulative" development: allows cells to acquire different functions.

SYNCYTIAL SPECIFICATION:

- ➢ Characteristic of most insect classes.
- ➢ Specification of body regions by interactions between cytoplasmic regions prior to cellularization of the blastoderm.
- ➢ Variable cleavage produces no rigid cell fates for particular nuclei.

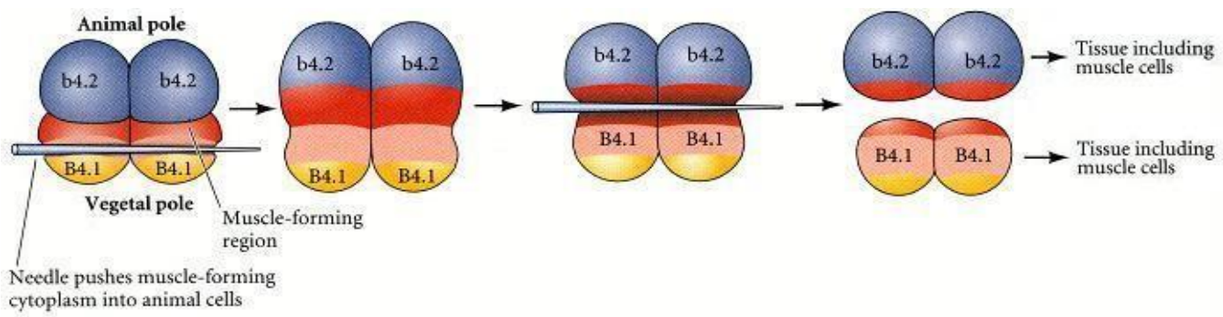
- After cellularization, conditional specification is most often seen.

Topic 07. AUTONOMOUS SPECIFICATION

Autonomous specification was first demonstrated in 1887 by a French medical student, **Laurent Chabry**. Chabry desired to know the causes of birth defects, and he reasoned that such malformations might be caused by the lack of certain cells.

Chabry set out to produce specific malformations by isolating or lancing specific blastomeres of the cleaving tunicate embryo.

He discovered that each blastomere was responsible for producing a particular set of larval tissues.



In the absence of particular blastomeres, the larva lacked just those structures normally formed by those cells. Moreover, he observed that when particular cells were isolated from the rest of the embryo, they formed their characteristic structure apart from the context of the other cells. Thus, each of the tunicate cells appeared to be developing autonomously.

Recent studies have confirmed that when particular cells of the 8-cell tunicate embryo are removed, the embryo lacks those structures normally produced by the missing cells, and the isolated cells produce these structures away from the embryo.

J. R. Whittaker provided dramatic biochemical confirmation of the cytoplasmic segregation of the morphogenetic determinants responsible for this pattern. He stained blastomeres for the presence of the enzyme acetylcholinesterase.

Topic 08. CONDITIONAL SPECIFICATION

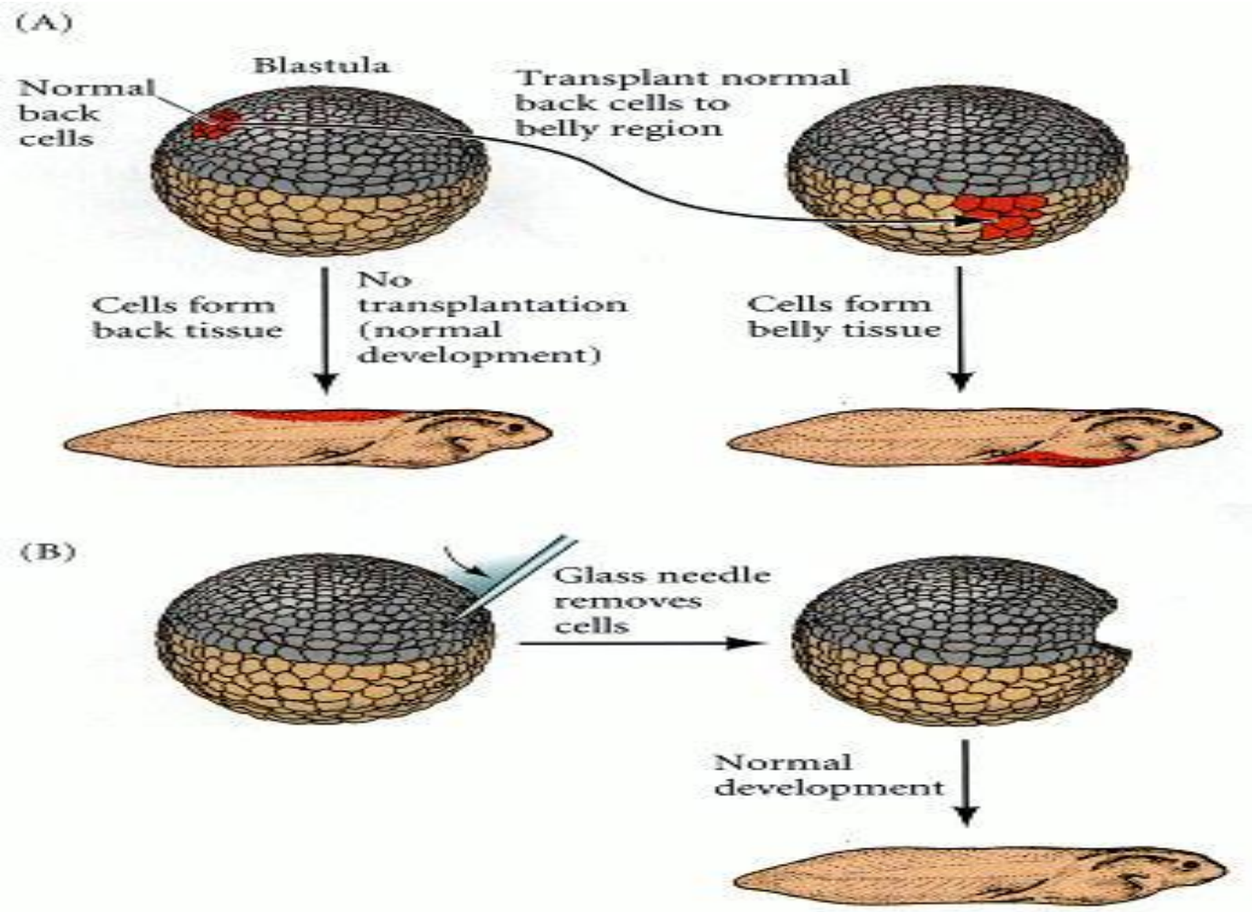
This mode of commitment is sometimes called **conditional specification**, because the fate of a cell depends upon the conditions in which the cell finds itself.

This mode of commitment involves interactions with neighboring cells.

In this type of specification, each cell originally has the ability to become many different cell types. However, the interactions of the cell with other cells restricts the fate of one or both of the participants.

If a blastomere is removed from an early embryo that uses conditional specification, the remaining embryonic cells alter their fates so that the roles of the missing cells can be taken over.

This ability of the embryonic cells to change their fates to compensate for the missing parts is called regulation.



The isolated blastomere can also give rise to a wide variety of cell types (and sometimes generates cell types that the cell would normally not have made if it were part of the embryo).

Thus, conditional specification gives rise to a pattern of embryogenesis called **regulative development**.

Regulative development is seen in most vertebrate embryos, and it is obviously critical in the development of identical twins. In the formation of such twins, the cleavage-stage cells of a single embryo divide into two groups, and each group of cells produces a fully developed individual.

Topic 09. SYNCYTIAL SPECIFICATION

Many insects also use a third means, known as **Syncytial specification**, to commit cells to their fates. Here, interactions occur not between cells, but between parts of one cell.

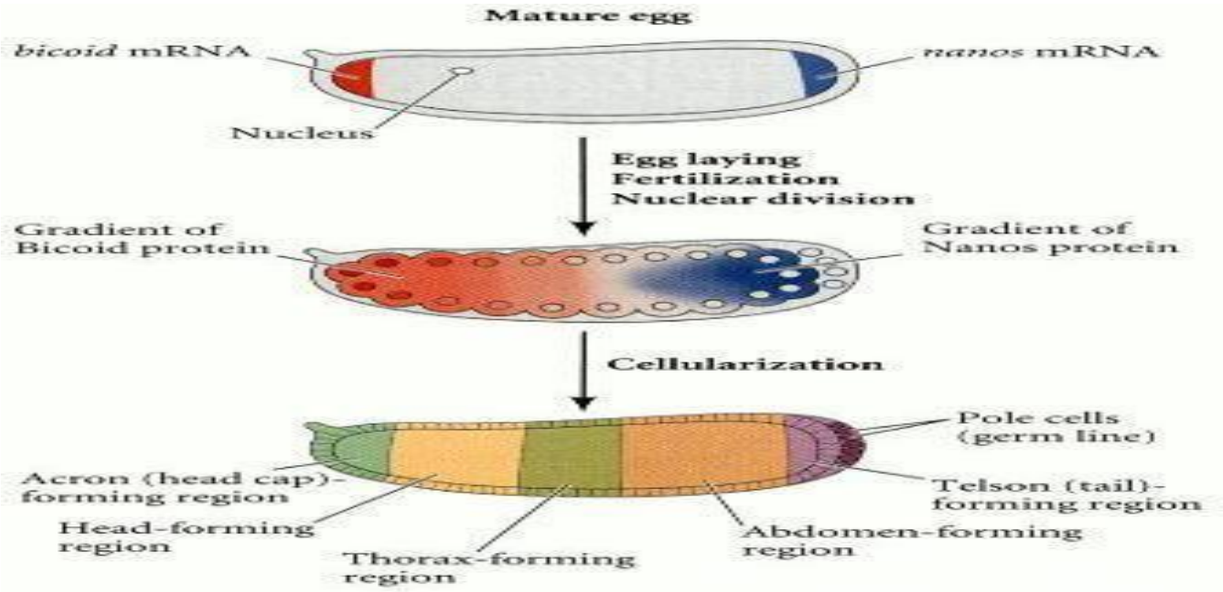
In early embryos of these insects, cell division is not complete. Rather, the nuclei divide within the egg cytoplasm. This creates many nuclei in the large egg cell.

A cytoplasm that contains many nuclei is called a **syncytium**.

The egg cytoplasm, however, is not uniform.

Rather, the anterior of the egg cytoplasm is markedly different from the posterior. In *Drosophila,* for instance, the anterior most portion of the egg contains an mRNA that encodes a protein called **Bicoid**.

The posterior most portion of the egg contains an mRNA that encodes a protein called **Nanos.**



When the egg is laid and fertilized, these two mRNAs are translated into their respective proteins.

The **Bicoid** and **Nanos** proteins form a coordinate system based on their ratios, such that each region of the embryo will be distinguished by a different ratio of the two proteins.

- Those nuclei in regions containing high amounts of Bicoid and little Nanos will be instructed to activate those genes necessary for producing the head.
- Those nuclei in regions with slightly less Bicoid but with a small amount of Nanos will be instructed to activate those genes that generate the thorax.
- Those nuclei in regions that have little or no Bicoid and plenty of Nanos will be instructed to form the abdominal Structures.

Insects such as *Drosophila* use all three modes of specification to commit their cells to particular fates.

Topic 10. PRINCIPLES OF DEVELOPMENT

Following are the some important points that are considered as principles of development.

- Organisms must function as they form their organs. They have to use one set of structures while constructing others.
- Preformation is not in the anatomical structures, but in the instructions to form them. The inheritance of the fertilized egg includes the genetic potentials of the organism.
- The preformed nuclear instructions include the ability to respond to environmental stimuli in specific ways.
- The **ectoderm** gives rise to the epidermis, nervous system, and pigment cells.
- The **mesoderm** generates the kidneys, gonads, bones, heart, and blood cells.
- The **endoderm** forms the lining of the digestive tube and the respiratory system.
- **Karl von Baer's principles** state that the general features of a large group of animals appear earlier in the embryo than do the specialized features of a smaller group. The early embryo of a "higher" animal species is not like the adult of a "lower" animal.

- Homologous structures in different species are those organs whose similarity is due to their sharing a common ancestral structure. Analogous structures are those organs whose similarity comes from their serving a similar function (but which are not derived from a common ancestral structure).
- "**Community of embryonic** structure reveals community of descent" (Charles Darwin).
- Congenital anomalies can be caused by genetic factors (mutations, aneuploidies, translocations) or by environmental agents (certain chemicals, certain viruses, radiation).
- **Syndromes** consists of sets of developmental abnormalities that "run together."
- Organs that are linked in developmental syndromes share either a common origin or a common mechanism of formation.
- **Allometric** growth can create dramatic changes in the structure of organisms.

Topic 11. THE STAGES OF ANIMAL DEVELOPMENT

CLEAVAGE:

Cleavage is a series of extremely rapid mitotic divisions wherein the enormous volume of zygote cytoplasm is divided into numerous smaller cells. These cells are called **blastomeres**, and by the end of cleavage, they generally form a sphere known as a **blastula**.

GASTRULATION:

A series of extensive cell rearrangements is called **gastrulation,** and the embryo is said to be in the **gastrula** stage. As a result of gastrulation, the embryo contains three **germ layers**: the ectoderm, the endoderm, and the mesoderm.

ORGANOGENESIS:

Once the three germ layers are established, the cells interact with one another and rearrange themselves to produce tissues and organs. This process is called **organogenesis**. Many organs contain cells from more than one germ layer.

For example, the outer layer of skin comes from the ectoderm, while the inner layer (the dermis) comes from the mesoderm. Also during organogenesis, certain cells undergo long migrations from their place of origin to their final location.

GERM CELLS AND SOMATIC CELLS:

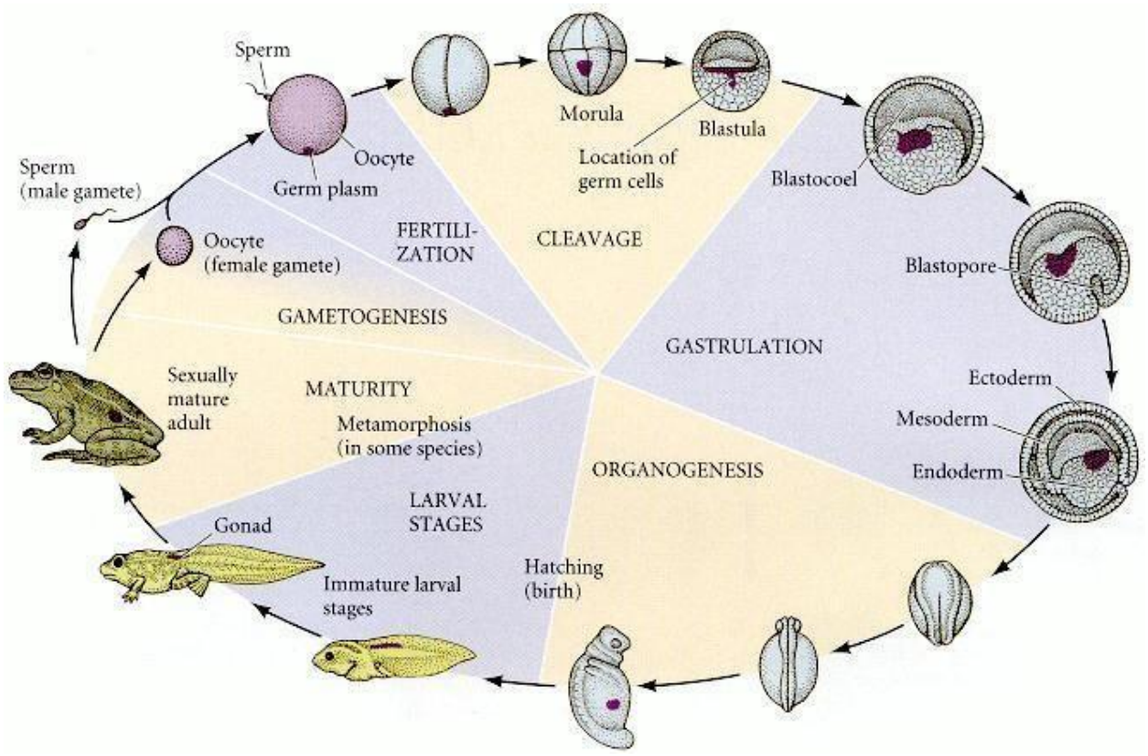
In many species a specialized portion of egg cytoplasm gives rise to cells that are the precursors of the **gametes** (the sperm and egg). The gametes and their precursor cells are collectively called **germ cells**, and they are set aside for reproductive function.

All the other cells of the body are called **Somatic cells**. This separation of somatic cells (which give rise to the individual body) and germ cells (which contribute to the formation of a new generation) is often one of the first differentiations to occur during animal development.

GAMETOGENESIS:

The germ cells eventually migrate to the gonads, where they differentiate into gametes. The development of gametes, called **gametogenesis**, is usually not completed until the organism has become physically mature.

In many species, the organism that hatches from the egg or is born into the world is not sexually mature. That young organism is a **larva** that may look significantly different from the adult.
Larvae often constitute the stage of life that is used for feeding or dispersal.

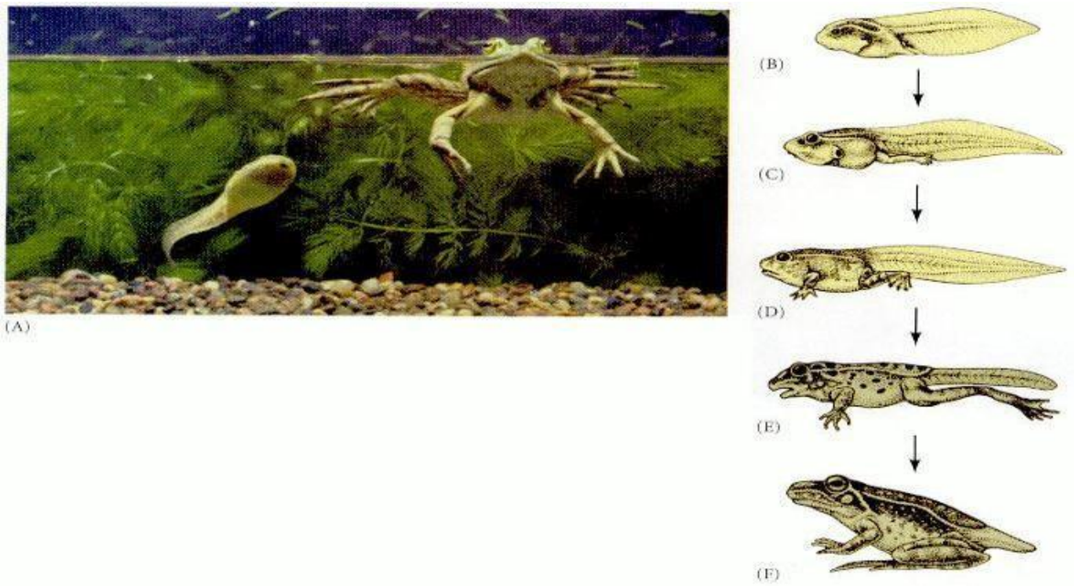


TOPIC 12. AMPHIBIAN DEVELOPMENT

In amphibians, metamorphosis is initiated by hormones from the tadpole's thyroid gland, and these changes prepare an aquatic organism for a terrestrial existence.

In anurans (frogs and toads), the metamorphic changes are most striking, and almost every organ is subject to modification.

- For locomotion, the hind limbs and forelimbs differentiate as the paddle tail withdraws.
- The gills regress, and the lungs enlarge.
- The horny teeth the tadpole uses to tear up pond plants disappear as the mouth and jaw take a new shape.
- Meanwhile the large intestine characteristic of herbivores shortens to suit the more carnivorous diet of the adult frog.
- As metamorphosis ends, the development of the first germ cells begins. In *Rana pipiens,* egg development lasts 3 years.
- The speed of metamorphosis is directly linked with environmental pressures.
- Since the bottom half of the egg usually contains the yolk, it divides more slowly (because the large yolk deposits interfere with cleavage). This portion is the vegetal hemisphere of the egg.
- Conversely, the upper half of the egg usually has less yolk and divides faster. This upper portion is called the animal hemisphere of the egg.



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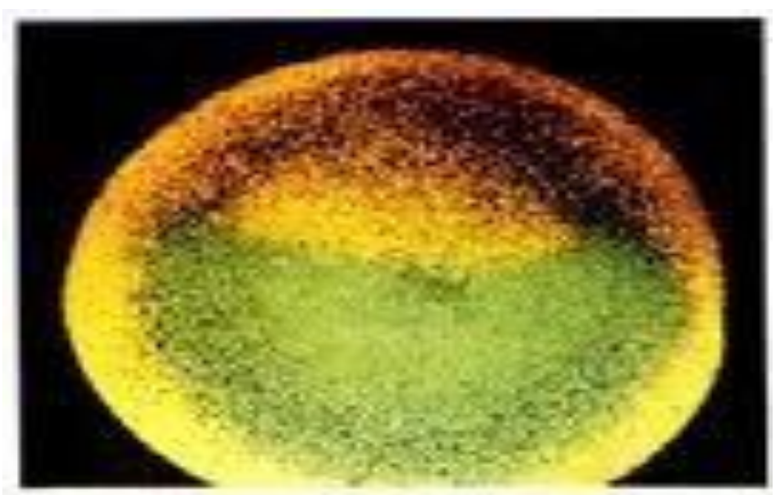
TOPIC 13. THE FROG LIFE CYCLE

If the frog is mature, the pituitary gland secretes hormones that stimulate the ovary to make estrogen. **Estrogen** is a hormone that can instruct the liver to make and secrete the yolk proteins, which are then transported through the blood into the enlarging eggs in the ovary. The yolk is transported into the bottom portion of the egg.



Another ovarian hormone, **progesterone**, signals the egg to resume its meiotic division. This is necessary because the egg had been "frozen" in the metaphase of its first meiosis. When it has completed this first meiotic division, the egg is released from the ovary and can be fertilized.

The male leopard frogs make their sperm in the summer, and by the time they begin hibernation in autumn, they have all the sperm that are to be available for the following spring's breeding season.

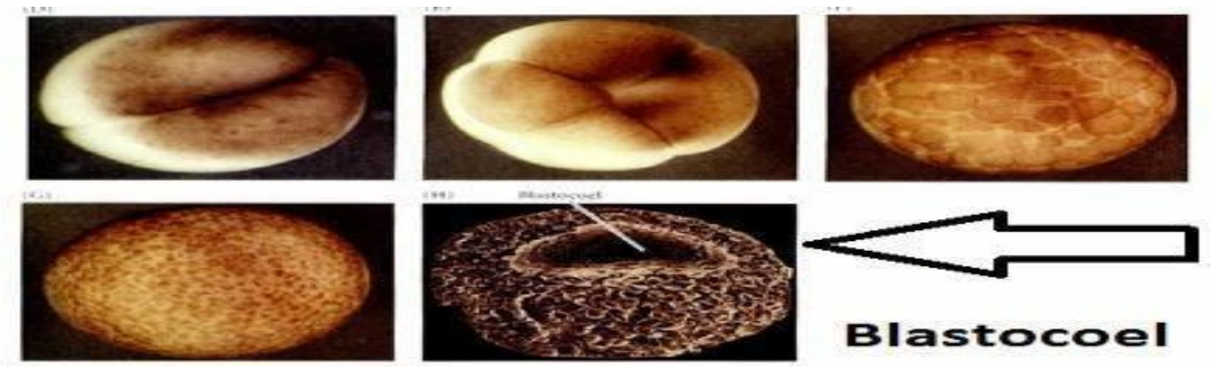
In most species of frogs, fertilization is external. The male frog grabs the female's back and fertilizes the eggs as the female frog releases them.

Rana pipiens usually lays around 2500 eggs, while the bullfrog, ***Rana catesbiana,*** can lay as many as 20,000. Some species lay their eggs in pond vegetation, and the jelly adheres to the plants and anchors the eggs.

Fertilization accomplishes several things.

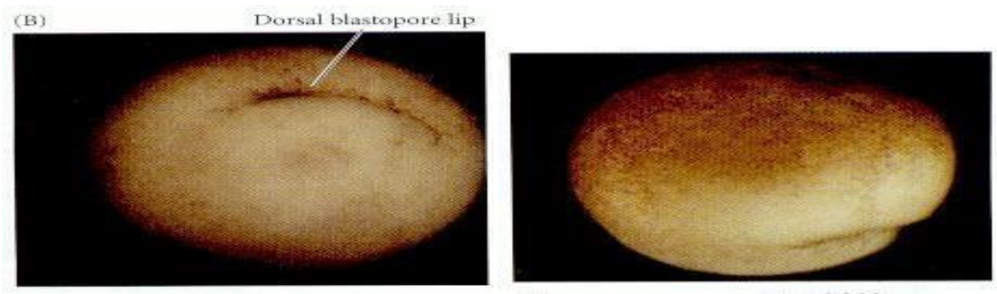
- First, it allows the egg to complete its second meiotic division, which provides the egg with a haploid **pronucleus**. The egg pronucleus and the sperm pronucleus will meet in the egg cytoplasm to form the diploid zygotic nucleus.
- Second, fertilization causes the cytoplasm of the egg to move such that different parts of the cytoplasm find themselves in new locations.

- Third, fertilization activates those molecules necessary to begin cell cleavage and development. The sperm and egg die quickly unless fertilization occurs.
- During cleavage, the volume of the frog egg stays the same, but it is divided into tens of thousands of cells. A fluid-filled cavity, the **blastocoel**, forms in the animal hemisphere. This cavity will be important for allowing cell movements to occur during gastrulation.



TOPIC 14. THE FROG LIFE CYCLE II

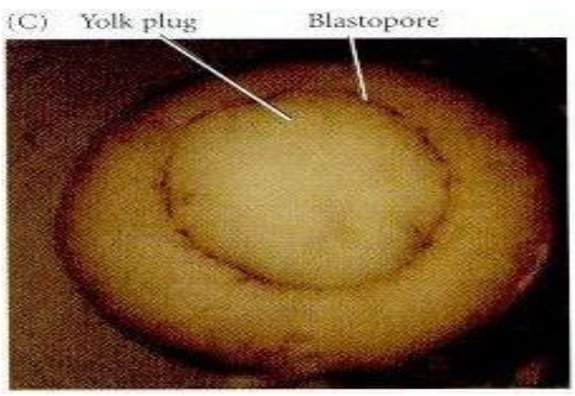
Gastrulation in the frog begins at a point on the embryo surface roughly 180 degrees opposite the point of sperm entry with the formation of a dimple, called the **blastopore.** Cells migrate through the blastopore and toward the animal pole. These cells become the dorsal mesoderm.



The blastopore expands into a circle and cells migrating through this circle become the **lateral and ventral mesoderm.**

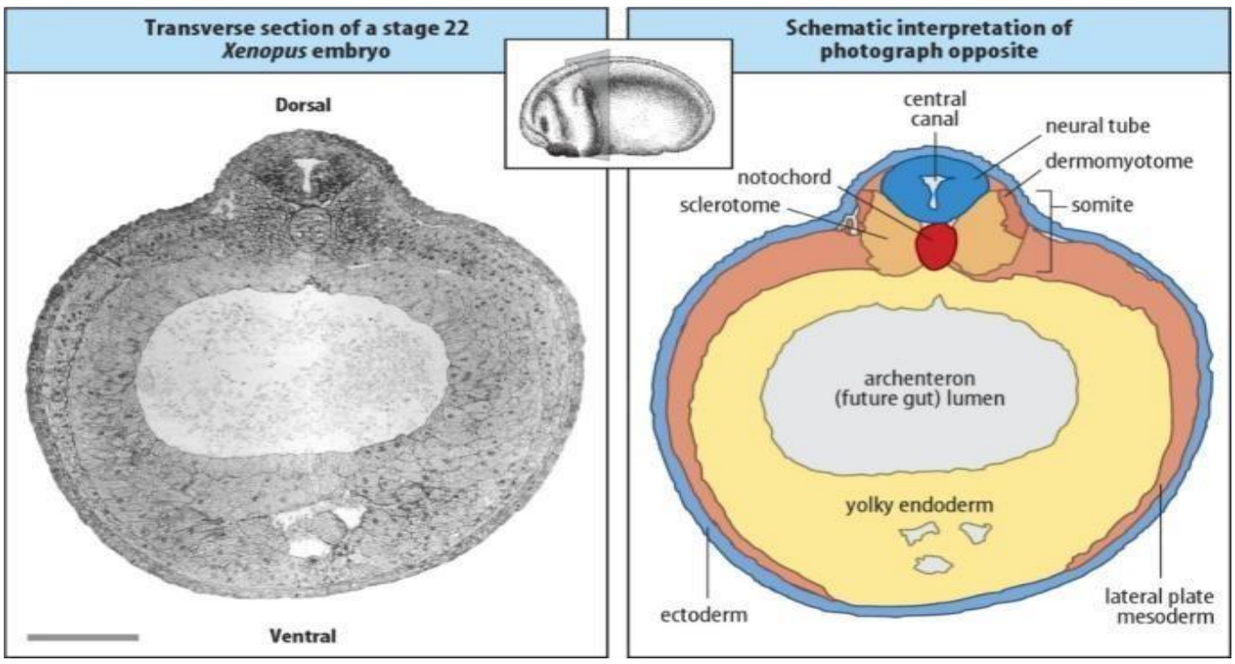
The cells remaining on the outside become the **ectoderm.**

The large yolky cells that remain at the vegetal hemisphere (until they are encircled by the ectoderm) become the **endoderm.**

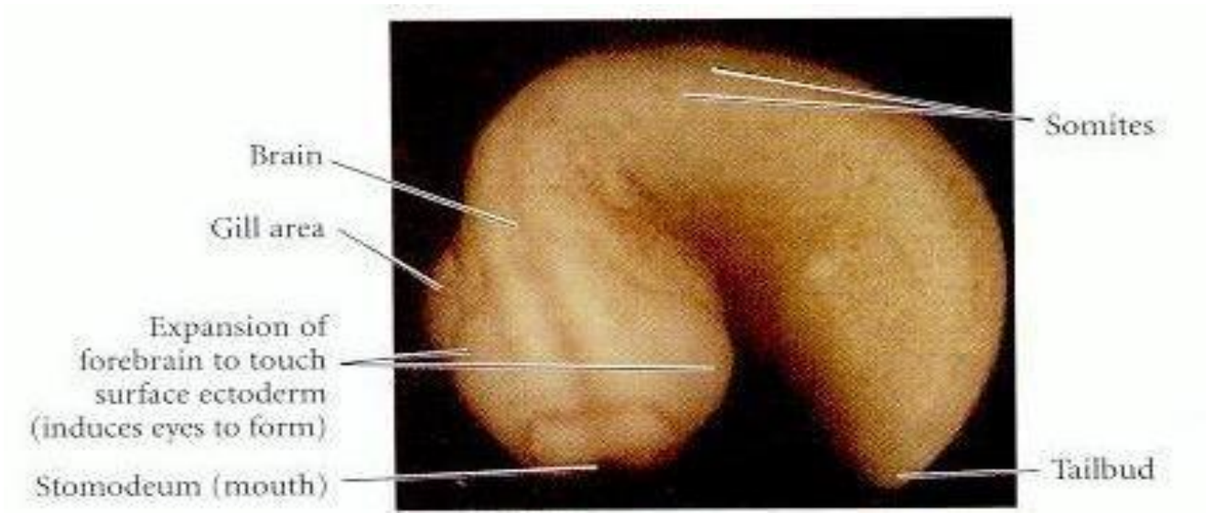


NOTOCHORD

A rod of mesodermal cells in the most dorsal portion of the embryo. At this stage, the embryo is called a **Neurula**. The neural precursor cells elongate, stretch, and fold into the embryo forming the **neural tube**.



Once the neural tube has formed, it induces changes in its neighbors, and organogenesis continues. The mesodermal tissue adjacent to the notochord becomes segmented into **somites**, the precursors of the frog's back muscles, spinal cord, and dermis (the inner portion of the skin).



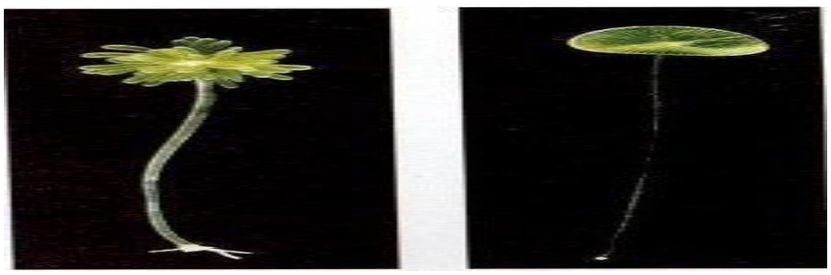
Tadpole:

Somites appear as blocks of mesodermal tissue. The embryo develops a mouth and an anus, and it elongates into the typical tadpole structure.

TOPIC 15. THE ROLE OF NUCLEUS IN MORPHOGENESIS

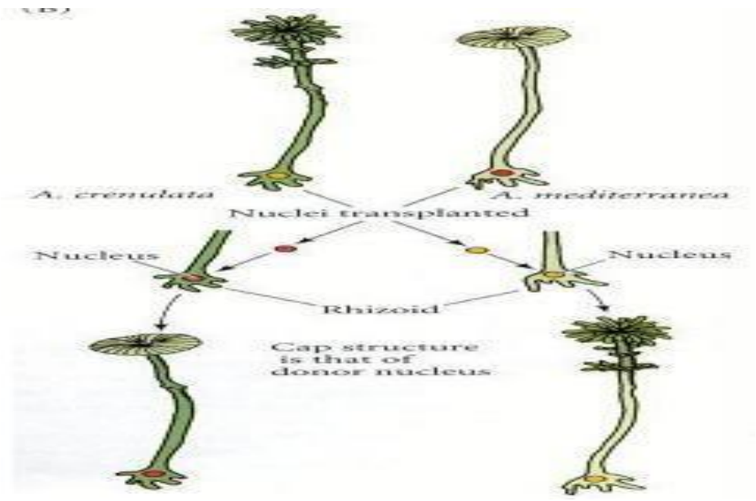
Nuclear control of cell morphogenesis and the interaction of nucleus and cytoplasm are well studied in *Acetabularia.* This enormous single cell (2-4 cm long) consists of three parts:

- a cap,
- a stalk,
- a rhizoid.



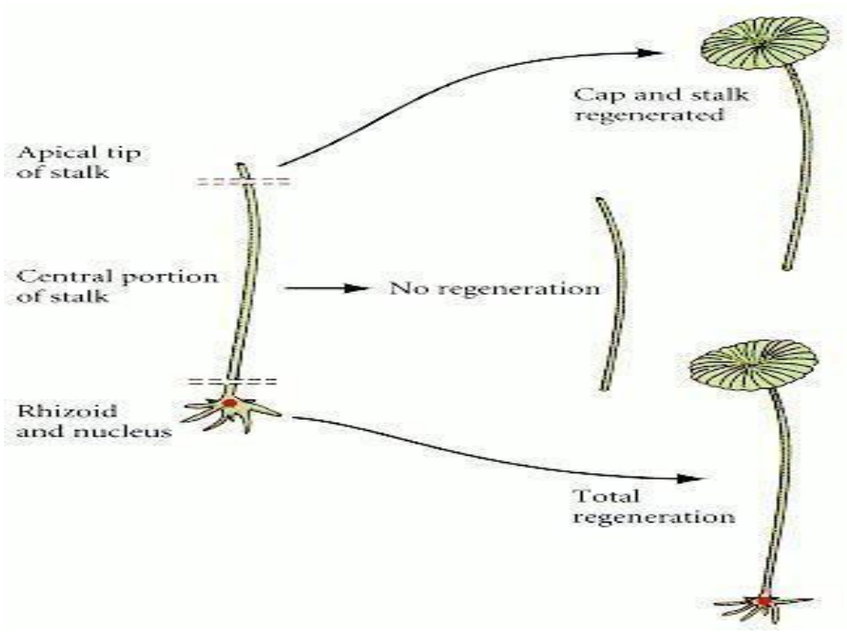
The single nucleus of the cell resides within the rhizoid. The size of *Acetabularia* and the location of its nucleus allow investigators to remove the nucleus from one cell and replace it with a nucleus from another cell.

J.Hammerling exchanged nuclei between two morphologically distinct species, *A. mediterranea* and *A. crenulata.* Figure shows that these two species have very different cap structures.



J. Hammerling found that when he transferred the nucleus from one species into the stalk of another species, the newly formed cap eventually assumed the form associated with the *donor* nucleus. Thus, the nucleus was seen to control *Acetabularia* development.

The formation of a cap is a complex morphogenic event involving the synthesis of numerous proteins, which must be accumulated in a certain portion of the cell and then assembled into complex, species-specific structures.



These studies suggest that

- Nucleus contains information specifying the type of cap produced (i.e., it contains the genetic information that specifies the proteins required for the production of a certain type of cap).
- Material containing this information enters the cytoplasm long before cap production occurs.
- One hypothesis proposed on the base of Hammerling study is that,
- “The nucleus synthesizes a stable mRNA that lies dormant in the cytoplasm until the time of cap formation”.
- Hence, the expression of the cap is controlled not only by nuclear transcription, but also by the translation of the cytoplasmic RNA. In this unicellular organism, "development" is controlled at both the transcriptional and translational levels.

TOPIC16. Unicellular protists and the origins of sexual reproduction

Sex and reproduction are two distinct and separable processes. **Reproduction** involves the creation of new individuals.

Sex involves the combining of genes from two different individuals into new

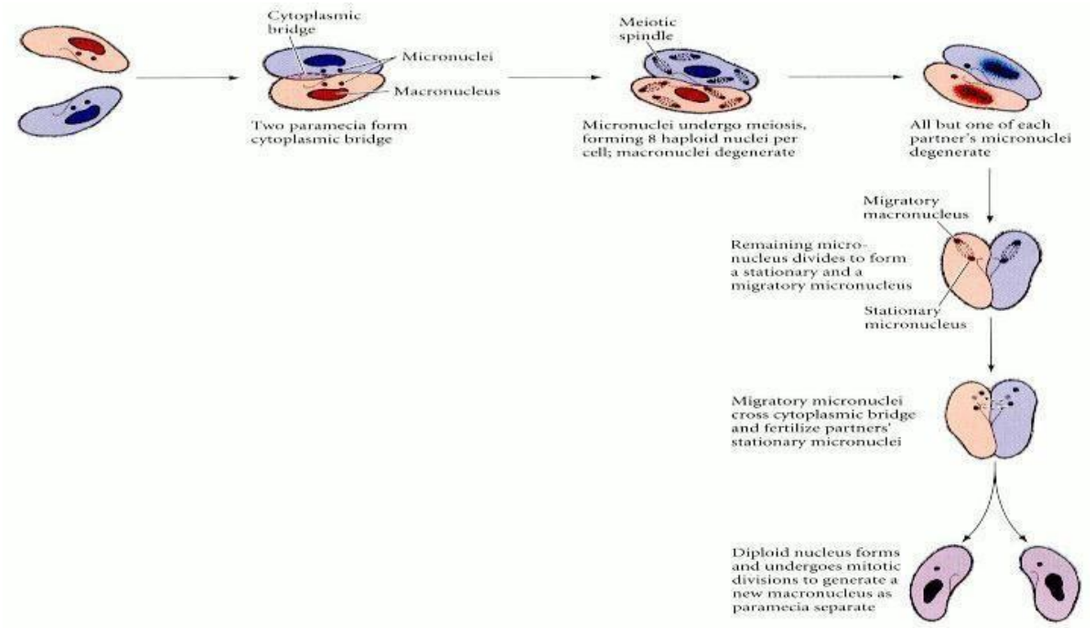
arrangements.

Reproduction in the absence of sex is characteristic of organisms that reproduce by fission (i.e., splitting into two); there is no sorting of genes when an amoeba divides or when a hydra buds off cells to form a new colony.

Sex without reproduction is also common among unicellular organisms. Bacteria are able to transmit genes from one individual to another by means of sex pili. This transmission is separate from reproduction.

Protists are also able to reassort genes without reproduction. Paramecia, for instance, reproduce by fission, but sex is accomplished by **conjugation**

When two paramecia join together, they link their oral apparatuses and form a cytoplasmic connection through which they can exchange genetic material



Each macronucleus (which controls the metabolism of the organism) degenerates, while each micronucleus undergoes meiosis to produce eight haploid micronuclei, of which all but one

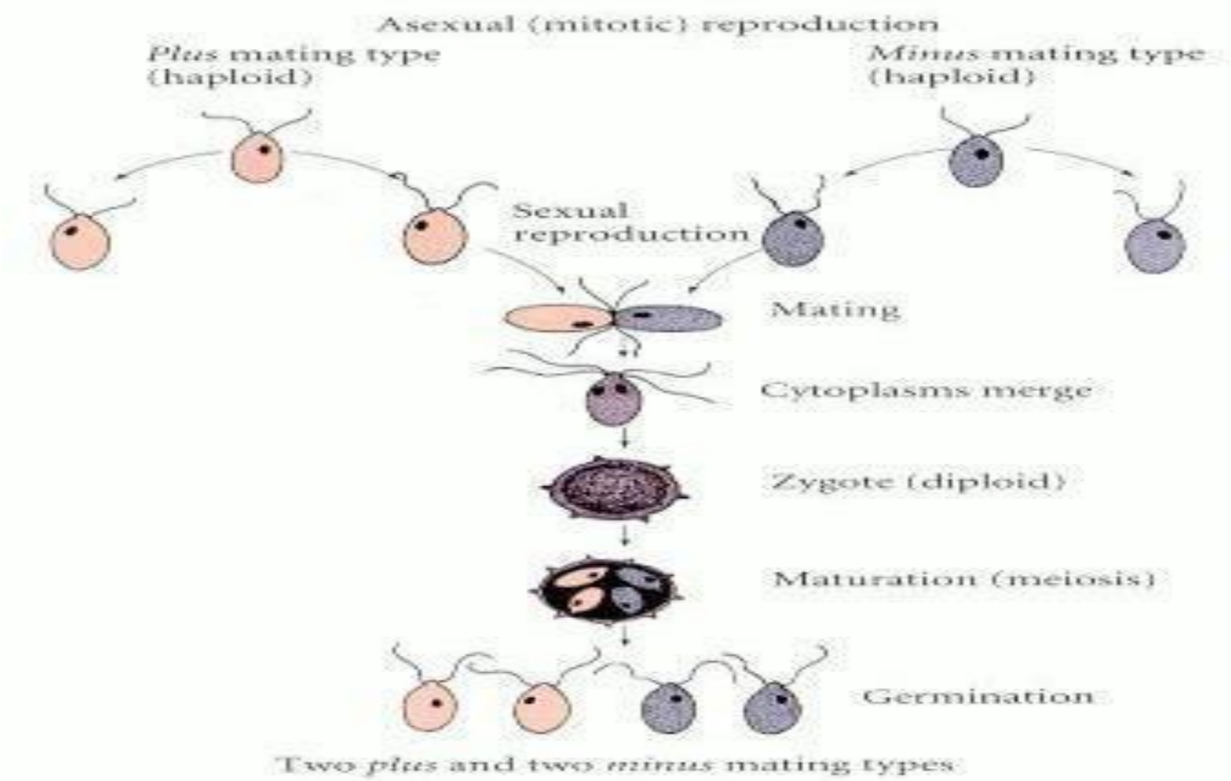
degenerate. The remaining micronucleus divides once more to form a stationary micronucleus and a migratory micronucleus.

Each migratory micronucleus crosses the cytoplasmic bridge and fuses with ("fertilizes") the stationary micronucleus, thereby creating a new diploid nucleus in each cell. This diploid nucleus then divides mitotically to give rise to a new micronucleus and a new macronucleus as the two partners disengage. Therefore, no reproduction has occurred, only sex.

TOPIC 17. SEXUAL REPRODUCTION IN UNICELLULAR EUKARYOTES

The union of these two distinct processes, sex and reproduction, into **sexual reproduction** is seen in unicellular eukaryotes. Let us take an example of the life cycle of *Chlamydomonas.* Which is usually haploid, having just one copy of each chromosome.

The individuals of each species, however, are divided into two mating types: plus and minus. When a plus and a minus meet, they join their cytoplasm, and their nuclei fuse to form a diploid zygote.



The flagella of two individuals twist around each other, enabling specific regions of the cell membranes to come together. These specialized regions contain mating type-specific components that enable the cytoplasms to fuse.

Following flagellar agglutination, the plus individuals initiate fusion by extending a fertilization tube.

This tube contacts and fuses with a specific site on the minus individual.

In evolving sexual reproduction, two important advances had to be achieved.

- The first was the mechanism of meiosis whereby the diploid complement of chromosomes is reduced to the haploid state.
- The second was a mechanism whereby the two different mating types could recognize each other.

TOPIC 18. DEVELOPMENTAL PATTERNS AMONG THE METAZOA

Metazoans belong to one of three major branches:

1) Diploblasts,

2) Protostomes,

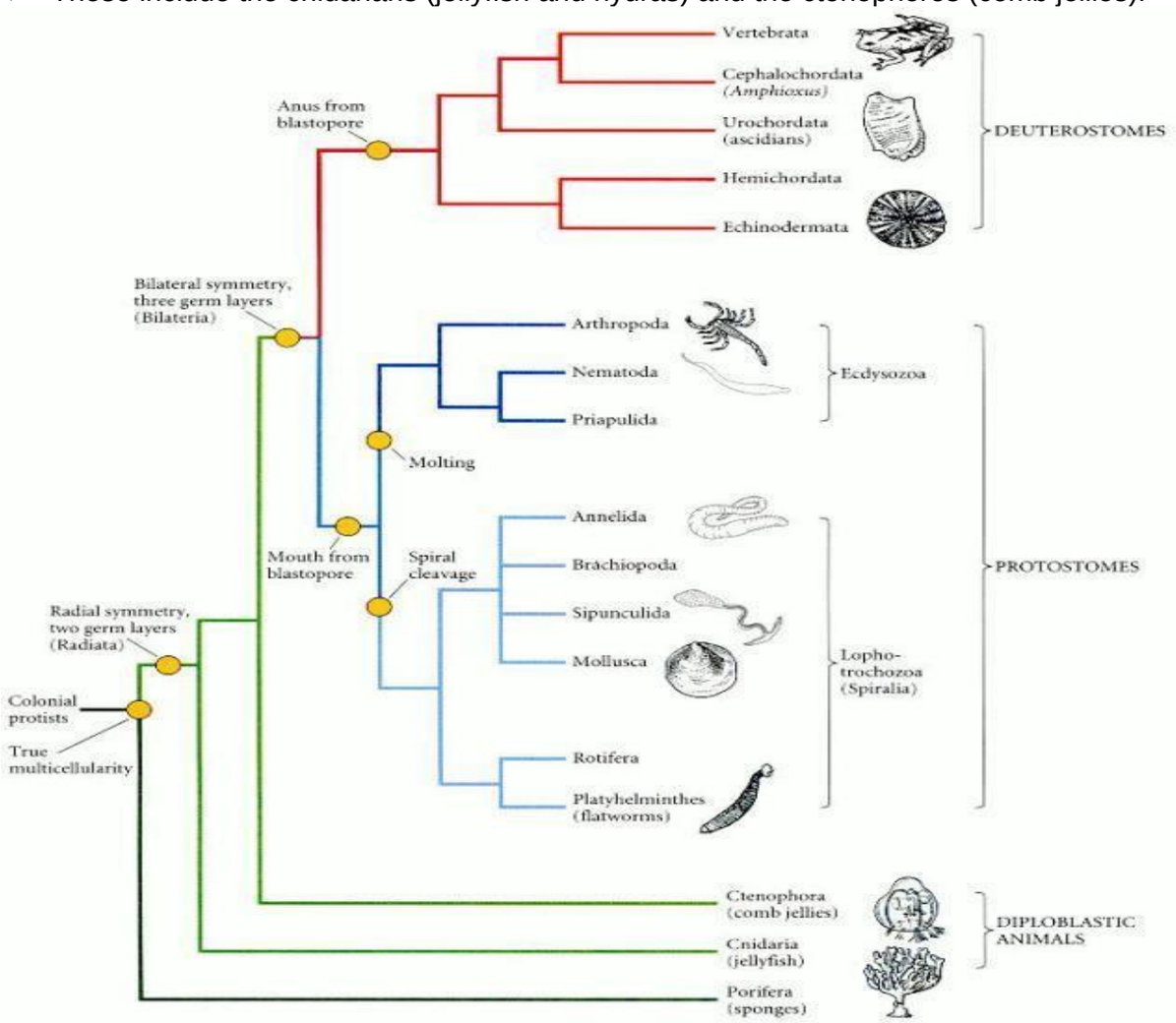
3) Deuterostomes.

Sponges develop in a manner so different from that of any other animal group that some taxonomists do not consider them metazoans at all, and call them "**Parazoans.**"

DIPLOBLASTS:

Diploblastic animals are those who have ectoderm and endoderm, but no true mesoderm.

- These include the cnidarians (jellyfish and hydras) and the ctenophores (comb jellies).



PROTOSTOMES:

(Greek, "mouth first"), which include the mollusc, arthropod, and worm phyla, are so called because the mouth is formed first, at or near the opening to the gut, which is produced during gastrulation. The anus forms later at another location.

There are two major branches of the protostomes.

- The **Ecdysozoa** includes those animals that molt. Its major constituent is Arthropoda, a phylum containing insects, arachnids, mites, crustaceans, and millipedes.

- The second major group of protostomes are the **Lophotrochozoa**. They are characterized by a common type of cleavage (spiral), a common larval form, and a distinctive feeding apparatus. These phyla include annelids, molluscs, and flatworms.

Deuterostomes:

- In Deuterostomes ("mouth second"), the mouth opening is formed after the anal opening.
- Phyla in the Deuterostomes lineage include the chordates and echinoderms.

TOPIC 19 .20 Cadherins and cell adhesion

Cadherins and cell adhesion

Recent evidence shows that boundaries between tissues can indeed be created both by,

- Different cell types having different types of cell adhesion molecules.
- Different cell types having different amounts of cell adhesion molecules.
- There are several classes of molecules that can mediate cell adhesion. The major cell adhesion molecules appear to be the **Cadherins**.
- As their name suggests, they are calcium-dependent adhesion molecules.
- Cadherins are critical for establishing and maintaining intercellular connections, and they appear to be crucial to the spatial segregation of cell types and to the organization of animal form.
- Cadherins interact with other Cadherins on adjacent cells, and they are anchored into the cell by a complex of proteins called **Catenins**. The cadherin-catenin complex forms the classic adherens junctions that connect epithelial cells together.

Types of cadherin

In vertebrate embryos, several major cadherin classes have been identified:

1. **E-cadherin**
2. **P-cadherin**
3. **N-cadherin**
4. **Protocadherins**

1) **E-cadherin:**
   a. Epithelial cadherin is expressed on all early mammalian embryonic cells, even at the 1cell stage. Later, this molecule is restricted to epithelial tissues of embryos and adults.
2) **P-cadherin:**
   a. Placental cadherin appears to be expressed primarily on the trophoblast cells (those placental cells of the mammalian embryo that contact the uterine wall) and on the uterine wall epithelium.
3) **N-cadherin:**
   a. Neural cadherin is first seen on mesodermal cells in the gastrulating embryo as they lose their E-cadherin expression. It is also highly expressed on the cells of the developing central nervous system.
4) **Protocadherins:**
   a. Calcium-dependent adhesion proteins that differ from the classic Cadherins in that they lack connections to the cytoskeleton through catenins. Protocadherins have been found to be very important in separating the notochord from the other mesodermal tissues during *Xenopus* gastrulation.

TOPIC 21 22. Genes and Development

Genes and Development

Following important points depict the relationship of genes and development.

- Development connects genotype and phenotype.
- Nuclear genes are not lost or mutated during development. The genome of every cell is equivalent.
- The exceptions to the rule of genomic equivalence are the lymphocytes. During differentiation, these cells rearrange their DNA to create new immunoglobulin and antigen receptor genes.
- The ability of nuclei from differentiated cells to direct the development of complete adult organisms has recently confirmed the principle of genomic equivalence.
- Only a small percentage of the genome is expressed in any particular cell.
- The cloning of human beings, as well as regenerating damaged organs or enhancing physical abilities, may soon be possible through cloning technology and the use of embryonic stem cells.
- Northern blots, in situ hybridization, and the polymerase chain reaction can show which cells are transcribing particular genes.
- The functions of a gene often can be ascertained by antisense mRNA, transgenic expression, or (in the case of mammals) gene knockouts.
- Knowledge of gene activity in humans can be obtained by candidate gene mapping or positional cloning.

TOPIC 23 Differential gene expression

Differential gene expression

Different cell types use different subsets of these genes. Red blood cells make globins, lens cells make crystallins, melanocytes make melanin, and endocrine glands make their specific hormones

Developmental genetics is the discipline that examines how the genotype is transformed into the phenotype, and the major paradigm of developmental genetics is differential gene expression from the same nuclear repertoire.

TOPIC 24. Regulation of gene expression

The regulation of gene expression can be accomplished at several levels:

- **Differential gene transcription:**

  It regulates that which of the nuclear genes are transcribed into RNA.

- **Selective nuclear RNA processing:**

  It regulating which of the transcribed RNAs (or which parts of such a nuclear RNA) enter into the cytoplasm to become messenger RNAs.

- **Selective messenger RNA translation:**

  It regulates that which of the mRNAs in the cytoplasm become translated into proteins.

- **Differential protein modification:**

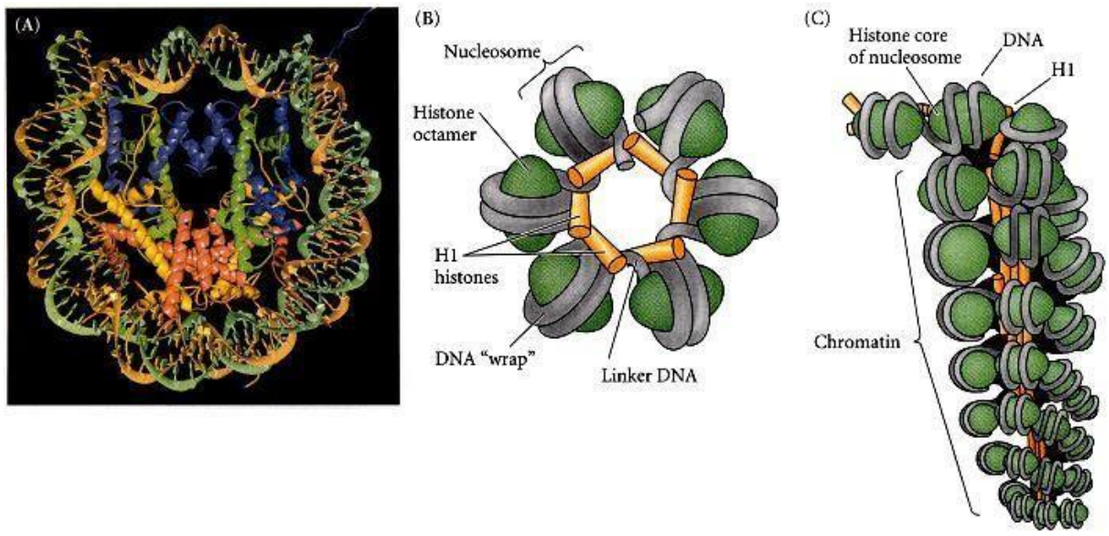
  It regulates that which proteins are allowed to remain or function in the cell.

TOPIC 25 . Anatomy of the gene

Anatomy of the gene

Eukaryotic genes are contained within a complex of DNA and protein called **chromatin**. The protein component constitutes about half the weight of chromatin and is composed largely of **nucleosomes**.

Nucleosome is composed of an octamer of **histone** proteins (two molecules each of histones H2A-H2B and histones H3-H4) wrapped with two loops containing approximately 140 base pairs of DNA.

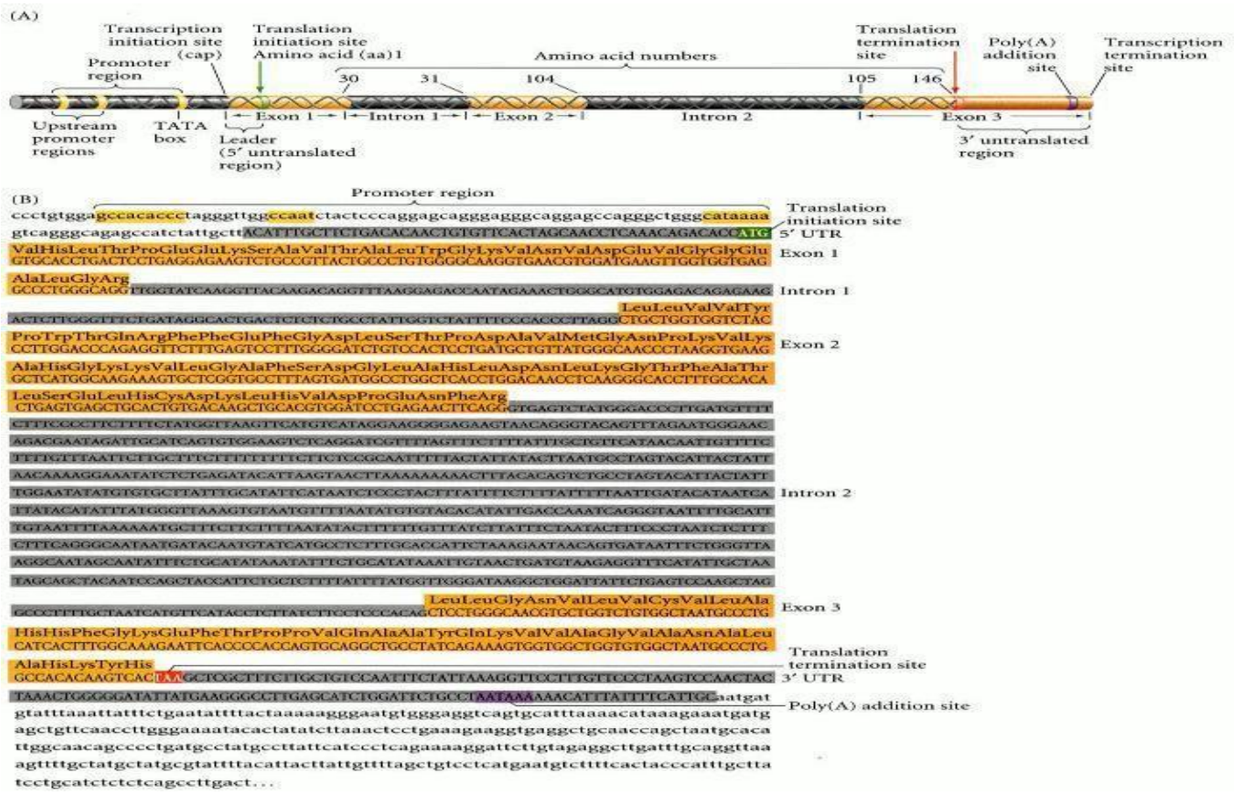


- **Exons:**

  Coding regions of DNA.

- **Introns:**

  Non-coding regions.



TOPIC 26.Anatomy of the gene

Promoter:

A region, which is responsible for the binding of RNA polymerase and for the subsequent initiation of transcription.

Enhancer: It is a DNA sequence that can activate the utilization of a promoter, controlling the efficiency and rate of transcription from that particular promoter.

The human β-globin gene has an enhancer in its 3´ UTR, roughly 700 base pairs.

Untranslated region (UTR):

A region that is not translated into protein.This region includes the sequence which is needed for **polyadenylation**(addition of a poly(A) tail to a messenger RNA).

The **poly(A) tail**

- Confers stability on the messenger RNA.
- Allows the mRNA to exit the nucleus.
- Permits the mRNA to be translated into protein.

TOPIC 27 28 Role of Enhancers in differential gene expression

Role of Enhancers in differential gene expression

An **enhancer** is a DNA sequence that can activate the utilization of a promoter, controlling the efficiency and rate of transcription from that particular promoter.Enhancers can regulate the temporal and tissue-specific expression of any differentially regulated gene, but different types of genes normally have different enhancers.

Enhancers are critical in the regulation of normal development. Over the past decade, six generalizations that emphasize their importance for differential gene expression have been made:

- Most genes require enhancers for their transcription.
- Enhancers are the major determinant of differential transcription in space (cell type) and time.
- A given gene can have several enhancer sites linked to it, and each enhancer can be bound by more than one transcription factor.
- The interaction between the proteins bound to the enhancer sites and the transcription initiation complex assembled at the promoter is thought to regulate transcription.
- Enhancers are modular. It is the combination of transcription factors that causes particular genes to be transcribed.
- A gene can have several enhancer elements, each turning it on in a different set of cells.
- Enhancers can also be used to inhibit transcription. In some cases, the same transcription factors that activate the transcription of one gene can be used to repress the transcription of other genes. These "negative enhancers " are also called **silencers**.

TOPIC 29.30.31 Transcription factors AND domains

Transcription factors

Transcription factors are proteins that bind to enhancer or promoter regions and interact to activate or repress the transcription of a particular gene. Most transcription factors can bind to specific DNA sequences. These proteins can be grouped together in families based on similarities in structure. The transcription factors within such a family share a common framework structure in their DNA-binding sites, and slight differences in the amino acids at the binding site can alter the sequence of the DNA to which the factor binds.

Transcription factors domains

Transcription factors have three major domains.

1) DNA-binding domain
2) Protein-protein interaction domain
3) *Trans*-activating domain

- **DNA-binding domain:**
  It recognizes a particular DNA sequence.

- **Protein-protein interaction domain:**
  It allows the transcription factor's activity to be modulated by TAFs or other transcription factors.

- **Trans-activating domain:**
  It activates or suppresses the transcription of the gene whose promoter or enhancer it has bound. Usually, this *trans*-activating domain enables the transcription factor to interact with proteins involved in binding RNA polymerase.

Examples of Transcription factors

. Examples,

MITF:

*(*Microphthalmia-Associated Transcription Factor*.* The microphthalmia (MITF) protein is necessary for the production of pigment cells and their pigments.

Pax6:

Paired box gene 6*.* The Pax6 transcription factor, which is needed for mammalian eye, nervous system, and pancreas development, contains two potential DNA-binding domains.

Pdx1:

Pancreatic And Duodenal Homeobox 1 gene.

It is specific for the pancreatic region of the endoderm.

TOPIC 32.33 Fertilization AND Steps in Fertilization

Fertilization

Fertilization is the process whereby two sex cells (gametes) fuse together to create a new individual with genetic potentials derived from both parents.

Fertilization accomplishes two separate processes:

- Sex (the combining of genes derived from the two parents) and
- Reproduction (the creation of new organisms)
- Thus, the first function of fertilization is to transmit genes from parent to offspring, and the second is to initiate in the egg cytoplasm those reactions that permit development to proceed.

Steps in Fertilization

Although the details of fertilization vary from species to species, conception generally consists of four major events:

- Contact and recognition between sperm and egg. In most cases, this ensures that the sperm and egg are of the same species.
- Regulation of sperm entry into the egg. Only one sperm can ultimately fertilize the egg.
- Fusion of the genetic material of sperm and egg.
- Activation of egg metabolism to start development.

TOPIC 34.35 Discovery of the Sperm AND Evidence for the importance of sperm in Reproduction

A.V Leeuwenhoek, the Dutch microscopist who co-discovered sperm in 1678, first believed them to be parasitic animals living within the semen (hence the term *spermatozoa,* meaning "sperm animals").

Leeuwenhoek (1685) wrote that sperm were seeds (both *sperm* and *semen* mean "seed") and that the female merely provided the nutrient soil in which the seeds were planted.

The first evidence suggesting the importance of sperm in reproduction came from a series of experiments performed by **Lazzaro Spallanzani** in the late 1700s. Spallanzani demonstrated that filtered toad semen devoid of sperm would not fertilize eggs.In 1824, **J. L. Prevost and J. B. Dumas** claimed that sperm were not parasites, but rather the active agents of fertilization.

“They proposed that the sperm entered the egg and contributed materially to the next generation”.

In 1876 Oscar Hertwig and Herman Fol independently demonstrated sperm entry into the egg and the union of the two cells' nuclei.

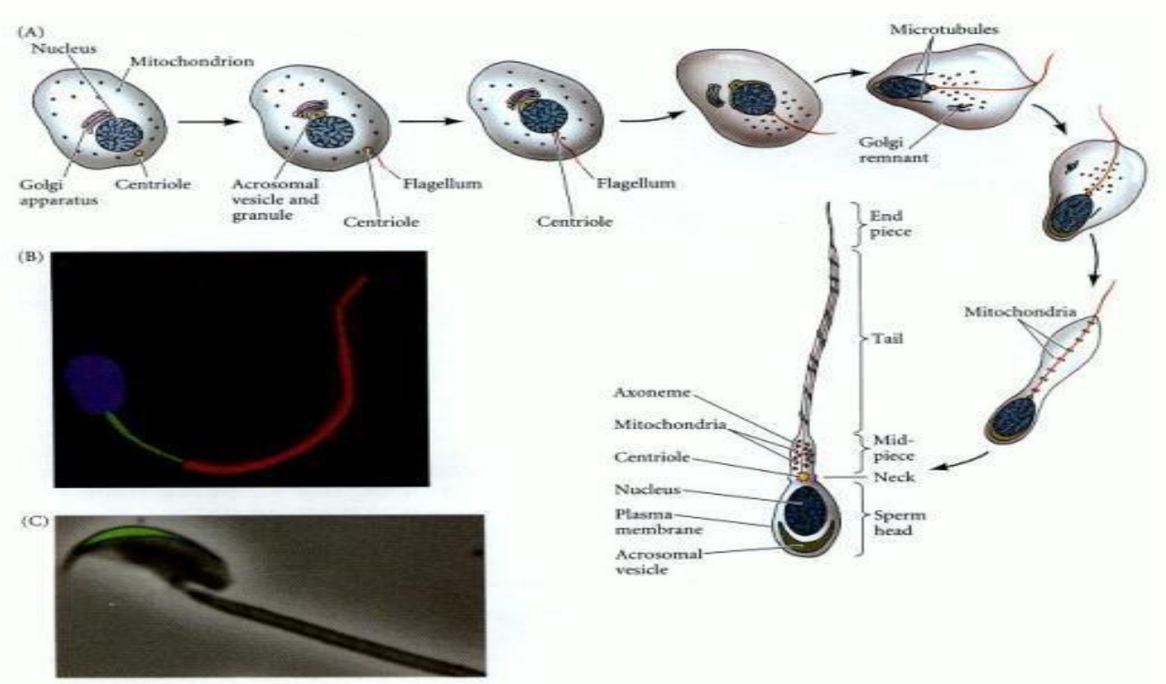
“Fertilization was at last recognized as the union of sperm and egg, and the union of sea urchin gametes remains one of the best-studied examples of fertilization”.

TOPIC 36.37 Structure of the Sperm

Each sperm consists of,

1) A haploid nucleus,
2) A propulsion system to move the nucleus, and
3) A sac of enzymes that enable the nucleus to enter the egg.

- During the course of sperm maturation, the haploid nucleus becomes very streamlined, and its DNA becomes tightly compressed



In front of this compressed haploid nucleus lies the **acrosomal vesicle**, or **acrosome**, which is derived from the Golgi apparatus and contains enzymes that digest proteins and complex sugars; thus, it can be considered a modified secretory vesicle.

These stored enzymes are used to lyse the outer coverings of the egg. In many species, such as sea urchins, a region of globular actin molecules lies between the nucleus and the acrosomal vesicle.

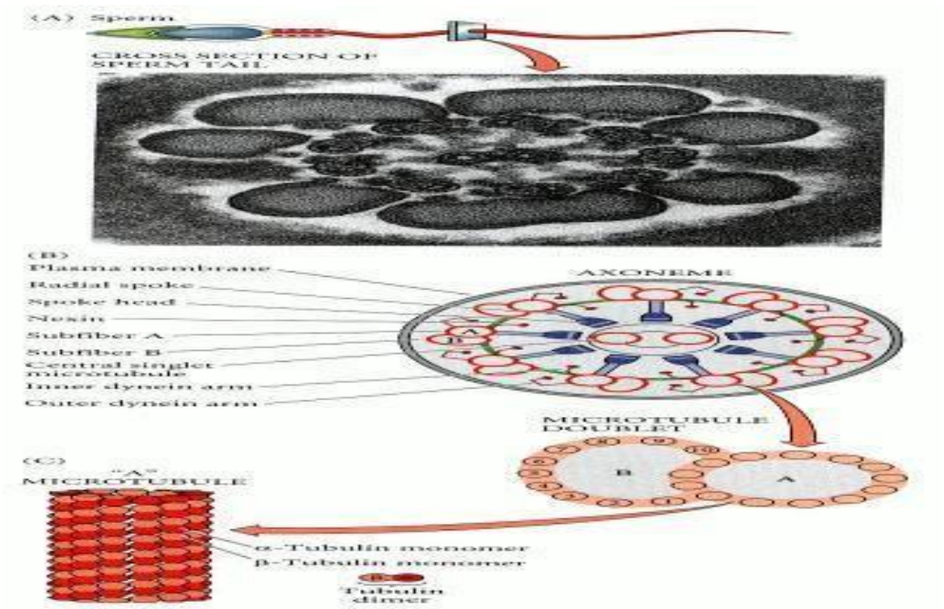
These proteins are used to extend a fingerlike **acrosomal process** from the sperm during the early stages of fertilization.

In sea urchins and several other species, recognition between sperm and egg involves molecules on the acrosomal process. Together, the acrosome and nucleus constitute the head of the sperm.

The means by which sperm are propelled vary according to how the species has adapted to environmental conditions. In some species (such as the **parasitic roundworm *Ascaris***), the sperm travel by the amoeboid motion of **lamellipodial extensions** of the cell membrane.

In most species, however, each sperm is able to travel long distances by whipping its **flagellum.** Flagella are complex structures. The major motor portion of the flagellum is called the **axoneme**. It is formed by microtubules emanating from the centriole at the base of the sperm nucleus.

The core of the axoneme consists of two central microtubules surrounded by a row of nine doublet microtubules.The force for sperm propulsion is provided by **dynein**, a protein that is attached to the microtubules. Dynein hydrolyzes molecules of ATP and can convert the released chemical energy into the mechanical energy that propels the sperm.

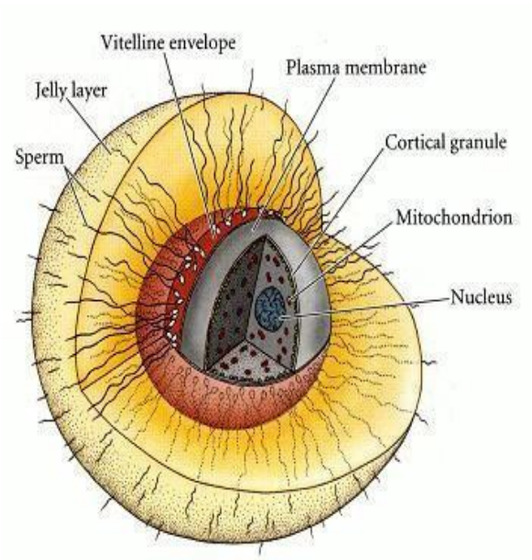


The sperm released during ejaculation are able to move, yet they do not have the capacity to bind to and fertilize an egg. These final stages of sperm maturation (**called capacitation**) do not occur until the sperm has been inside the female reproductive tract for a certain period of time.

TOPIC 38 Composition of Egg

Structure of Egg

The volume of a sea urchin egg is about 200 picoliters (**2** $\times$**10**$^{-4}$ mm3, more than 10,000 times the volume of the sperm)



Composition of Egg

The egg also has a remarkable cytoplasmic storehouse that it has accumulated during its maturation. This cytoplasmic storehouse includes the following,

Proteins:

The early embryonic cells need a supply of energy and amino acids which is provided by yolk proteins in the egg. Many of the yolk proteins are made in other organs (liver, fat body) and travel through the maternal blood to the egg.

Ribosomes and tRNA:

Protein synthesis is accomplished by ribosomes and tRNA, which exist in the egg. The developing egg has special mechanisms to synthesize ribosomes, and certain amphibian oocytes produce as many as $\mathbf{10^{12}}$ ribosomes.

Messenger RNA:

The instructions for proteins made during early development are already packaged in the oocyte. It is estimated that the eggs of sea urchins contain 25,000 to 50,000 different types of mRNA.

Morphogenetic factors:

Molecules that direct the differentiation of cells into certain cell types are localized in different regions of the egg and become segregated into different cells during cleavage.

Protective chemicals:

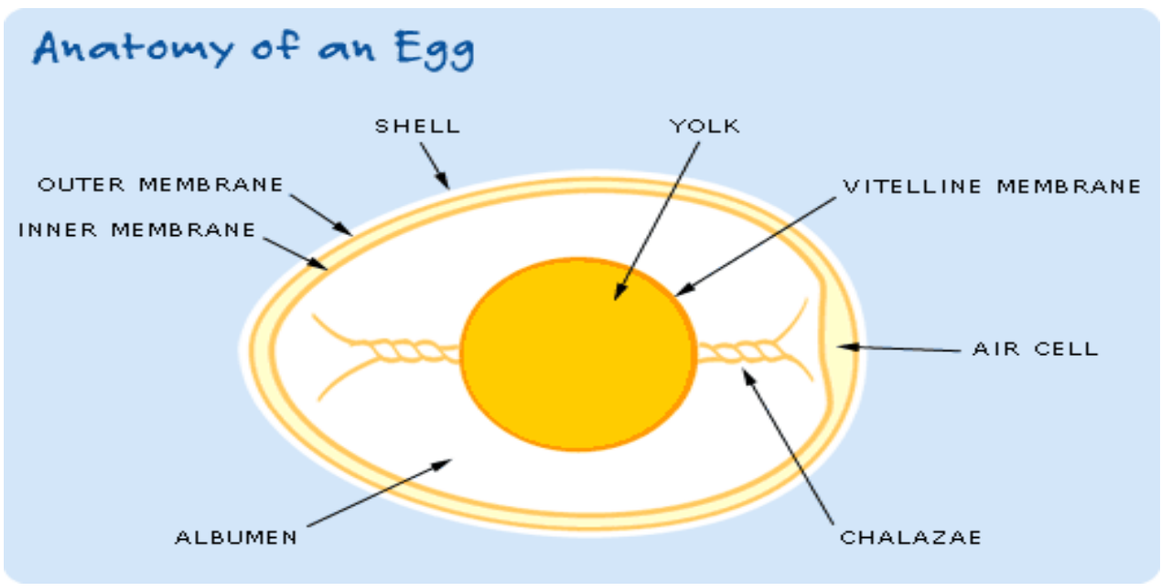
Many eggs contain ultraviolet filters and DNA repair enzymes that protect them from sunlight; some eggs contain molecules that potential predators find distasteful; and the yolk of bird eggs even contains antibodies. Many types of eggs have glycoprotein meshwork called **egg jelly** outside the vitelline envelope which is used either to attract or to activate sperm. The egg, then, is a cell specialized for receiving sperm and initiating development.

TOPIC 39. Composition of Egg

Anatomy of an Egg The egg is composed of many parts, namely:

- Yolk
- Albumen or egg white
- Shell membranes
- Shell

The following figure provides a better understanding of its physical structure.



The Shell

The shell is the egg's outer covering, accounting for about 9 to 12% of its tota weight depending on egg size. The shell is the egg's first line of defense against bacterial contamination. This bumpy and grainy outer covering has approximately 17,000 tiny pores and is made almost entirely of calcium carbonate. It is semipermeable, which means that air and moisture can pass through its pores.

The Shell Membranes

Lying between the eggshell and albumen, these two transparent protein membranes provide efficient defense against bacterial invasion. These tough membranes are made partly of keratin, a protein also found in human hair.

Air Cell

An air space forms when the contents of the egg cool and contract after the egg is laid. The air cell usually rests between the outer and inner membranes at the egg's larger end.

Albumen

The egg white is known as the albumen, which comes from *albus, the Latin word* for "white." Albumen accounts for most of an egg's liquid weight, about 67%. It contains more than half the egg's total protein.

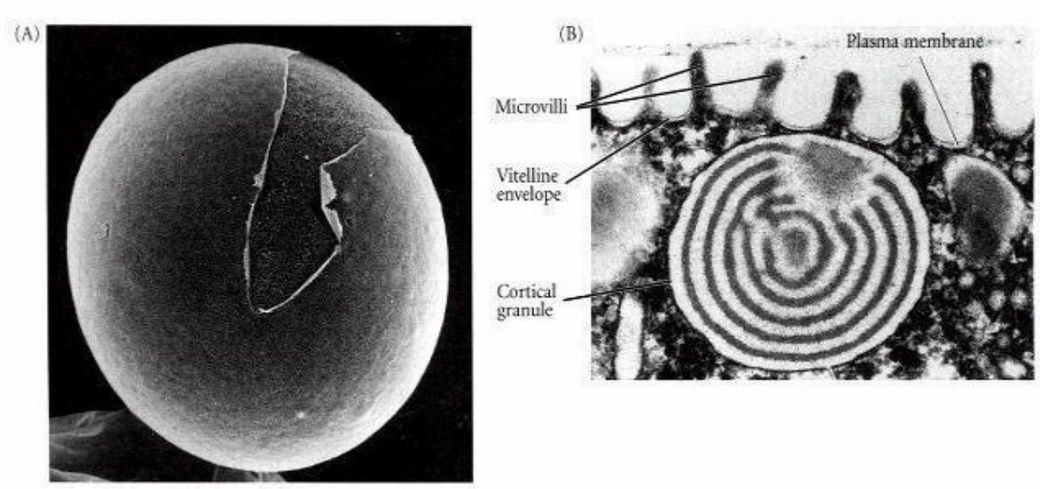
Yolk

Yolk color ranges from just a hint of yellow to a magnificent deep orange, according to the feed and breed of the hen. The yolk has important nutritional and functional qualities. From a nutritional perspective, the yolk contains less water and more fat than the white, a little less than half of the protein of the white, and most of the vitamins and minerals of the egg. These include iron, vitamin A, vitamin D, phosphorus, calcium, thiamine, and riboflavin.

TOPIC 40 Structure of Egg II

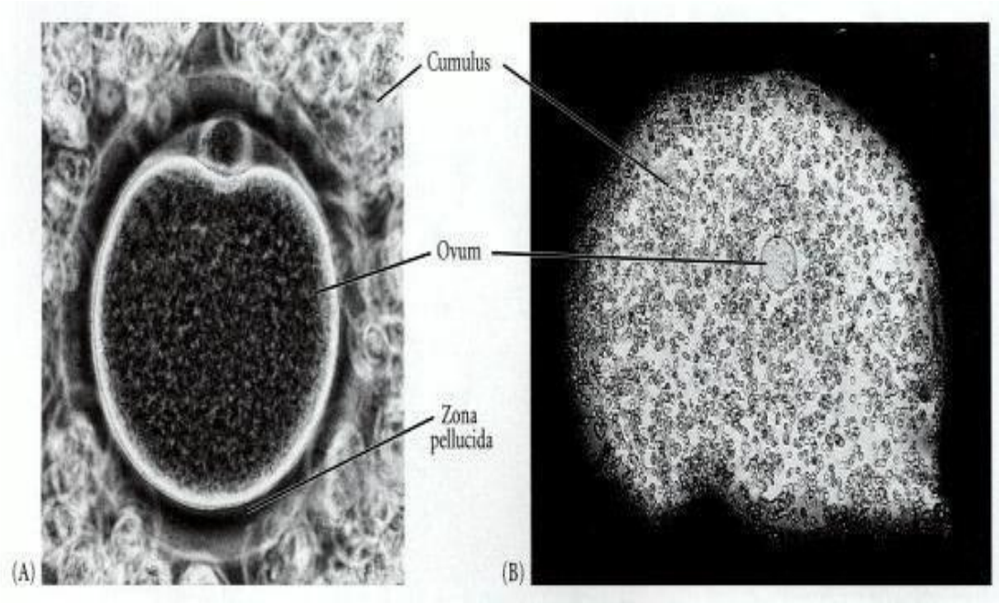
Structure of Egg II

Enclosing the cytoplasm is the egg **plasma membrane** that regulates the flow of certain ions during fertilization and must be capable of fusing with the sperm plasma membrane.



Outside the plasma membrane is the **vitelline envelope**, which forms a fibrous mat around the egg. This envelope contains at least eight different glycoproteins and is often involved in spermegg recognition.

The vitelline envelope is essential for the species-specific binding of sperm. In mammals, the vitelline envelope is a separate and thick extracellular matrix called the **zona pellucid.**



The mammalian egg is also surrounded by a layer of cells called the cumulus.

The innermost layer of cumulus cells, immediately adjacent to the zona pellucida, is called the **corona radiata.**

Lying immediately beneath the plasma membrane of the egg is a thin shell (about 5 μm) of gellike cytoplasm called the **cortex**. The cytoplasm in this region is stiffer than the internal cytoplasm and contains high concentrations of globular actin molecules.

During fertilization, these actin molecules polymerize to form long cables of actin known as **microfilaments**.

Microfilaments are necessary for cell division, and they also are used to extend the egg surface into small projections called **microvilli**, which may aid sperm entry into the cell.

Cortical granules are membrane-bound structures containing proteolytic enzymes.

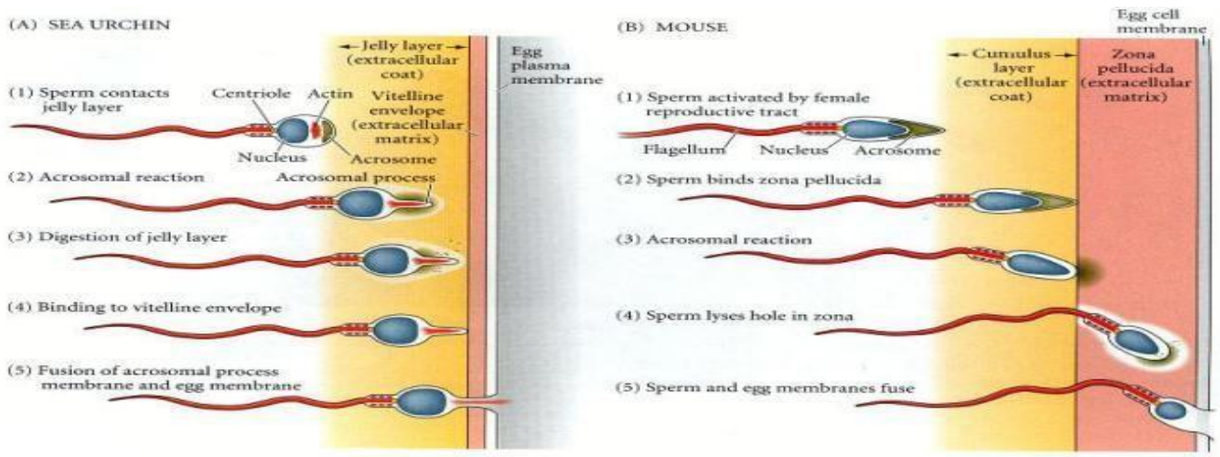
However, whereas each sperm contains one acrosomal vesicle, each sea urchin egg contains approximately 15,000 cortical granules.

TOPIC 41 42. Recognition of Egg and Sperm

Recognition of Egg and Sperm

The interaction of sperm and egg generally proceeds according to five basic step:

- The chemo-attraction of the sperm to the egg by soluble molecules secreted by the egg.
- The exocytosis of the acrosomal vesicle to release its enzymes.
- The binding of the sperm to the extracellular envelope (vitelline layer or zona pellucida) of the egg.
- The passing of the sperm through this extracellular envelope.
- Fusion of egg and sperm cell plasma membranes.



In many species, the meeting of sperm and egg is not a simple matter. Many marine organisms release their gametes into the environment. That environment may be as small as a tide pool or as large as an ocean.

These organisms are faced with two problems:

- How can sperm and eggs meet in such a dilute concentration?
- How can sperm be prevented from trying to fertilize eggs of another species?

Two major mechanisms have evolved to solve these problems:

- Species specific attraction of sperm.
- Species-specific sperm activation.

TOPIC 43.44 Sperm attraction

Sperm attraction

Species-specific sperm attraction has been documented in numerous species, including cnidarians, molluscs, echinoderms, and urochordates. In many species, sperm are attracted toward eggs of their species by **chemotaxis**, that is, by following a gradient of a chemical secreted by the egg.

In 1978, Miller demonstrated that the eggs of the cnidarian ***Orthopyxis caliculata*** not only secrete a chemotactic factor but also regulate the timing of its release.

Miller found that when sperm were added to oocytes that had not yet completed their second meiotic division, there was no attraction of sperm to eggs. However, after the second meiotic division was finished and the eggs were ready to be fertilized, the sperm migrated toward them.Thus, these oocytes control not only the type of sperm they attract, but also the time at which they attract them.

The mechanisms of **chemotaxis** differ among species. One chemotactic molecule, a 14-amino acid peptide called **resact,** has been isolated from the egg jelly of the sea urchin *Arbacia punctulata.*

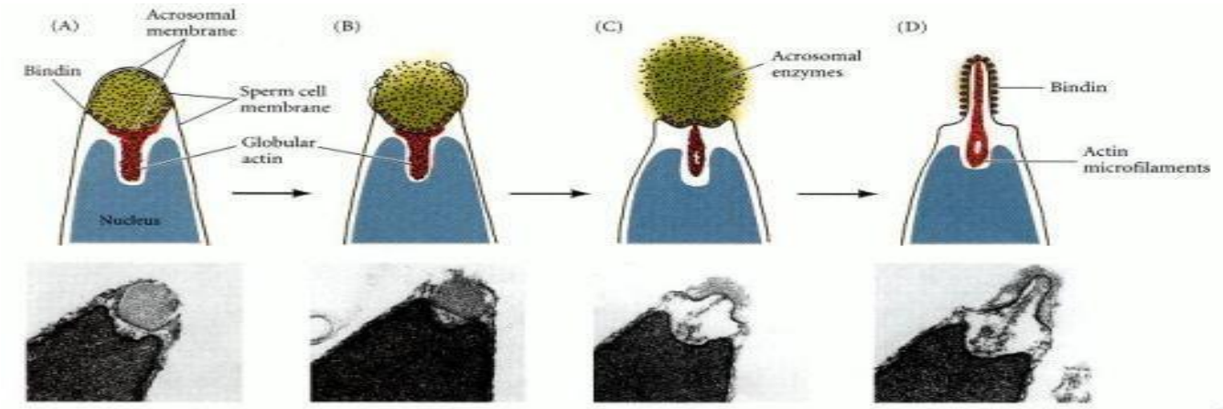
Resact diffuses readily in seawater and has a profound effect at very low concentrations when added to a suspension of *Arbacia* sperm.**Resact** is specific for ***A. punctulata*** and does not attract sperm of other species. *A. punctulata* sperm have receptors in their plasma membranes that bind resact and can swim up a concentration gradient of this compound until they reach the egg.Resact also acts as a **sperm-activating peptide**.**The sperm receptor for Resact** is a trans membrane protein, and when it binds Resact on the extracellular side, a conformational change on the cytoplasmic side activates the receptor's enzymatic activity.This activates the mitochondrial ATP-generating apparatus as well as the dynein ATPase that stimulates flagellar movement in the sperm.

TOPIC 45 The acrosomal reaction in sea urchins

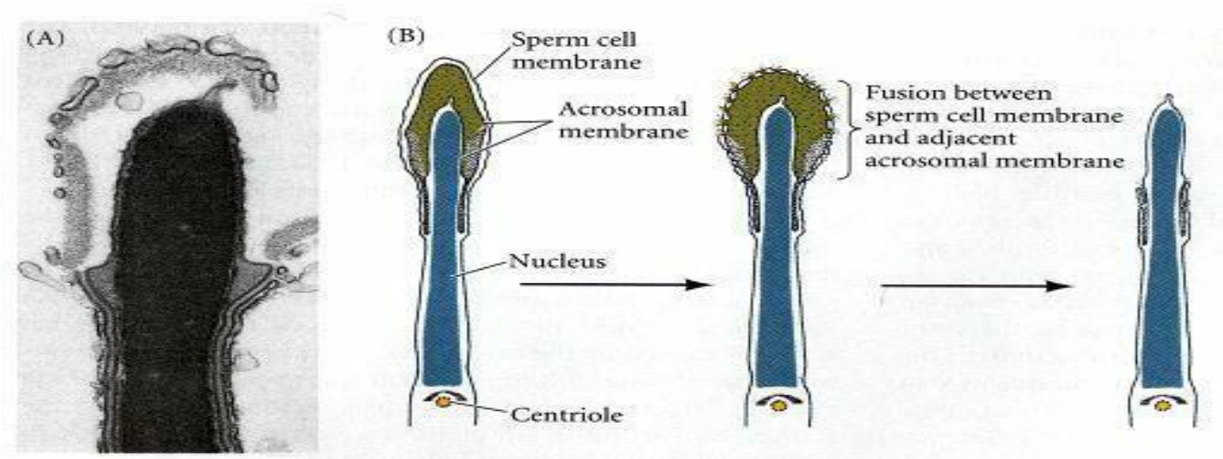
The acrosomal reaction in sea urchins

In most marine invertebrates, the acrosomal reaction has two components:

The fusion of the acrosomal vesicle with the sperm plasma membrane (an exocytosis that results in the release of the contents of the acrosomal vesicle).The extension of the acrosomal process. The acrosomal reaction in sea urchins is initiated by contact of the sperm with the egg jelly. Contact with egg jelly causes the exocytosis of the sperm's acrosomal vesicle and the release of proteolytic enzymes that can digest a path through the jelly coat to the egg surface**.** In sea urchins, the acrosomal reaction is thought to be initiated by a fucose-containing polysaccharide in the egg jelly that binds to the sperm and allows calcium to enter into the sperm head. The exocytosis of the acrosomal vesicle is caused by the calcium-mediated fusion of the acrosomal membrane with the adjacent sperm plasma membrane.



The second part of the acrosomal reaction involves the extension of the acrosomal process. This protrusion arises through the polymerization of globular actin molecules into actin filaments. The egg jelly factors that initiate the acrosomal reaction in sea urchins are often highly specific to each species.

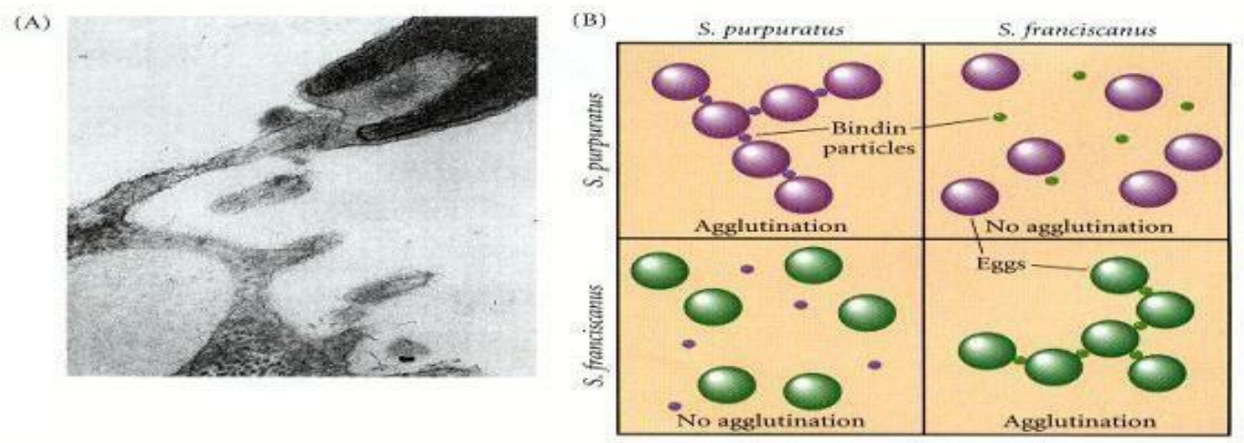


TOPIC 46. Species-specific recognition in sea urchins

Species-specific recognition in sea urchins

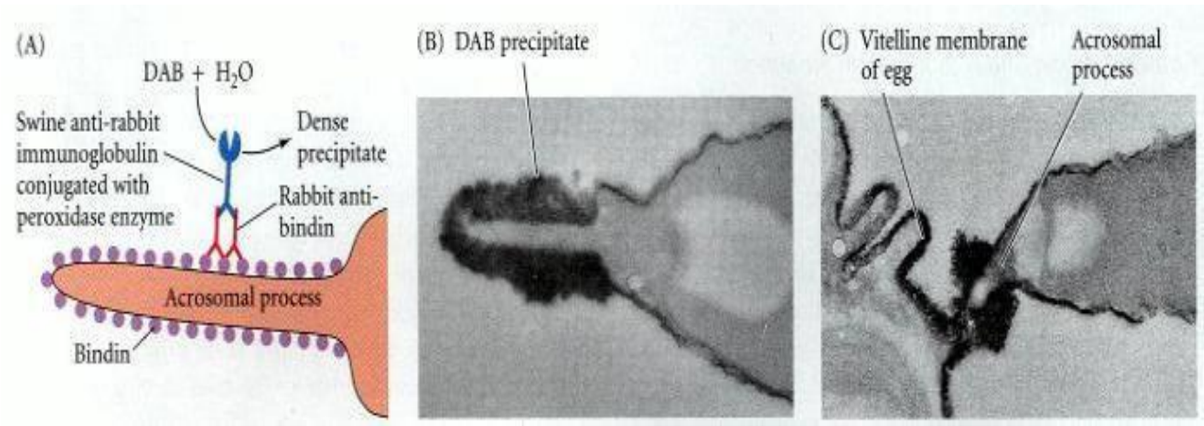
Once the sea urchin sperm has penetrated the egg jelly, the acrosomal process of the sperm contacts the surface of the egg . A major species-specific recognition step occurs at this point.The acrosomal protein mediating this recognition is called **bindin**. In 1977, Vacquier and co-workers isolated this nonsoluble 30,500-Da (Dalton) protein from the acrosome of

Strongylocentrotus purpuratus and found it to be capable of binding to dejellied eggs of the same species. The interaction bindin with eggs is relatively species-specific. **Bindin** isolated from the acrosomes of *S. purpuratus* binds to its own dejellied eggs, but not to those of *Arbacia punctulata.*



Using immunological techniques, **Moy and Vacquier** demonstrated that **bindin** is located specifically on the acrosomal process exactly where it should be for sperm-egg recognition.

As seen in Figure, sperm binding does not occur over the entire egg surface.



Thus, species-specific recognition of sea urchin gametes occurs at the levels of:

- Sperm attraction
- Sperm activation
- Sperm adhesion to the egg surface.

TOPIC 47. Gamete binding and recognition in mammals

Gamete binding and recognition in mammals

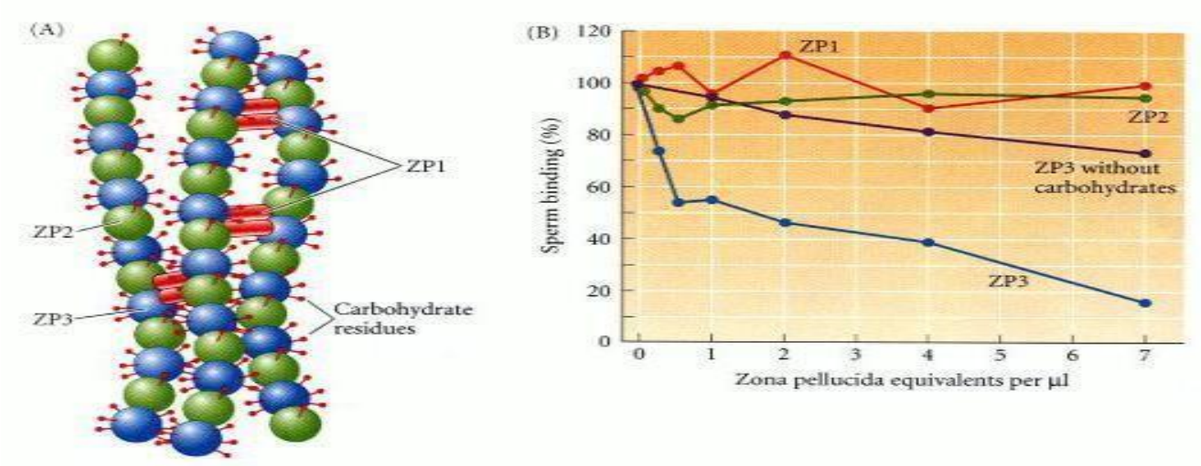
The zona pellucida is a glycoprotein matrix, which is synthesized and secreted by the growing oocyte.

In mammals the zona pellucida plays a role analogous to that of the vitelline envelope in invertebrates.

The zona pellucida plays two major roles during fertilization:

- It binds the sperm.
- It initiates the acrosomal reaction after the sperm is bound.

- The binding of sperm to the zona is relatively, but not absolutely, species-specific.
- Species specific gamete recognition is not a major problem when fertilization occurs internally.
- Bleil and Wassarman (1988) isolated an 83-kDa glycoprotein, **ZP3**, from the mouse zona that was the active site for sperm binding.
- The other two zona glycoproteins they found, **ZP1** and **ZP2**, failed to compete for sperm binding.
- Thus, **ZP3** is the specific glycoprotein in the mouse zona pellucida to which the sperm bind.



ZP3 also initiates the acrosomal reaction after sperm have bound to it. The mouse sperm can thereby concentrate its proteolytic enzymes directly at the point of attachment at the zona pellucida.

TOPIC 48. Induction of the mammalian acrosomal reaction

Induction of the mammalian acrosomal reaction

Unlike the sea urchin acrosomal reaction, the acrosomal reaction in mammals occurs only after the sperm has bound to the zona pellucida. The opening of the calcium channels involve the receptor's activating a cation channel (for sodium, potassium, or calcium), which would change the resting potential of the sperm plasma membrane. The calcium channels in the membrane would be sensitive to this change in membrane potential, allowing calcium to enter the sperm.The difference between the acrosomal reaction in sea urchins and mammals may be due to the thickness of the extracellular envelopes surrounding the egg. In the sea urchin, the vitelline envelope is very thin and porous. Once a sperm has bound there, it is very close to the egg plasma membrane, and, indeed, the bindin receptor may extend through the vitelline envelope. In mammals, however, the zona pellucida is a very thick matrix, so the sperm is far removed from the egg.

By undergoing the acrosomal reaction directly on the zona, the sperm is able to concentrate its proteolytic enzymes to lyse a hole in this envelope. Indeed, sperm that undergo the acrosomal reaction before they reach the zona pellucida are unable to penetrate it.

It appears that the acrosome-reacted sperm transfer their binding from ZP3 to the adjacent ZP2 molecules. After a mouse sperm has entered the egg, the egg cortical granules release their contents.

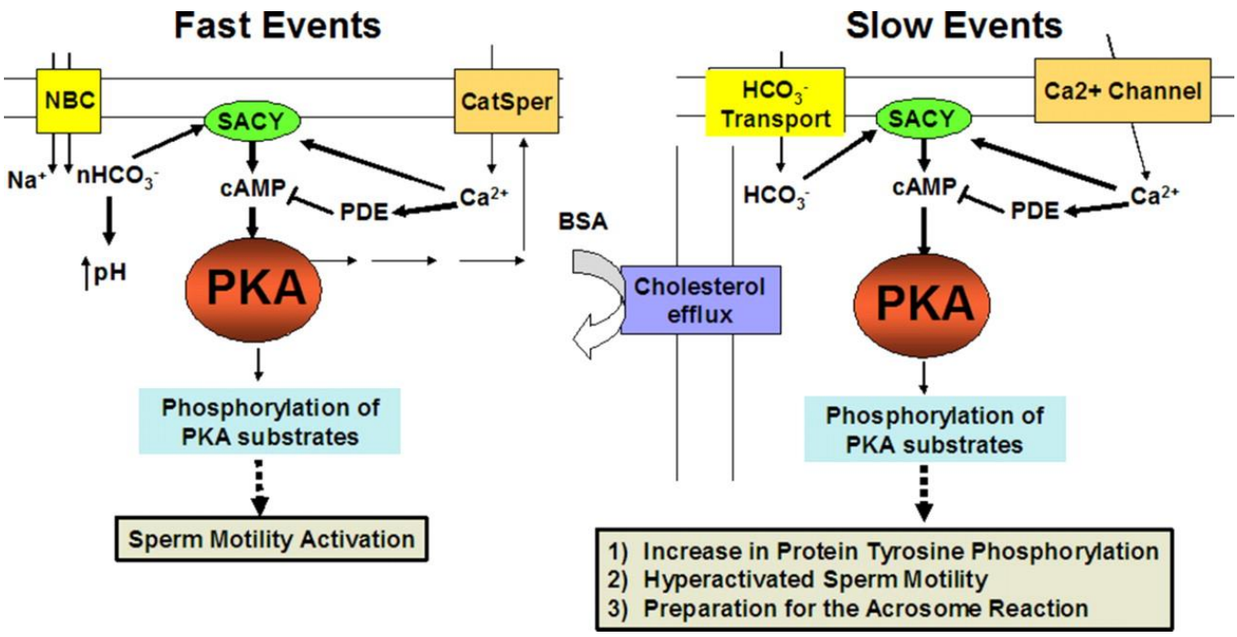
One of the proteins released by these granules is a protease that specifically alters **ZP2** (Moller and Wassarman 1989). This inhibits other acrosome-reacted sperm from moving closer toward the egg.

TOPIC 49. 50. Capacitation AND Changes that account for capacitation

Capacitation

The set of physiological changes that allow the sperm to be competent to fertilize the egg is called **capacitation**. The requirement for capacitation varies from species to species.

Wilcox and colleagues (1995) found that nearly all human pregnancies result from sexual intercourse during a **6-day period** ending on the day of ovulation.This means that the fertilizing sperm could have taken as long as 6 days to make the journey.



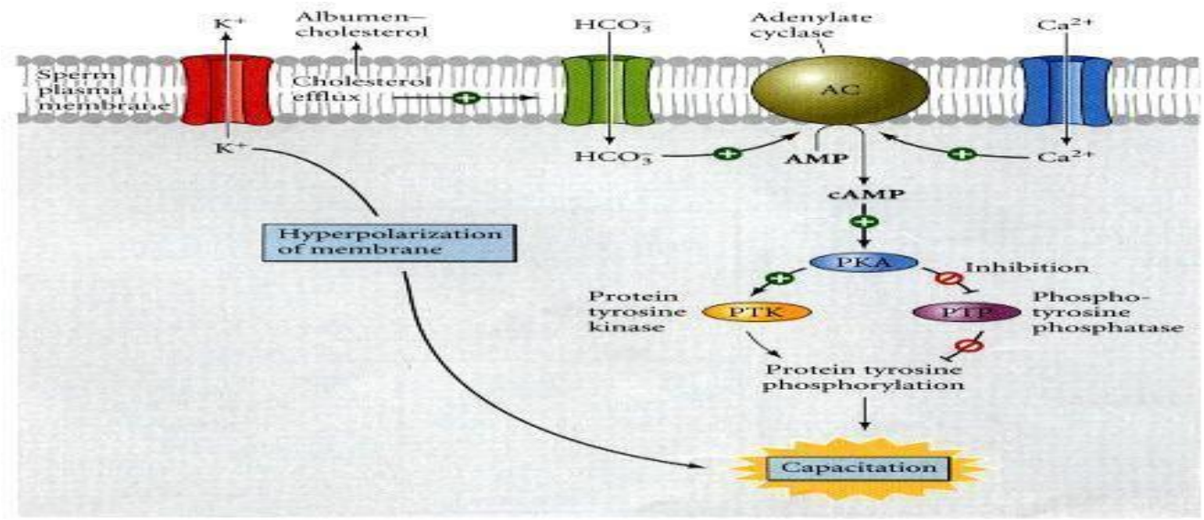
The molecular changes that account for capacitation are,

- **First**, the fluidity of the sperm plasma membrane is altered by the removal of cholesterol by albumin proteins found in the female reproductive tract.
- **Second**, particular proteins or carbohydrates on the sperm surface are lost during capacitation.

  It is possible that these compounds block the recognition sites for the proteins that bind to the zona pellucida.
- **Third,** the membrane potential of the sperm becomes more negative as potassium ions leave the sperm.

  This change in membrane potential may allow calcium channels to be opened and permit calcium to enter the sperm.

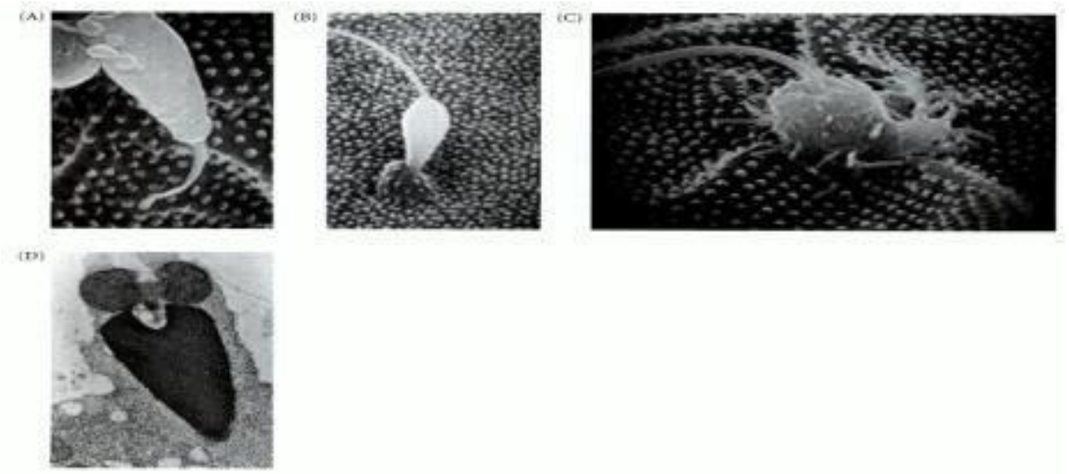
  Calcium and bicarbonate ions may be critical in activating **cAMP** (Cyclic adenosine monophosphate) production and in facilitating the membrane fusion events of the acrosomal reaction.
- **Fourth**, protein phosphorylation occurs.



TOPIC 51.52. Gamete Fusion AND Gamete Fusion in Mammals

Gamete Fusion

Recognition of sperm by the vitelline envelope or zona pellucida is followed by the lysis of that portion of the envelope or zona in the region of the sperm head by the acrosomal enzymes.This lysis is followed by the fusion of the sperm plasma membrane with the plasma membrane of the egg.The entry of a sperm into a sea urchin egg is illustrated in this Figure



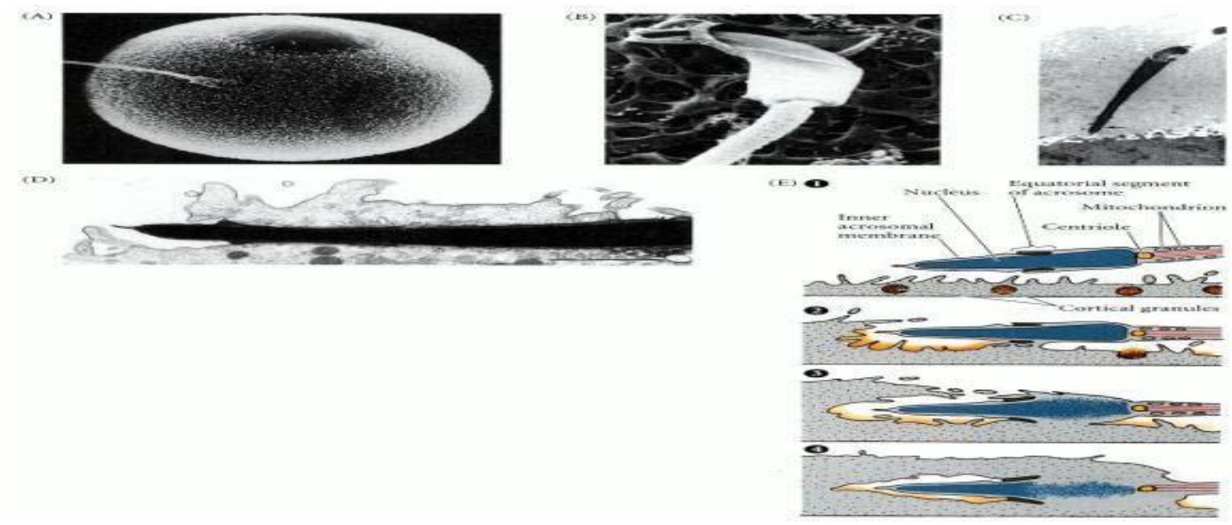
The sperm and egg plasma membranes then join together, and material from the sperm membrane can later be found on the egg membrane. The sperm nucleus and tail pass through the resulting cytoplasmic bridge, which is widened by the actin polymerization.

In the sea urchin, all regions of the egg plasma membrane are capable of fusing with sperm. In several other species, certain regions of the membrane are specialized for sperm recognition and fusion. Fusion is an active process, often mediated by specific "**fusogenic" proteins**.

Gamete Fusion in Mammals

In mammals, the **fertilin** proteins in the sperm plasma membrane are essential for sperm membrane-egg membrane fusion. Mouse fertilin is localized to the posterior plasma membrane of the sperm head. It adheres the sperm to the egg by binding to the **6β1 integrin protein** on the egg plasma membrane. Moreover, like sea urchin bindin (to which it is not structurally related), fertilin has a hydrophobic region that could potentially mediate the union of the two membranes.

Thus, **fertilin** appears to bind the sperm plasma membrane to the egg plasma membrane and then to fuse the two of them together. When the membranes are fused, the sperm nucleus, mitochondria, centriole, and flagellum can enter the egg.



TOPIC 53.54. The prevention of polyspermy

The prevention of polyspermy

Difference B/W Mono and Poly Spermy

monospermy

In normal **monospermy,** in which only one sperm enters the egg, a haploid sperm nucleus and a haploid egg nucleus combine to form the diploid nucleus of the fertilized egg (zygote). The centriole, which is provided by the sperm, will divide to form the two poles of the mitotic spindle during cleavage.

polyspermy

The entrance of multiple sperm **polyspermy** leads to disastrous consequences in most organisms. In the sea urchin, fertilization by two sperm results in a triploid nucleus, in which each chromosome is represented three times rather than twice (Worse condition).

Species have evolved ways to prevent the union of more than two haploid nuclei. The most common way is to prevent the entry of more than one sperm into the egg.

The sea urchin egg has two mechanisms to avoid polyspermy:

- A fast reaction, accomplished by an electric change in the egg plasma membrane.
- A slower reaction, caused by the exocytosis of the cortical granules.

TOPIC 55.56. The fast block to polyspermy AND Maintance of fast block to polyspermy

The fast block to polyspermy

The **Fast block to polyspermy** is achieved by changing the electric potential of the egg plasma membrane. This membrane provides a selective barrier between the egg cytoplasm and the outside environment, and the ionic concentration of the egg differs greatly from that of

its surroundings.This concentration difference is especially significant for **sodium and potassium ions.** Seawater has a particularly high sodium ion concentration, whereas the egg cytoplasm contains relatively little sodium. The reverse is the case with **potassium ions.**

Maintance of fast block to polyspermy

This condition is maintained by the plasma membrane, which steadfastly inhibits the entry of sodium ions into the oocyte and prevents potassium ions from leaking out into the environment.

If we insert an electrode into an egg and place a second electrode outside it, we can measure the constant difference in charge across the egg plasma membrane.This **resting membrane potential** is generally about **70 mV**, usually expressed as 70 mV because the inside of the cell is negatively charged with respect to the exterior.

TOPIC 57. 58. The slow block to polyspermy Cortical granule reaction to prevent polyspermy

The fast block to polyspermy

The eggs of sea urchins (and many other animals) have a second mechanism to ensure that multiple sperm do not enter the egg cytoplasm. **The fast block to polyspermy is transient,** since the membrane potential of the sea urchin egg remains positive for only about a minute.

The slow block to polyspermy

This brief potential shift is not sufficient to prevent polyspermy, which can still occur if the sperm bound to the vitelline envelope are not somehow removed .

Cortical granule reaction to prevent polyspermy

This removal is accomplished by the **cortical granule reaction**, a slower, mechanical block to polyspermy that becomes active about a minute after the first successful sperm-egg attachment.

If we insert an electrode into an egg and place a second electrode outside it, we can measure the constant difference in charge across the egg plasma membrane.

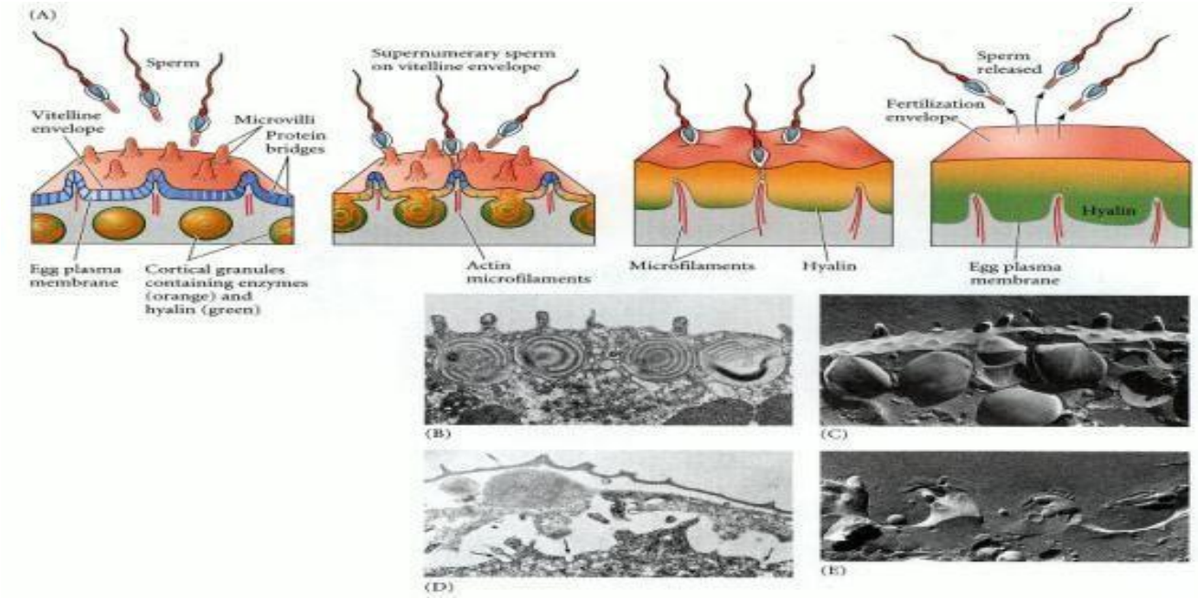
Directly beneath the sea urchin egg plasma membrane are about 15,000 cortical granules. Upon sperm entry, these cortical granules fuse with the egg plasma membrane and release their contents into the space between the plasma membrane and the fibrous mat of vitelline envelope proteins.

TOPIC 59. Cortical granule reaction in mammals

These enzymes dissolve the protein posts that connect the vitelline envelope proteins to the cell membrane, and they clip off the bindin receptor and any sperm attached to it.

Mucopolysaccharides released by the cortical granules produce an osmotic gradient that causes water to rush into the space between the plasma membrane and the vitelline envelope, causing the envelope to expand and become the **fertilization envelope.**

A third protein released by the cortical granules, a peroxidase enzyme, hardens the fertilization envelope by crosslinking tyrosine residues on adjacent proteins.

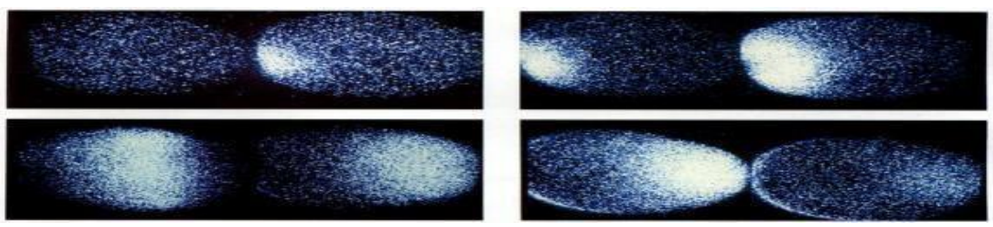


Finally, a fourth cortical granule protein, hyalin, forms a coating around the egg. The egg extends elongated microvilli whose tips attach to this **hyaline layer**. This layer provides support for the blastomeres during cleavage. In mammals, the cortical granule reaction does not create a fertilization envelope, but its ultimate effect is the same. Released enzymes modify the zona pellucida sperm receptors such that they can no longer bind sperm.

TOPIC 60. 61. Role of calcium in cortical reaction

Role of calcium in cortical reaction

Upon fertilization, the intracellular calcium ion concentration of the egg increases greatly. In this high-calcium environment, the cortical granule membranes fuse with the egg plasma membrane, releasing their contents. Once the fusion of the cortical granules begins near the point of sperm entry, a wave of cortical granule exocytosis propagates around the cortex to the opposite side of the egg.



The release of calcium from intracellular storage can be monitored visually using calciumactivated luminescent dyes such as aequorin (isolated from luminescent jellyfish) or fluorescent dyes such as fura-2. These dyes emit light when they bind free calcium ions.

Several experiments have demonstrated that calcium ions are directly responsible for propagating the cortical granule reaction, and that these calcium ions are stored within the egg itself.

The drug A23187 is a calcium ionophore (a compound that transports free calcium ions across lipid membranes, allowing these cations to traverse otherwise impermeable barriers).

Placing unfertilized sea urchin eggs into seawater containing A23187 causes the cortical granule reaction and the elevation of the fertilization envelope.

Therefore, A23187 must be causing the release of calcium ions already sequestered in organelles within the egg.

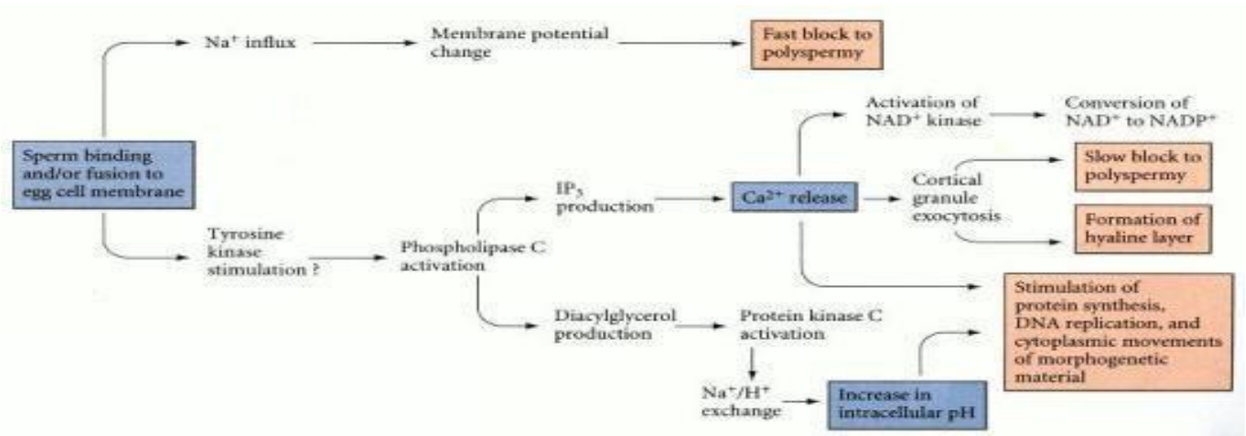
The calcium ions responsible for the cortical granule reaction are stored in the endoplasmic reticulum of the egg. In sea urchins and frogs, this reticulum is pronounced in the cortex and surrounds the cortical granules.

TOPIC 62.63. The Activation of Egg Metabolism AND Late responses in the Activation of Egg Metabolism

The Activation of Egg Metabolism

Although fertilization is often depicted as merely the means to merge two haploid nuclei, it has an equally important role in initiating the processes that begin development. These events happen in the cytoplasm and occur without the involvement of the nuclei.

The mature sea urchin egg is a metabolically sluggish cell that is activated by the sperm. This activation is merely a stimulus, however; it sets into action a preprogrammed set of metabolic events.



The responses of the egg to the sperm can be divided into "early" responses, which occur within seconds of the cortical reaction, and "late" responses, which take place several minutes after fertilization begins.

Early responses:

Contact between sea urchin sperm and egg activates the two major blocks to polyspermy: the fast block, initiated by sodium influx into the cell, and the slow block, initiated by the intracellular release of calcium ions. The activation of all eggs appears to depend on an increase in the concentration of free calcium ions within the egg. Such an increase can occur in two ways:
calcium ions can enter the egg from outside, or calcium ions can be released from the endoplasmic reticulum within the egg.

Late responses:

Shortly after the calcium ion levels rise in a sea urchin egg, its intracellular pH also increases. The rise in intracellular pH begins with a second influx of sodium ions, which causes a 1:1 exchange between sodium ions from the seawater and hydrogen ions from the egg.

This loss of hydrogen ions causes the pH to rise. It is thought that the pH increase and the calcium ion elevation act together to stimulate new protein synthesis and DNA synthesis.

The late responses of fertilization brought about by these ionic changes include the activation of DNA synthesis and protein synthesis.

TOPIC 64.65. Introduction to Early Developmental Processes AND Role of MPF in Early Developmental Processes

Introduction to Early Developmental Processes

Cleavage

After fertilization, the development of a multicellular organism proceeds by a process called **cleavage**, a series of mitotic divisions whereby the enormous volume of egg cytoplasm is divided into numerous smaller, nucleated cells. These cleavage-stage cells are called **blastomeres**.

In most species (mammals being the chief exception), the rate of cell division and the placement of the blastomeres with respect to one another is completely under the control of the proteins and mRNAs stored in the oocyte by the mother.

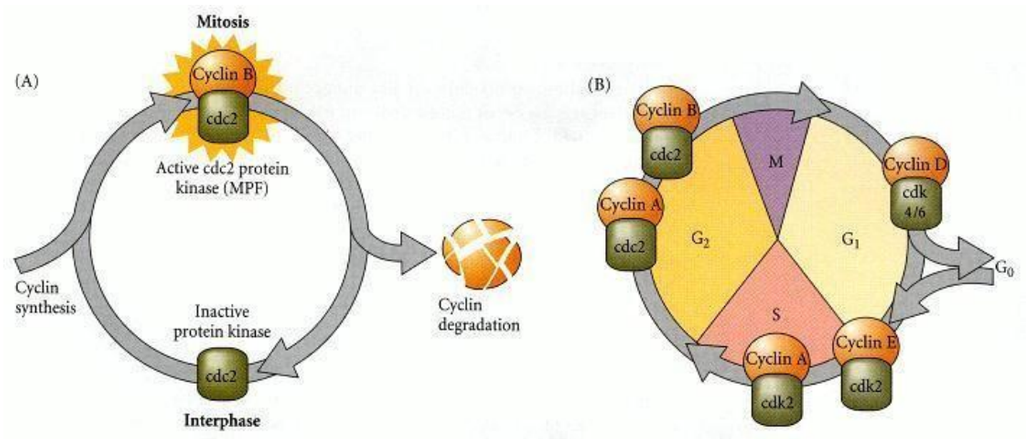
The mitotic spindle and contractile ring are perpendicular to each other, and the spindle is internal to the contractile ring. The contractile ring creates a cleavage furrow, which eventually bisects the plane of mitosis, thereby creating two genetically equivalent blastomeres.

Role of MPF in Early Developmental Processes

The transition from fertilization to cleavage is caused by the activation of mitosis promoting factor (MPF).

MPF was first discovered as the major factor responsible for the resumption of meiotic cell divisions in the ovulated frog egg.

Blastomeres generally progress through a cell cycle consisting of just two steps: M (mitosis) and S (DNA synthesis).



Cleaving cells can be experimentally trapped in S phase by incubating them in an inhibitor of protein synthesis. When MPF is microinjected into these cells, they enter M. Their nuclear envelope breaks down and their chromatin condenses into chromosomes. After an hour, MPF is degraded and the chromosomes return to S phase.

TOPIC 66.67.Causes of cyclic activity of MPF AND Differnce b/w Karyokinesis and Cytokinesis

What causes this cyclic activity of MPF?

Mitosis-promoting factor contains two subunits.

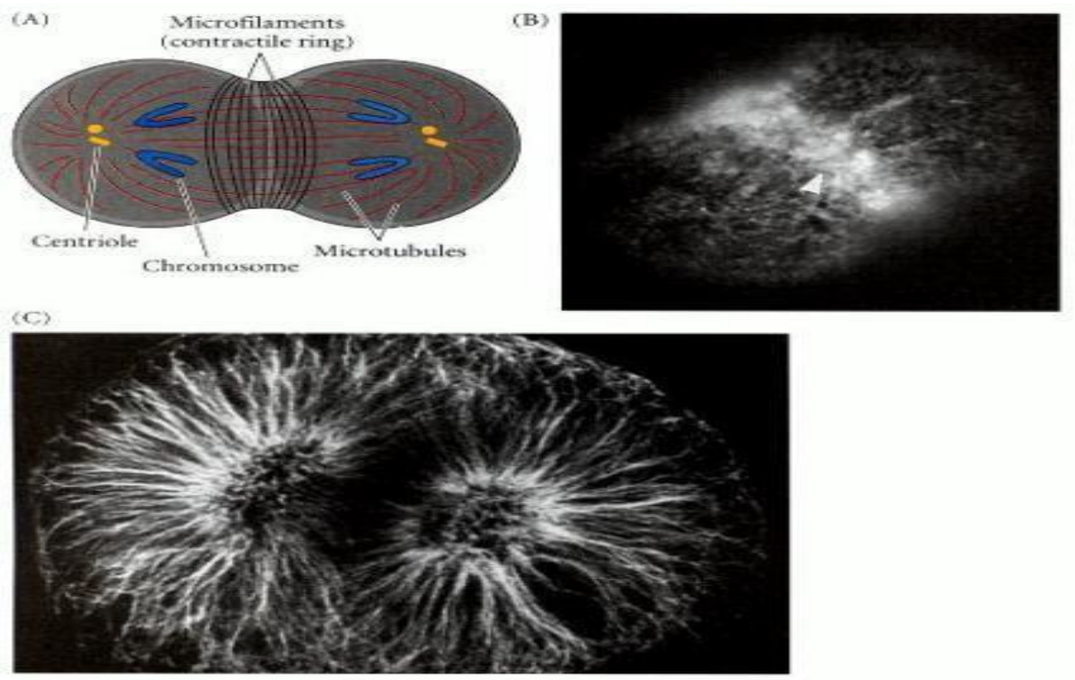
- The large subunit is called **cyclin B**.
- Small subunit of MPF, the **cyclin-dependent kinase**
- **Cyclin B** is often encoded by mRNAs stored in the oocyte cytoplasm, and if the translation of this message is specifically inhibited, the cell will not enter mitosis. The presence of cyclin B depends upon its synthesis and its degradation. Cyclin B regulates the small subunit of MPF, the **cyclin-dependent kinase**.

Cleavage is actually the result of two coordinated processes.

- **karyokinesis**
- **Cytokinesis**

Differnce b/w Karyokinesis and Cytokinesis

- **karyokinesis** the mitotic division of the nucleus.
- The mechanical agent of this division is the mitotic spindle, with its **microtubules** composed of **tubulin** (the same type of protein that makes up the sperm flagellum).
- The second process is **cytokinesis** the division of the cell.
- The mechanical agent of cytokinesis is a **contractile ring** of **microfilaments** made of **actin** (the same type of protein that extends the egg microvilli and the sperm acrosomal process).
- The relationship and coordination between these two systems during cleavage is depicted in Figure, in which a sea urchin egg is shown undergoing first cleavage.



The mitotic spindle and contractile ring are perpendicular to each other, and the spindle is internal to the contractile ring. The contractile ring creates a **cleavage furrow**, which eventually bisects the plane of mitosis, thereby creating two genetically equivalent blastomeres.

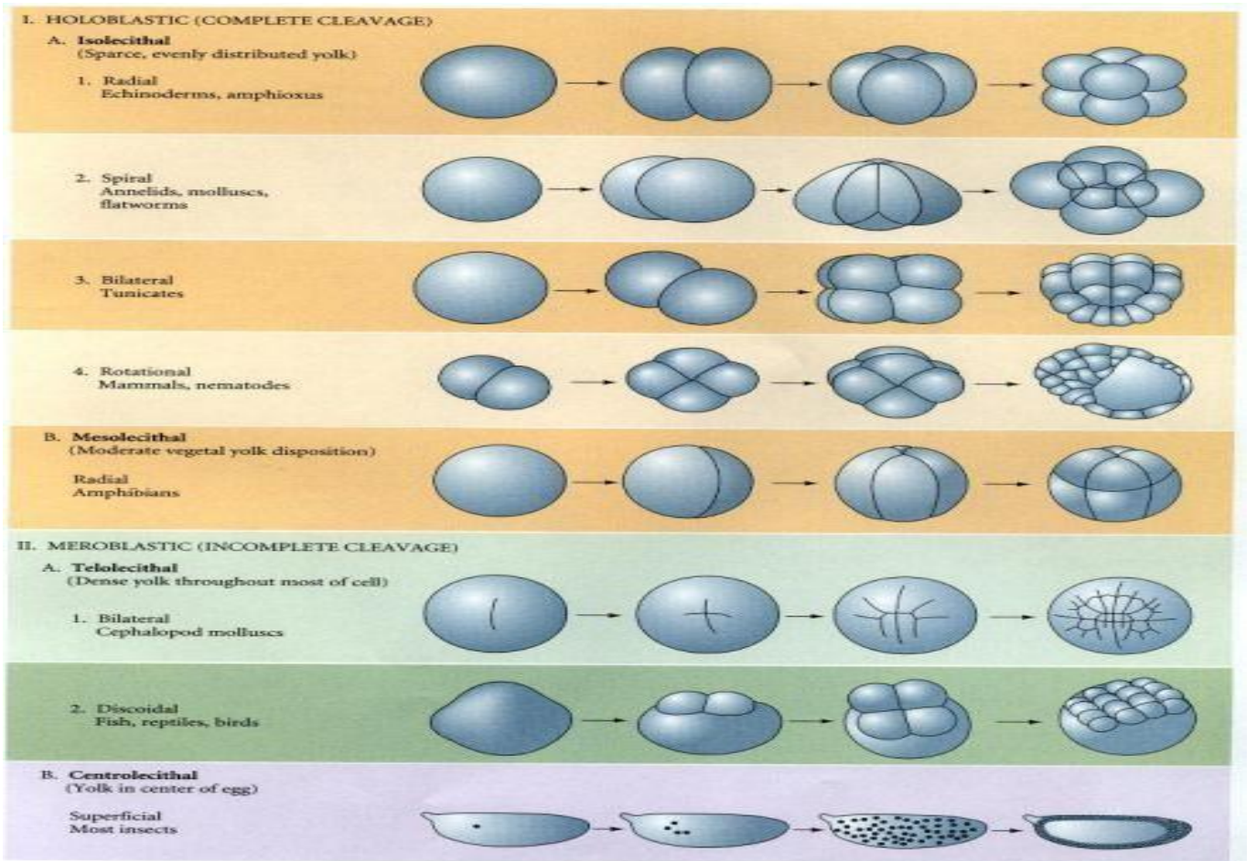
TOPIC 68. Patterns of embryonic cleavage

Patterns of embryonic cleavage

Indeed, different organisms undergo cleavage in distinctly different ways. The pattern of embryonic cleavage particular to a species is determined by two major parameters:

1. The amount and distribution of yolk protein within the cytoplasm.
2. The factors in the egg cytoplasm that influence the angle of the mitotic spindle and the timing of its formation.
3. The yolk-rich pole is referred to as the **vegetal pole**; the yolk concentration in the **animal pole** is relatively low. The zygote nucleus is frequently displaced toward the animal pole. In general, yolk inhibits cleavage.

This diagram provides a classification of cleavage types and shows the influence of yolk on cleavage symmetry and pattern



TOPIC 69. Patterns of embryonic cleavage

Early responses:

Contact between sea urchin sperm and egg activates the two major blocks to polyspermy: the fast block, initiated by sodium influx into the cell, and the slow block, initiated by the intracellular release of calcium ions.

The activation of all eggs appears to depend on an increase in the concentration of free calcium ions within the egg. Such an increase can occur in two ways: calcium ions can enter the egg from outside, or calcium ions can be released from the endoplasmic reticulum within the egg.

TOPIC 70. Classification of cleavage types

At one extreme are the eggs of sea urchins, mammals, and snails. These eggs have sparse, equally spaced yolk and are thus **isolecithal** (Greek, "equal yolk"). In these species, cleavage is **holoblastic** (Greek *holos,* "complete"). meaning that the cleavage furrow extends through the entire egg.

Zygotes containing large accumulations of yolk undergo **meroblastic** cleavage, wherein only a portion of the cytoplasm is cleaved. The cleavage furrow does not penetrate into the yolky portion of the cytoplasm.

The eggs of insects have their yolk in the center (i.e., they are **centrolecithal**), and the divisions of the cytoplasm occur only in the rim of cytoplasm around the periphery of the cell (i.e., **superficial** cleavage).

The eggs of birds and fishes have only one small area of the egg that is free of yolk (**telolecithal** eggs), and therefore, the cell divisions occur only in this small disc of cytoplasm, giving rise to the **discoidal** pattern of cleavage.

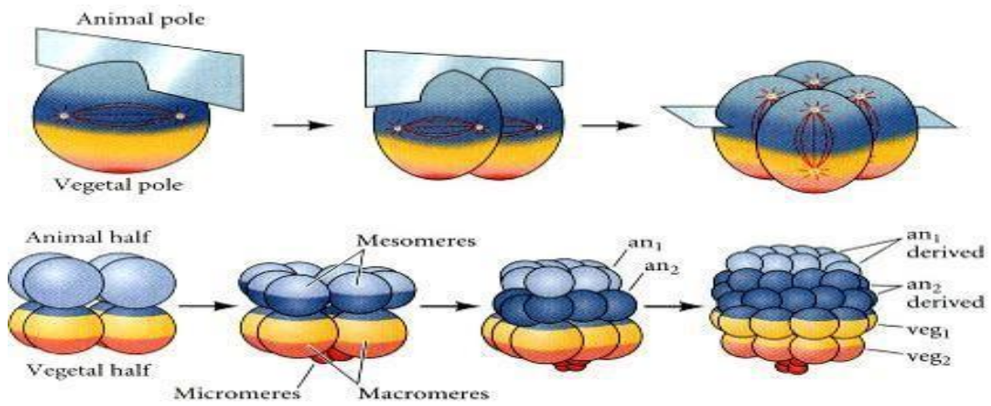
TOPIC 71..72. Cleavage in Sea Urchins AND Cleavage in Sea Urchins

The Early Development of Sea Urchins

Cleavage in Sea Urchins

Sea urchins exhibit **radial holoblastic cleavage**. The first and second cleavages are both meridional and are perpendicular to each other. That is to say, the cleavage furrows pass through the animal and vegetal poles.

The third cleavage is equatorial, perpendicular to the first two cleavage planes, and separates the animal and vegetal hemispheres from one another. The fourth cleavage, however, is very different from the first three.



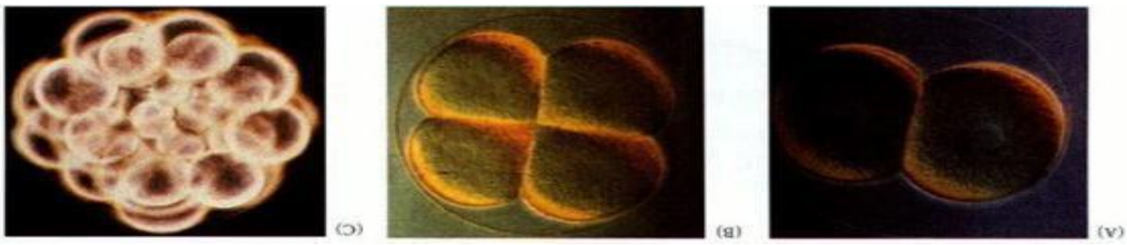
The four cells of the animal tier divide meridionally into eight blastomeres, each with the same volume. These cells are called **mesomeres**. The vegetal tier, however, undergoes an unequal equatorial cleavage to produce four large cells, the **macromeres**, and four smaller **micromeres** at the vegetal pole.

Cleavage in Sea Urchins

As the 16-cell embryo cleaves, the eight mesomeres divide to produce two "animal" tiers, an1 and an2, one staggered above the other.The macromeres divide meridionally, forming a tier of eight cells below an2.

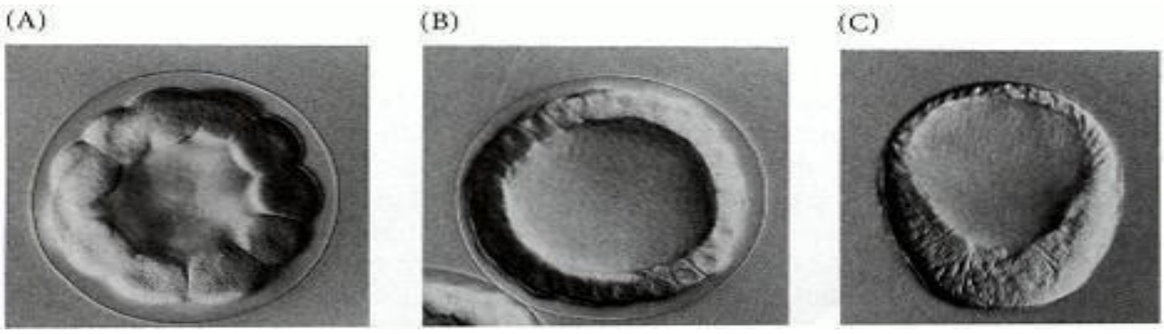
Blastula formation

The **blastula** stage of sea urchin development begins at the 128-cell stage. Here the cells form a hollow sphere surrounding a central cavity, or **blastocoel.**



At this time, tight junctions unite the once loosely connected blastomeres into a seamless epithelial sheet that completely encircles the blastocoel. As the cells continue to divide, the blastula remains one cell layer thick, thinning out as it expands.

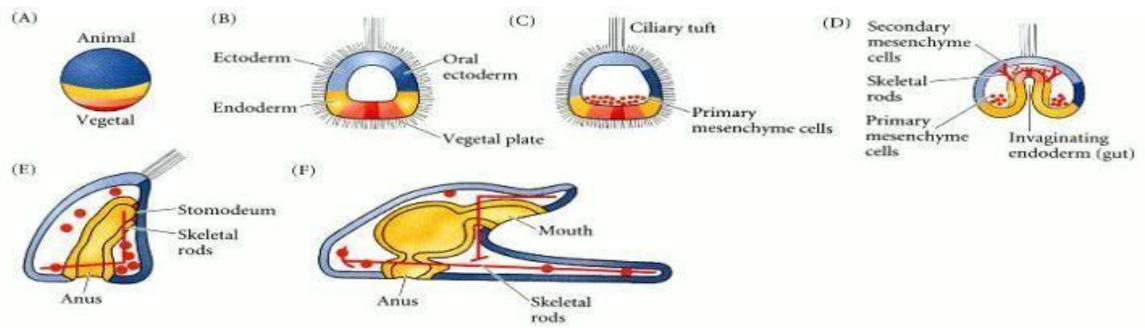
The cells at the vegetal pole of the blastula begin to thicken, forming a vegetal plate. The cells of the animal half synthesize and secrete a hatching enzyme that digests the fertilization envelope The embryo is now a free-swimming hatched blastula.



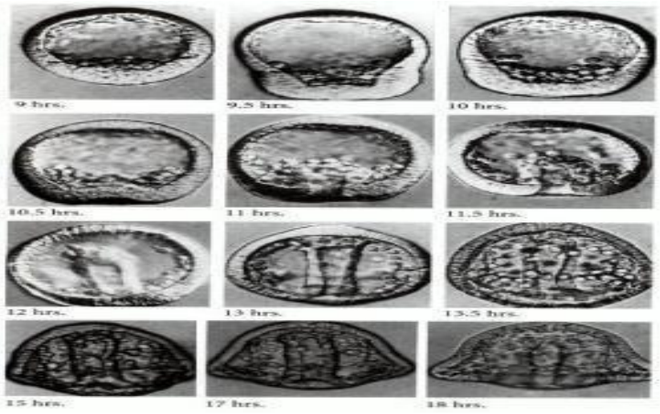
TOPIC 73.74. Sea Urchin Gastrulation

Sea Urchin Gastrulation

Figure show the fates of the various regions of the blastula as it develops through gastrulation to the **Pluteus larva** stage characteristic of sea urchins.

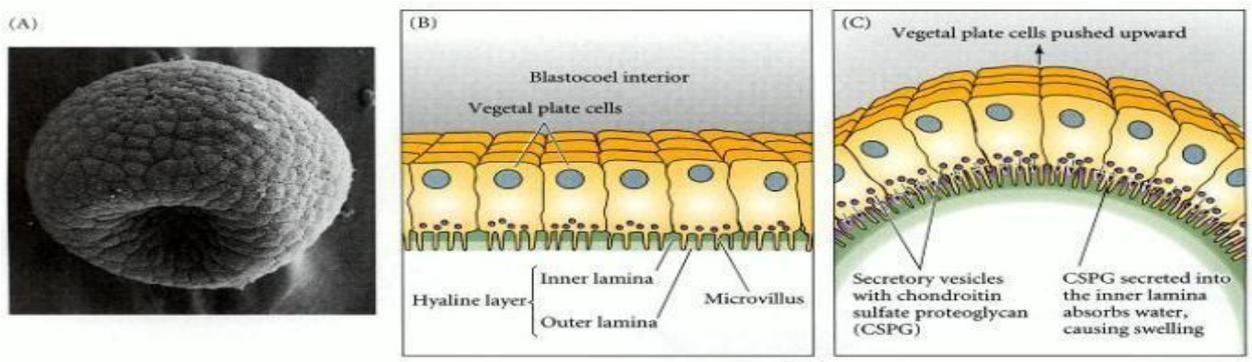


Shortly after the blastula hatches from its fertilization envelope, the vegetal side of the spherical blastula begins to thicken and flatten. At the centre of this flat vegetal plate, a cluster of small cells begins to change. These cells begin extending and contracting long, thin (30 × 5 μm) processes called **filopodia** from their inner surfaces.



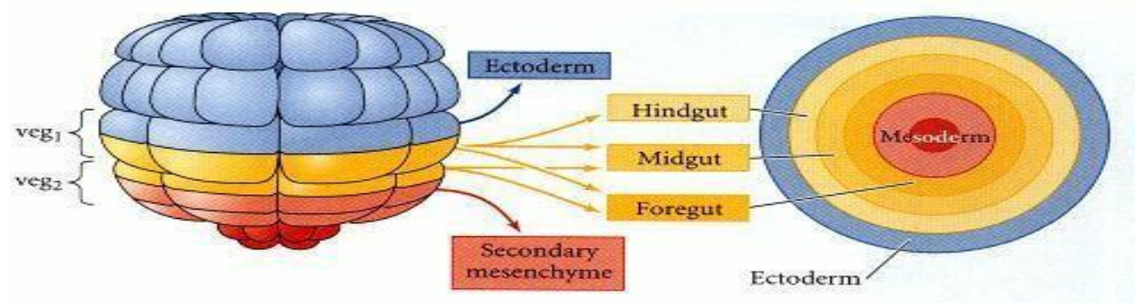
These cells, derived from the micromeres, are called the **primary mesenchyme**. They will form the larval skeleton, so they are sometimes called the **skeletogenic mesenchyme**.

The vegetal plate bends inward and invaginates about one-fourth to one half the way into the blastocoel. Then invagination suddenly ceases. The invaginated region is called the **archenteron** (primitive gut), and the opening of the archenteron at the vegetal region is called the **blastopore**.



The hyaline layer is actually made up of two layers, an outer lamina made primarily of **hyalin protein** and an inner lamina composed of **fibropellin proteins.**

The endodermal cells adjacent to the micromere-derived mesenchyme become foregut, migrating the farthest distance into the blastocoel. The next layer of endodermal cells becomes midgut, and the last circumferential row to invaginate forms the hindgut and anus.



TOPIC 75.76. Cleavage in Snail Eggs AND Sinistral coiling in Snail

The Early Development of Snails

Cleavage in Snail Eggs

Spiral holoblastic cleavage is characteristic of several animal groups, including annelid worms, some flatworms, and most molluscs. It differs from radial cleavage in numerous ways..

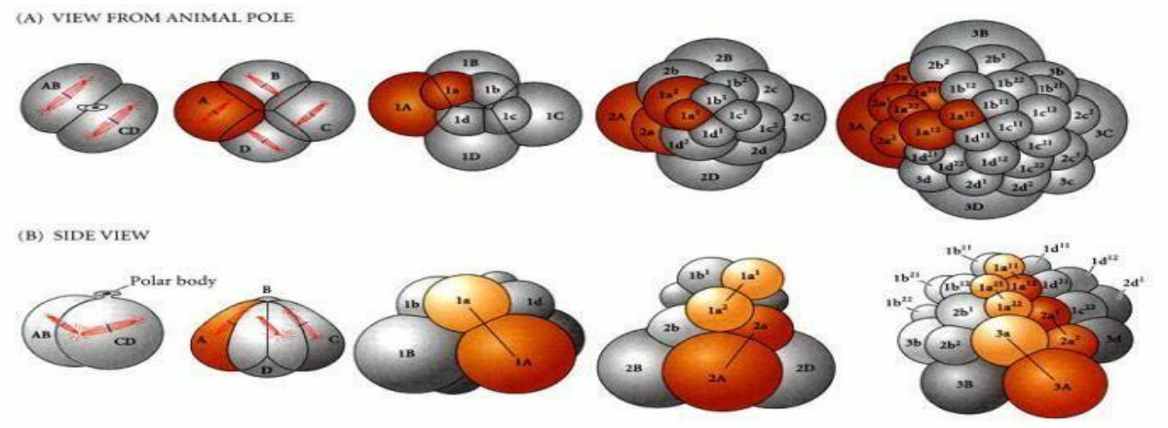
First, the cleavage planes are not parallel or perpendicular to the animal-vegetal axis of the egg; rather, cleavage is at oblique angles, forming a "spiral" arrangement of daughter blastomeres.

Second, the cells touch one another at more places than do those of radially cleaving embryos. In fact, they assume the most thermodynamically stable packing orientation, much like that of adjacent soap bubbles.

Third, spirally cleaving embryos usually undergo fewer divisions before they begin gastrulation, making it possible to follow the fate of each cell of the blastula.

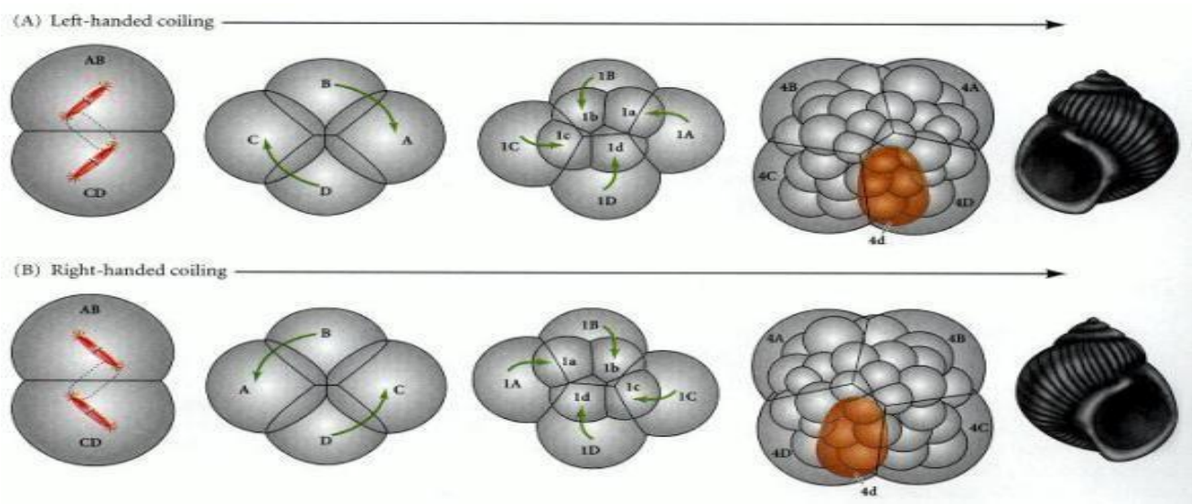
Blastulae produced by radial cleavage have no blastocoel and are called **stereoblastulae**.

Figure depicts the cleavage of mollusc embryos. The first two cleavages are nearly meridional, producing four large macromeres (labeled A, B, C, and D). In each successive cleavage, each macromere buds off a small micromere at its animal pole.



Sinistral coiling in Snail

The orientation of the cleavage plane to the left or to the right is controlled by cytoplasmic factors within the oocyte. Some snails have their coils opening to the right of their shells (**dextral** coiling), whereas other snails have their coils opening to the left (**sinistral** coiling).



Experiments have demonstrated that the non-diffusible polar lobe cytoplasm is extremely important in normal mollusc development for a number of reasons.

- It contains the determinants for the proper cleavage rhythm and cleavage orientation of the D blastomere.
- It contains certain determinants (those entering the 4d blastomere and hence leading to the **mesentoblasts**) for mesodermal and intestinal differentiation.

- It is responsible for permitting the inductive interactions (through the material entering the 3d blastomere) leading to the formation of the shell gland and eye.
- It contains determinants needed for specifying the dorsal-ventral axis of the embryo.

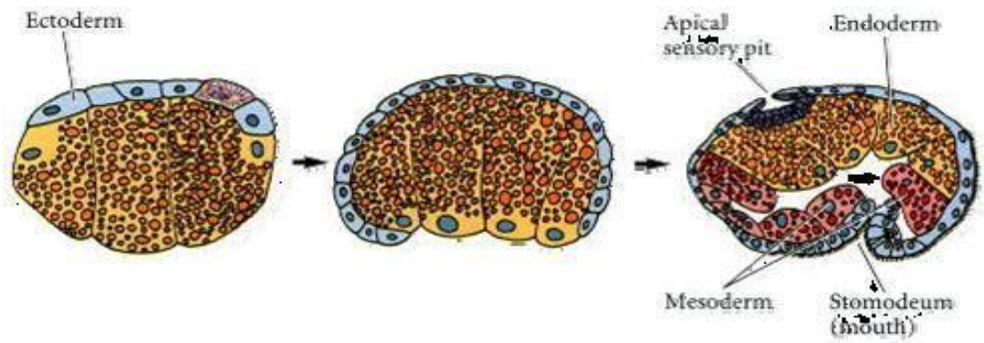
TOPIC 77.78. Gastrulation in Snails AND Shell formation during gastrulation in snails

Gastrulation in Snails

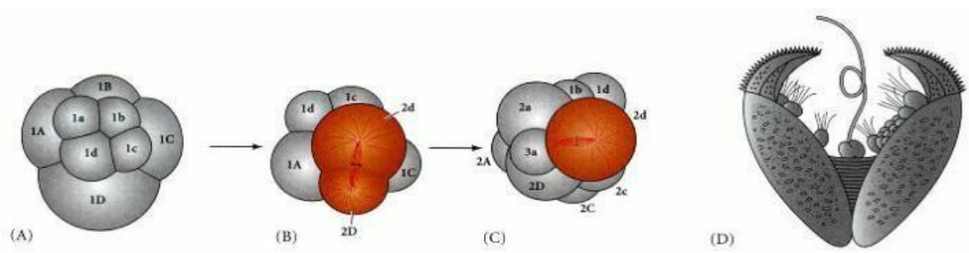
The snail stereoblastula is relatively small.

Gastrulation in Snails is accomplished primarily by epiboly, wherein the micromeres at the animal cap multiply and "overgrow" the vegetal macromeres.

Eventually, the micromeres will cover the entire embryo, leaving a small slit at the vegetal pole.



In the typical cleavage of molluscs, cells divides to produce most of the larval structures, including a gland capable of producing a large shell.



Shell formation during gastrulation in snails

The **Shell gland** is an ectodermal organ formed through induction by mesodermal cells. Without the mesoderm, no cells are present to induce the competent ectoderm. Here we see an example of limited induction within a mosaic embryo.

The resulting larvae (called **glochidia**) resemble tiny bear traps; they have sensitive hairs that cause the valves of the shell to snap shut when they are touched by the gills or fins of a wandering fish. They attach themselves to the fish and "hitchhike" with it until they are ready to drop off and metamorphose into adult clams. In this manner, they can spread upstream.

TOPIC 79.80. Early Development in Tunicates Tunicate Cleavage AND Blastula formation in Tunicate

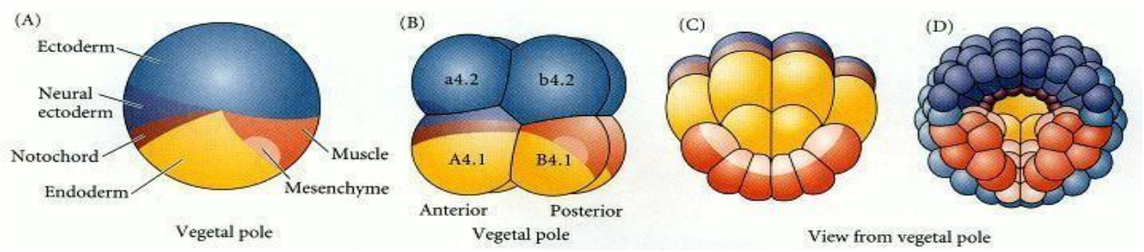
Early Development in Tunicates

Tunicate Cleavage

Ascidians, members of the tunicate subphylum, are fascinating animals for several reasons, but the foremost is that they are invertebrate chordates. They have a notochord as larvae (and therefore are chordates), but they lack vertebrae.

As larvae, they are free-swimming tadpoles; but when the tadpole undergoes metamorphosis, it sticks to the sea floor, its nerve cord and notochord degenerate, and it secrete a cellulose tunic (which gave the name "tunicates" to these creatures).

These animals are characterized by **bilateral holoblastic cleavage**, a pattern found primarily in tunicates.



Blastula formation in Tunicate

Each successive division orients itself to this plane of symmetry, and the half-embryo formed on one side of the first cleavage plane is the mirror image of the half-embryo on the other side. The second cleavage is meridional, like the first, but unlike the first division, it does not pass through the centre of the egg.

Rather, it creates two large anterior cells (the A and D blastomeres) and two smaller posterior cells (blastomeres B and C). Each side now has a large and a small blastomere.

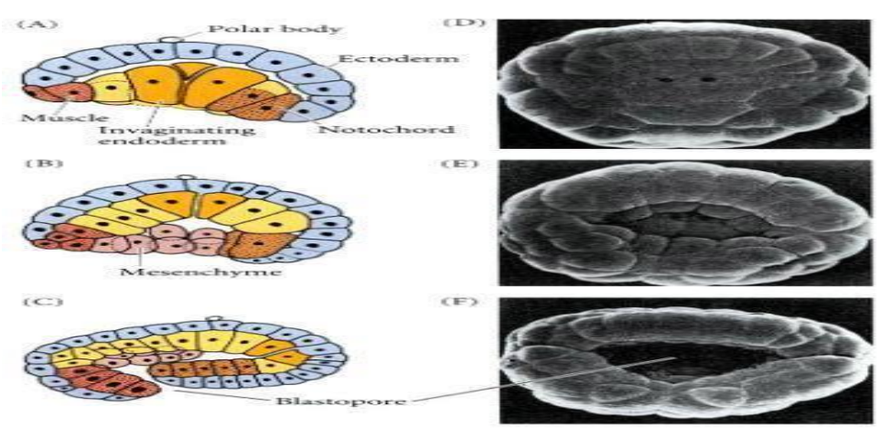
During the next three divisions, differences in cell size and shape highlight the bilateral symmetry of these embryos. At the 32-cell stage, a small blastocoel is formed, and gastrulation begins.

TOPIC 81.82. Gastrulation in Tunicates

Gastrulation in Tunicates

Tunicate gastrulation is characterized by the invagination of the endoderm, the involution of the mesoderm, and the epiboly of the ectoderm. About 4 5 hours after fertilization, the vegetal (endoderm) cells assume a wedge shape, expanding their apical margins and contracting near their vegetal margins.

Pairs of blastomeres appear to lead this invagination into the centre of the embryo. This invagination forms a blastopore whose lips will become the mesodermal cells.



The presumptive notochord cells are now on the anterior portion of the blastopore lip, while the presumptive tail muscle cells (from the yellow crescent) are on the posterior lip of the blastopore.

The lateral lips of the blastopore comprise those cells that will become mesenchyme.

The second step of gastrulation involves the involution of the mesoderm. The presumptive mesoderm cells involute over the lips of the blastopore, and by migrating upon the basal surfaces of the ectodermal cells, move inside the embryo.

The dorsal ectodermal cells that are the precursors of the neural tube invaginate into the embryo and are enclosed by neural folds. This forms the neural tube, which will form a brain anteriorly and a spinal chord posteriorly.

Meanwhile, the presumptive notochord cells on the right and left sides of the embryo migrate to the midline and interdigitate to form the notochord, a single row of 40 cells. The muscle cells of the tail differentiate on either side of the neural tube and notochord.

TOPIC 83.84. Summary Early Invertebrate Development AND Summary of Invertebrate cleavage

Summary

Early Invertebrate Development

- During cleavage, most cells do not grow. Rather, the volume of the oocyte is cleaved into numerous cells. The major exceptions to this rule are mammals.
- The blastomere cell cycle is governed by the synthesis and degradation of cyclin. Cyclin synthesis promotes the formation of MPF, and MPF promotes mitosis.
- A blastomere is a cell derived from cleavage in an early embryo. A blastula is an embryonic structure composed of blastomeres. The cavity in the blastula is the blastocoel. A mammalian blastula is called a blastocyst.
- The movements of gastrulation include invagination, involution, ingression, delamination, and epiboly.
- Three axes are the foundations of the body: the anterior-posterior axis (head to tail or mouth to anus), the dorsal-ventral axis (back to belly), and the right-left axis (between the two lateral sides of the body).
- **Summary of Invertebrate cleavage**
- In all four invertebrates described here, cleavage is holoblastic. In the sea urchin, cleavage is radial; in the snail, spiral; in the tunicate, bilateral; and in the nematode, rotational.
- In the tunicate, snail, and nematode, gastrulation occurs when there are relatively few cells, and the blastopore becomes the mouth. This is the protostome mode of gastrulation.
- In the sea urchin, gastrulation occurs only after thousands of cells have formed, and the blastopore becomes the anus. This is the deuterostome mode of gastrulation, and is characteristic only of echinoderms and chordates.
- In sea urchins, cell fates are determined by signaling. The micromeres constitute a major signaling center. β-catenin is important for the inducing capacity of the micromeres.
- In the sea urchin, gastrulation occurs only after thousands of cells have formed, and the blastopore becomes the anus. This is the deuterostome mode of gastrulation, and is characteristic only of echinoderms and chordates.

- In sea urchins, cell fates are determined by signalling. The micromeres constitute a major signalling center. β-catenin is important for the inducing capacity of the micromeres.

TOPIC 85. 86. Cleavage in Fish

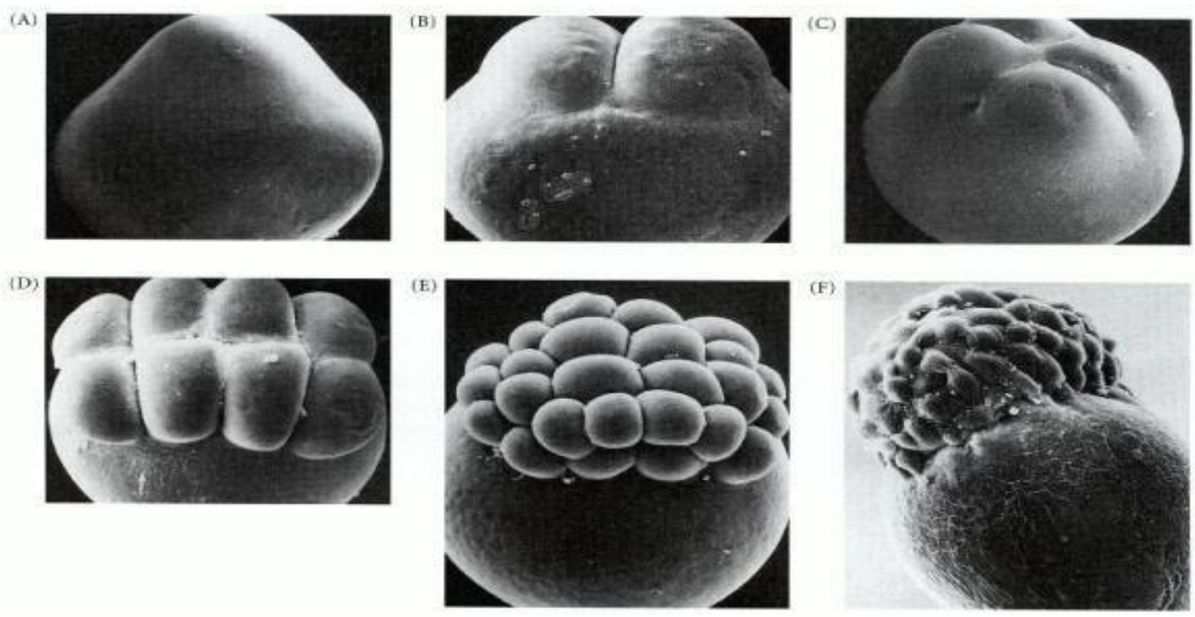
Early Development of Vertebrates

Cleavage in Fishes

In fish eggs, cleavage occurs only in the **blastodisc**, a thin region of yolk-free cytoplasm at the animal cap of the egg. Most of the egg cell is full of yolk. The cell divisions do not completely divide the egg, so this type of cleavage is called **meroblastic.**

Since only the cytoplasm of the blastodisc becomes the embryo, this type of meroblastic cleavage is called **discoidal**. The calcium waves initiated at fertilization stimulate the contraction of the actin cytoskeleton to squeeze non-yolky cytoplasm into the animal pole of the egg.

Scanning electron micrographs show beautifully the incomplete nature of discoidal meroblastic cleavage in fish eggs.

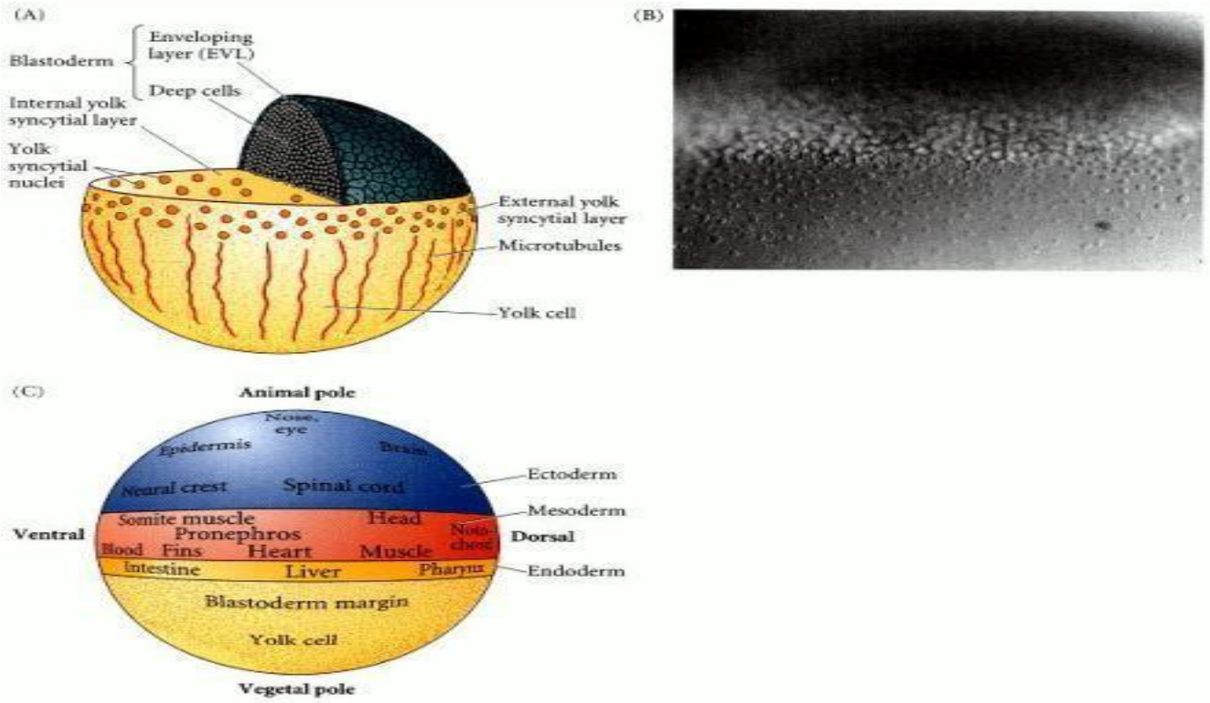


These divisions are rapid, taking about 15 minutes each. The first 12 divisions occur synchronously, forming a mound of cells that sits at the animal pole of a large **yolk cell**. These cells constitute the **blastoderm**.

Three distinct cell populations can be distinguished. The first of these is the **yolk syncytial layer** (**YSL**). The YSL is formed at the ninth or tenth cell cycle, when the cells at the vegetal edge of the blastoderm fuse with the underlying yolk cell.

Later, as the blastoderm expands vegetally to surround the yolk cell, some of the yolk syncytial nuclei will move under the blastoderm to form the **internal YSL**, and some of the nuclei will move vegetally, staying ahead of the blastoderm margin, to form **external YSL.**

The second cell population distinguished at the midblastula transition is the **enveloping layer** (**EVL).**



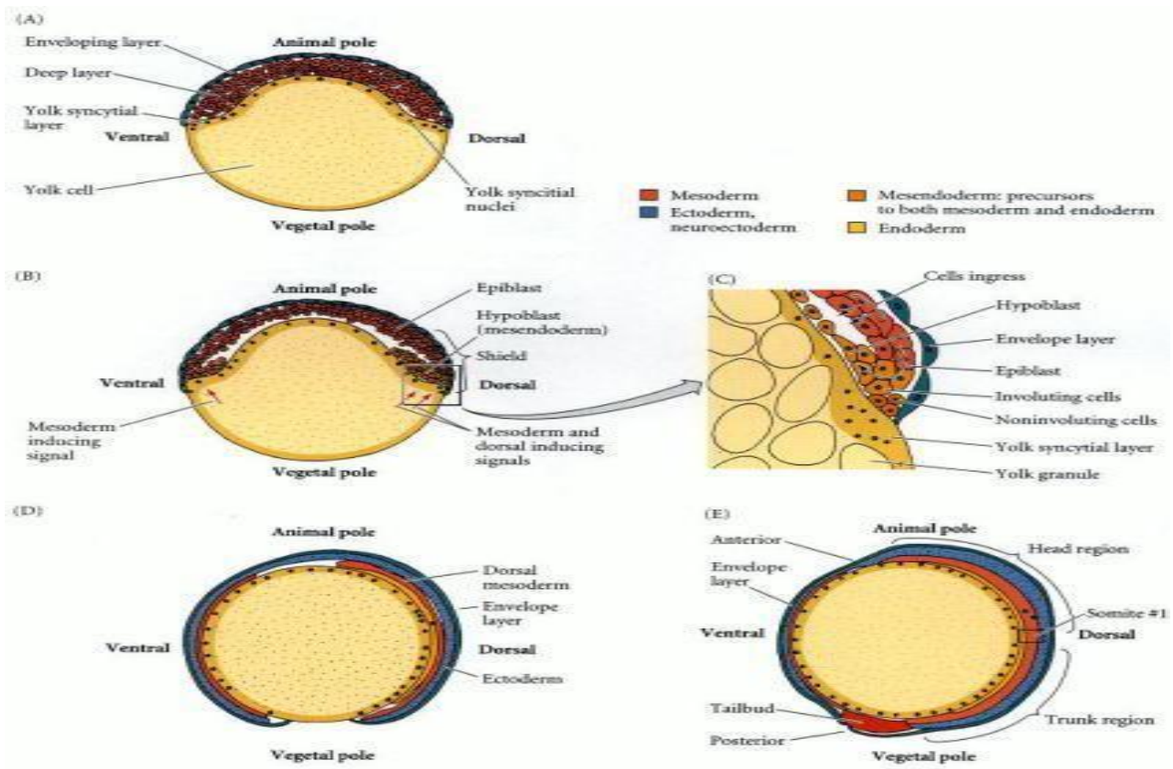
It is made up of the most superficial cells of the blastoderm, which form an epithelial sheet a single cell layer thick. The EVL eventually becomes the **periderm**, an extra-embryonic protective covering that is sloughed off during later development.

Between the EVL and the YSL are the **deep cells**. These are the cells that give rise to the embryo proper. The fates of the early blastoderm cells are not determined, and cell lineage studies show that there is much cell mixing during cleavage.

TOPIC 87.88. Early Gastrulation in Fish Embryos AND Layers formation during Gastrulation in Fish Embryos

Gastrulation in Fish Embryos

The first cell movement of fish gastrulation is the epiboly of the blastoderm cells over the yolk.



This movement is not due to the active crawling of the blastomeres, however. Rather, the movement is provided by the autonomously expanding YSL "within" the animal pole yolk cytoplasm.The EVL is tightly joined to the YSL and is dragged along with it. The deep cells of the blastoderm then fill in the space between the **YSL** and the **EVL** as epiboly proceeds. This can be demonstrated by severing the attachments between the **YSL** and the **EVL.** When this is done, the **EVL** and deep cells spring back to the top of the yolk, while the **YSL** continues its expansion around the yolk cell. The expansion of the **YSL** depends on a network of microtubules in the **YSL**, and radiation or drugs that block the polymerization of tubulin inhibit epiboly

During migration, one side of the blastoderm becomes noticeably thicker than the other. Celllabeling experiments indicate that the thicker side marks the site of the future dorsal surface of the embryo

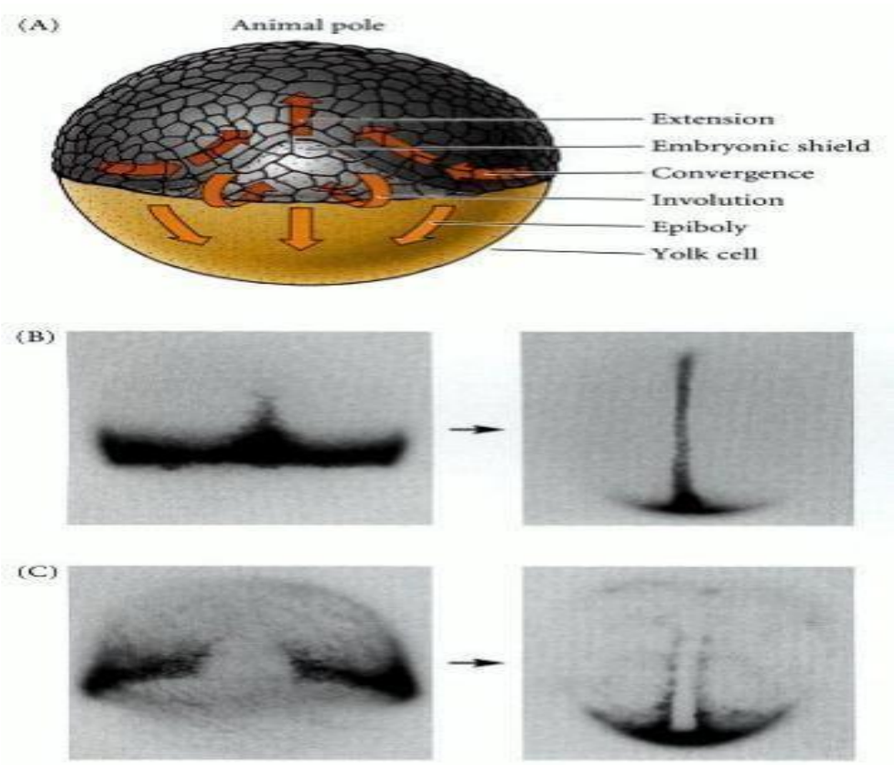
After the blastoderm cells have covered about half the zebra fish yolk cell, a thickening occurs throughout the margin of the epibolizing blastoderm. This thickening is called the **germ ring**, and it is composed of a superficial layer, the **epiblast**, and an inner layer, the **hypoblast**.

TOPIC 89. Gastrulation in Fish Embryos II

Gastrulation in Fish Embryos II

After the blastoderm cells have covered about half the zebra fish yolk cell, a thickening occurs throughout the margin of the epibolizing blastoderm. This thickening is called the **germ ring**, and it is composed of a superficial layer, the **epiblast**, and an inner layer, the **hypoblast**.

The cells of both the epiblast and hypoblast intercalate on the future dorsal side of the embryo to form a localized thickening, the **embryonic shield.**



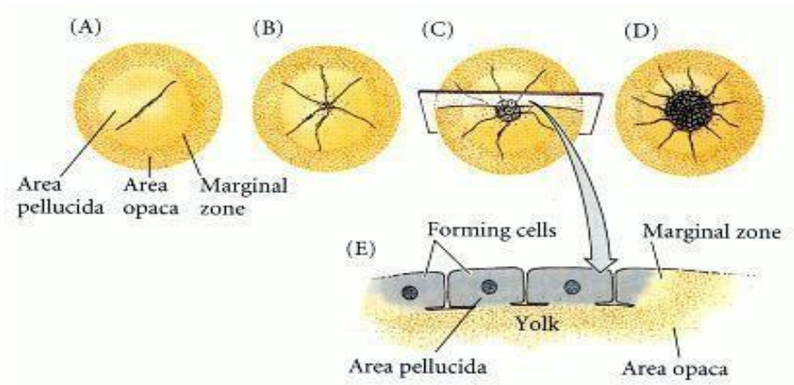
The hypoblast cells of the embryonic shield converge and extend anteriorly, eventually narrowing along the dorsal midline of the hypoblast. This movement forms the **chorda mesoderm**, the precursor of the **notochord.** The cells adjacent to the chordamesoderm, the **paraxial mesoderm** cells, are the precursors of the mesodermal somites.

The concomitant convergence and extension in the epiblast brings the presumptive neural cells from all over the epiblast into the dorsal midline, where they form the **neural keel**. The rest of the epiblast becomes the skin of the fish.

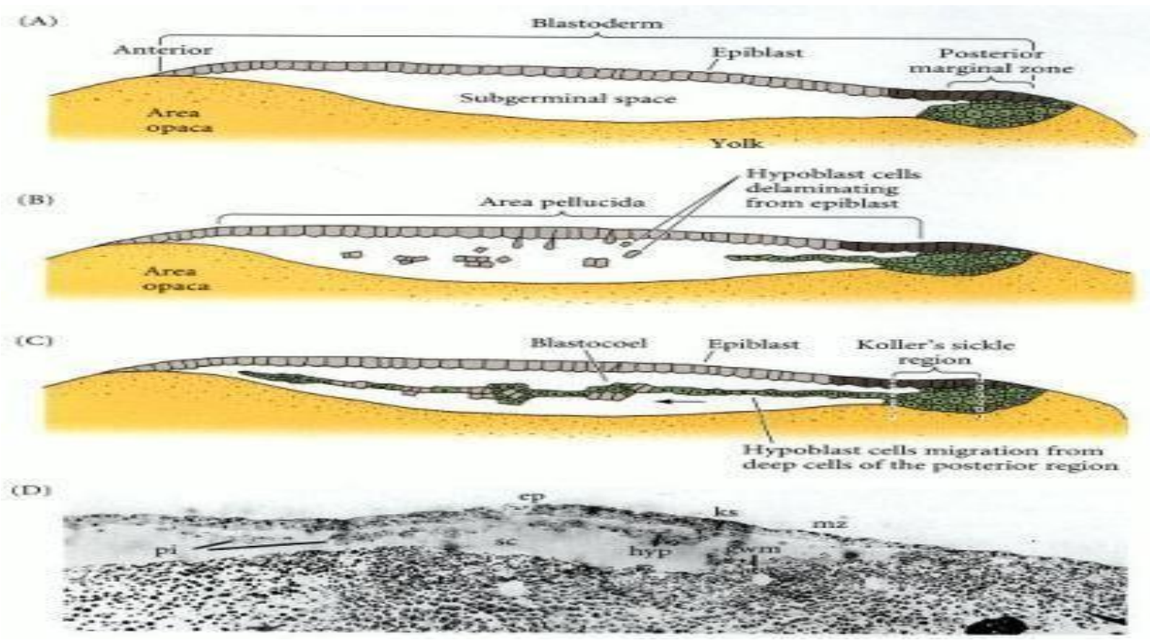
TOPIC 90. 91. Cleavage in Bird Eggs

Cleavage in Bird Eggs

Fertilization of the chick egg occurs in the oviduct, before the albumen and the shell are secreted upon it. The egg is telo-lecithal (like that of the fish),with a small disc of cytoplasm sitting atop a large yolk.Cleavage occurs only in the blastodisc, a small disc of cytoplasm 2-3 mm in diameter at the animal pole of the egg cell. The first cleavage furrow appears centrally in the blastodisc, and other cleavages follow to create a single-layered blastoderm.



Between the blastoderm and the yolk is a space called the **sub-germinal cavity**. This space is created when the blastoderm cells absorb fluid from the albumin ("egg white") and secrete it between themselves and the yolk. At this stage, the deep cells in the center of the blastoderm are shed and die, leaving behind a onecell- thick **area pellucida**. This part of the blastoderm forms most of the actual embryo. The peripheral ring of blastoderm cells that have not shed their deep cells constitutes the **area opaca**.Between the area pellucida and the area opaca is a thin layer of cells called the **marginal zone** (or **marginal belt**).

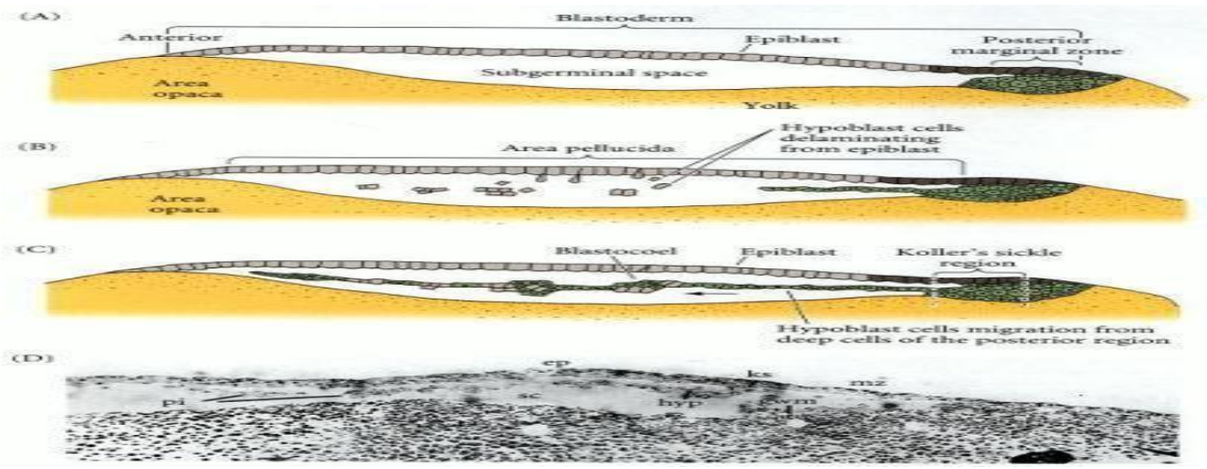


TOPIC 92.93. Gastrulation of the Avian Embryo

Gastrulation of the Avian Embryo

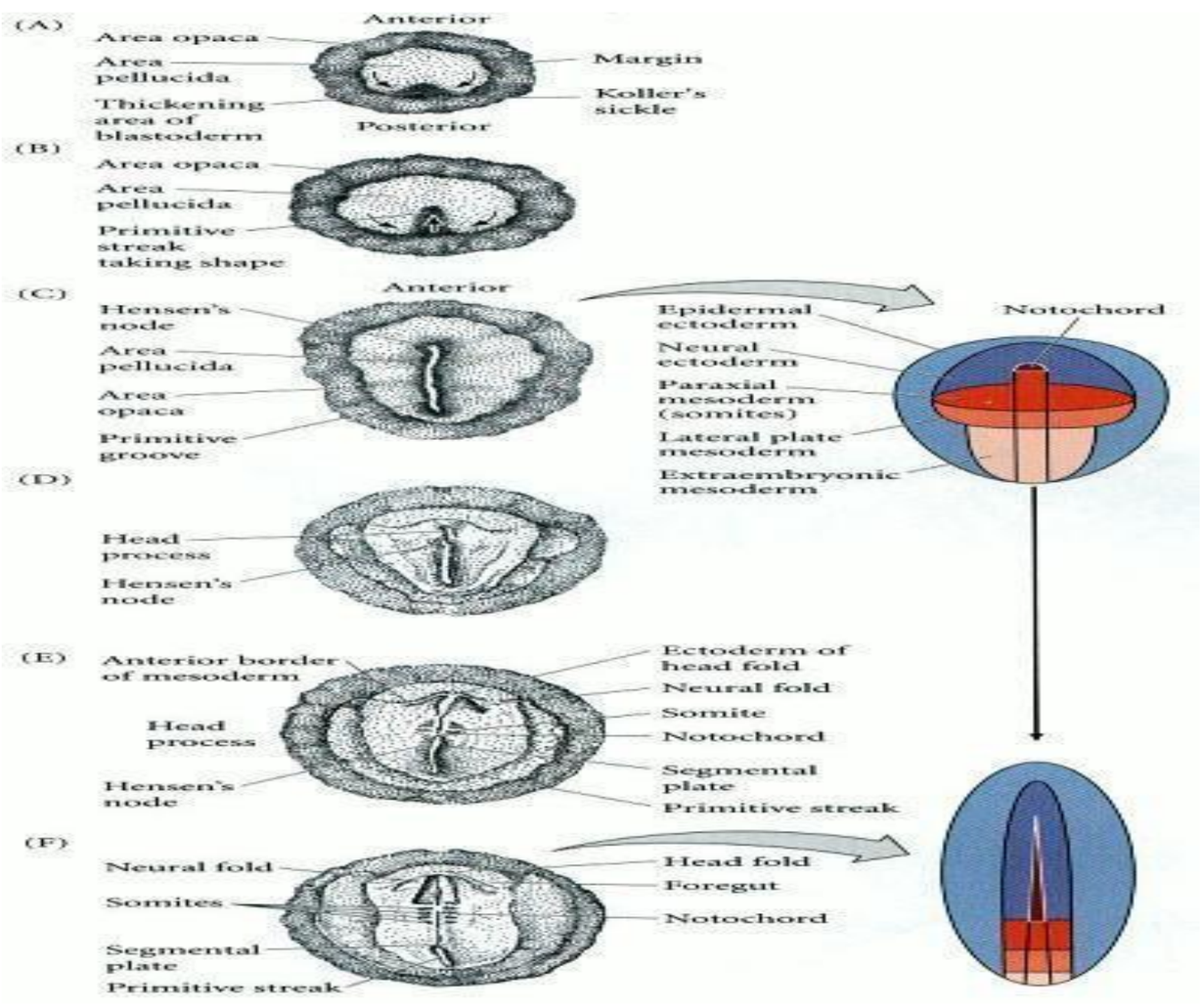
The hypoblast

Area pellucida cells migrate individually into the subgerminal cavity to form the polyinvagination islands (primary hypoblast).



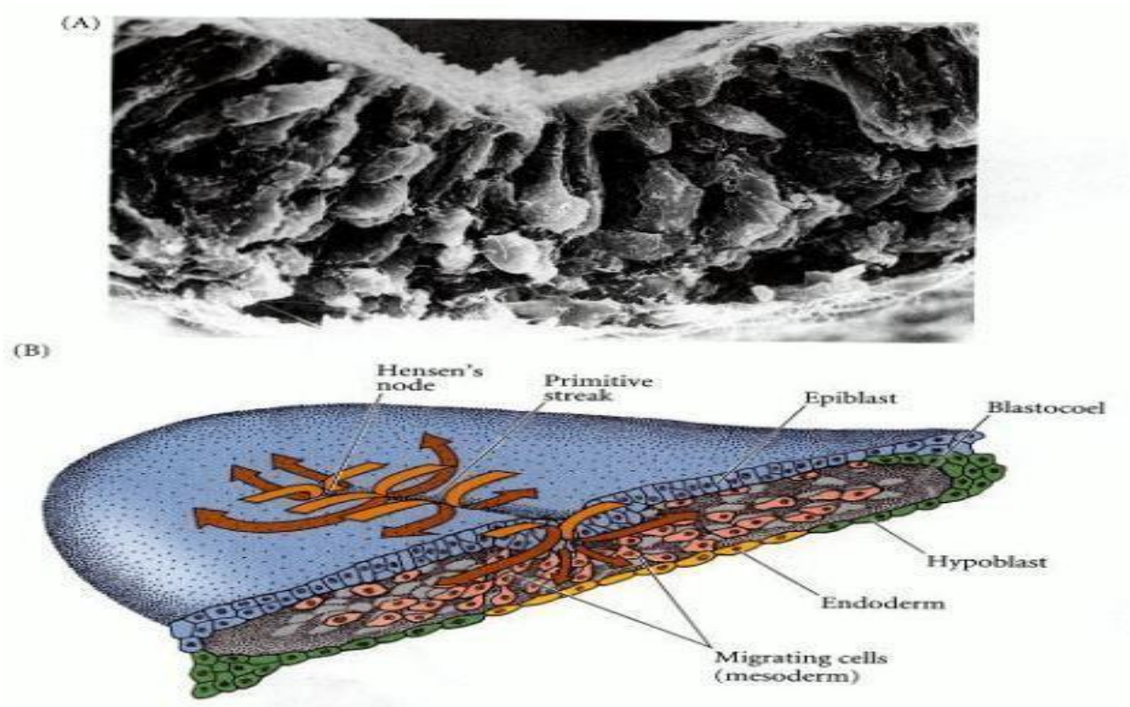
Shortly thereafter, a sheet of cells from the *posterior* margin of the blastoderm (distinguished from the other regions of the margin by **Koller's sickle**, a local thickening) migrates anteriorly to join the polyinvagination islands, thereby forming the **secondary hypoblast.**

The two-layered blastoderm (epiblast and hypoblast) is joined together at the margin of the area opaca, and the space between the layers forms a blastocoel.



The primitive streak

The major structural characteristic of avian, reptilian, and mammalian gastrulation is the **primitive streak**. This streak is first visible as a thickening of the epiblast at the posterior region of the embryo, just anterior to Koller's sickle As cells converge to form the primitive streak, a depression forms within the streak. This depression is called the **primitive groove**, and it serves as an opening through which migrating cells pass into the blastocoel.

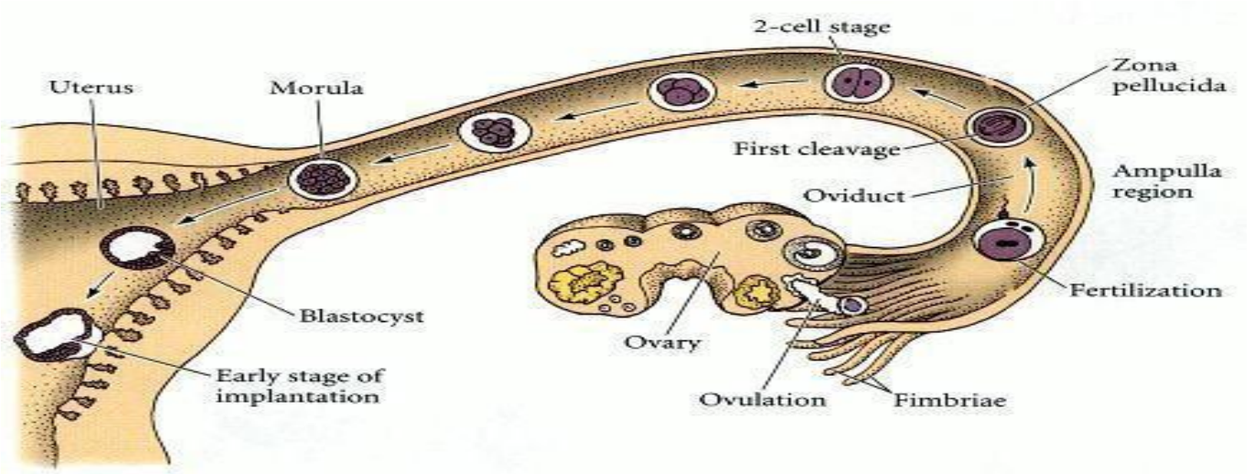


At the anterior end of the primitive streak is a regional thickening of cells called the **primitive knot** or **Hensen's node**. The center of this node contains a funnel-shaped depression (sometimes called the **primitive pit**) through which cells can pass into the blastocoel. Cells migrating through **Hensen's node** pass down into the blastocoel and migrate anteriorly, forming foregut, head mesoderm, and notochord; cells passing through the lateral portions of the primitive streak give rise to the majority of endodermal and mesodermal tissues.

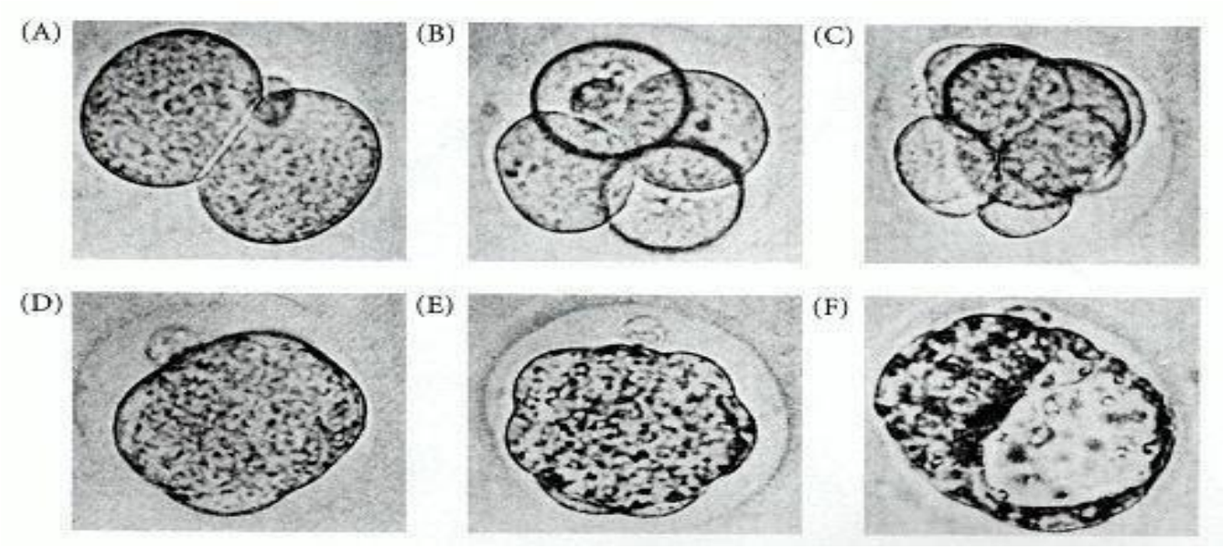
TOPIC 94.95.96. Cleavage in Mammals

Cleavage in Mammals

The mammalian oocyte is released from the ovary and swept by the fimbriae into the oviduct. Fertilization occurs in the ampulla of the oviduct, a region close to the ovary. Meiosis is completed at this time, and first cleavage begins about a day later. Cleavages in mammalian eggs are among the slowest in the animal kingdom about 12-24 hours apart. Meanwhile, the cilia in the oviduct push the embryo toward the uterus; the first cleavages occur along this journey.



The most crucial, difference between mammalian cleavage and all other types involves the phenomenon of **compaction**.



The cells of the compacted 8-cell embryo divide to produce a 16-cell **morula**. The morula consists of a small group of internal cells surrounded by a larger group of external cells.

Most of the descendants of the external cells become the **trophoblast** (**trophectoderm**) cells. This group of cells produces no embryonic structures. Rather, it forms the tissue of the **chorion**, the embryonic portion of the **placenta**.

These cells generate the **inner cell mass** (**ICM**), which will give rise to the embryo and its associated yolk sac, allantois, and amnion. By the 64-cell stage, the inner cell mass (approximately 13 cells) and the trophoblast cells have become separate cell layers, neither contributing cells to the other group. During a process called **cavitation**, the trophoblast cells secrete fluid into the morula to create a blastocoel. The inner cell mass is positioned on one side of the ring of trophoblast cells. The resulting structure, called the **blastocyst**, is another hallmark of mammalian cleavage.

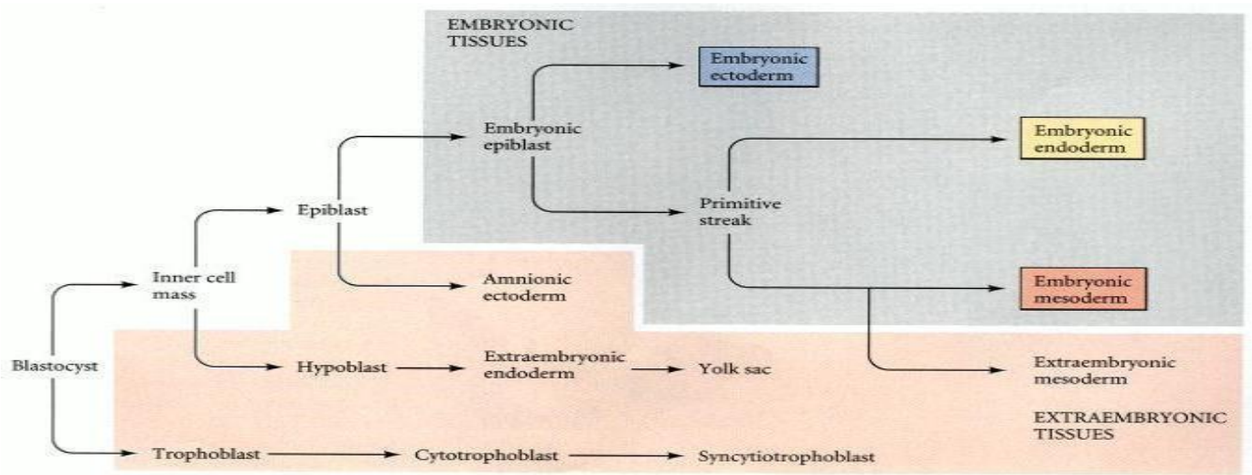
Once out, the blastocyst can make direct contact with the uterus. The uterine epithelium (**endometrium**) "catches" the blastocyst on an extracellular matrix containing collagen, laminin, fibronectin, hyaluronic acid, and heparin sulfate receptors.

TOPIC 97 TO 100. Gastrulation in Mammals

Gastrulation in Mammals

The mammalian embryo obtains nutrients directly from its mother and does not rely on stored yolk. This adaptation has entailed a dramatic restructuring of the maternal anatomy (such as expansion of the oviduct to form the uterus) as well as the development of a fetal organ capable of absorbing maternal nutrients.This fetal organ the **Chorion** is derived primarily from embryonic trophoblast cells, supplemented with mesodermal cells derived from the inner cell mass. The chorion forms the fetal portion of the placenta.

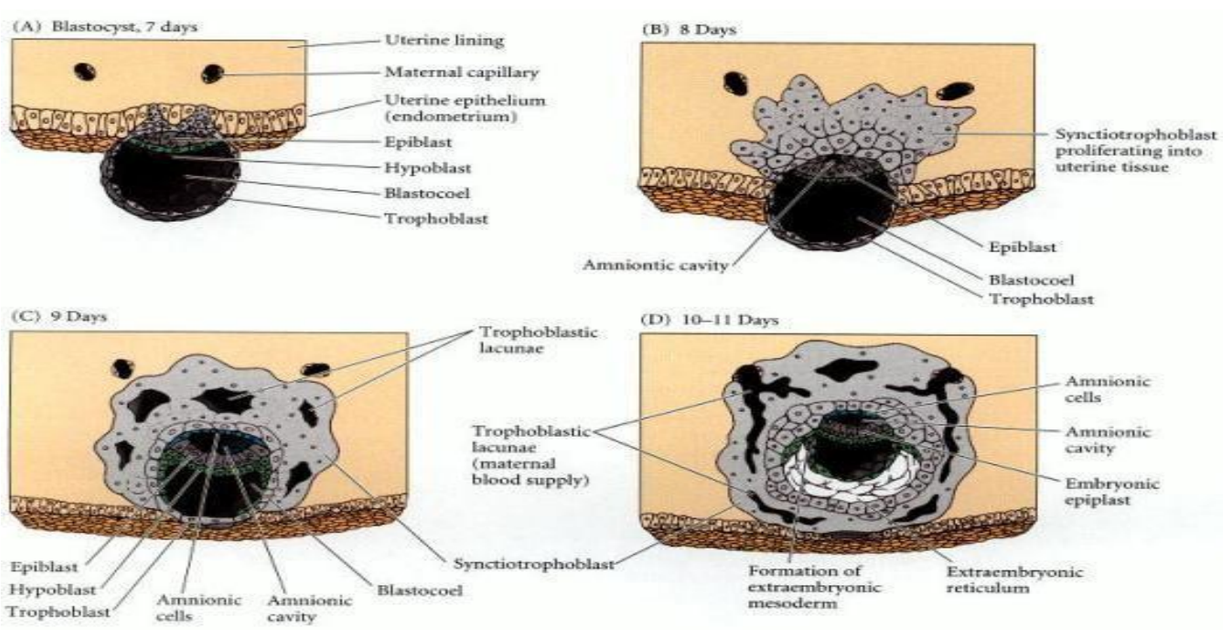
It will induce the uterine cells to form the maternal portion of the placenta, the **decidua**. The decidua becomes rich in the blood vessels that will provide oxygen and nutrients to the embryo.



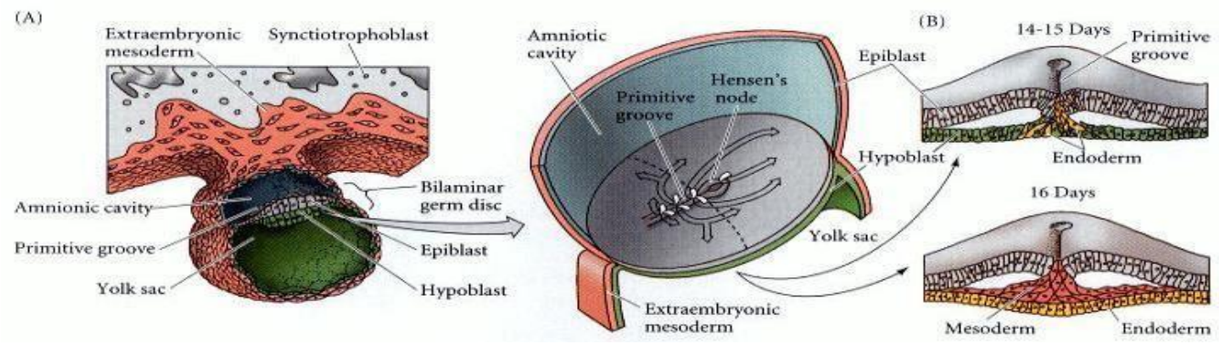
The first segregation of cells within the inner cell mass results in the formation of the hypoblast (sometimes called the **primitive endoderm**) layer. The hypoblast cells delaminate from the inner cell mass to line the blastocoel cavity, where they give rise to the **extra-embryonic endoderm**, which forms the yolk sac.

The epiblast cell layer is split by small clefts that eventually coalesce to separate the **embryonic epiblast** from the other epiblast cells, which form the **amnionic cavity**. Once the lining of the amnion is completed, it fills with a secretion called **amnionic** (**amniotic**) **fluid**, which serves as a shock absorber for the developing embryo while preventing its desiccation.

Gastrulation begins at the posterior end of the embryo, and this is where the **node** forms.



Those cells migrating through the node give rise to the notochord.



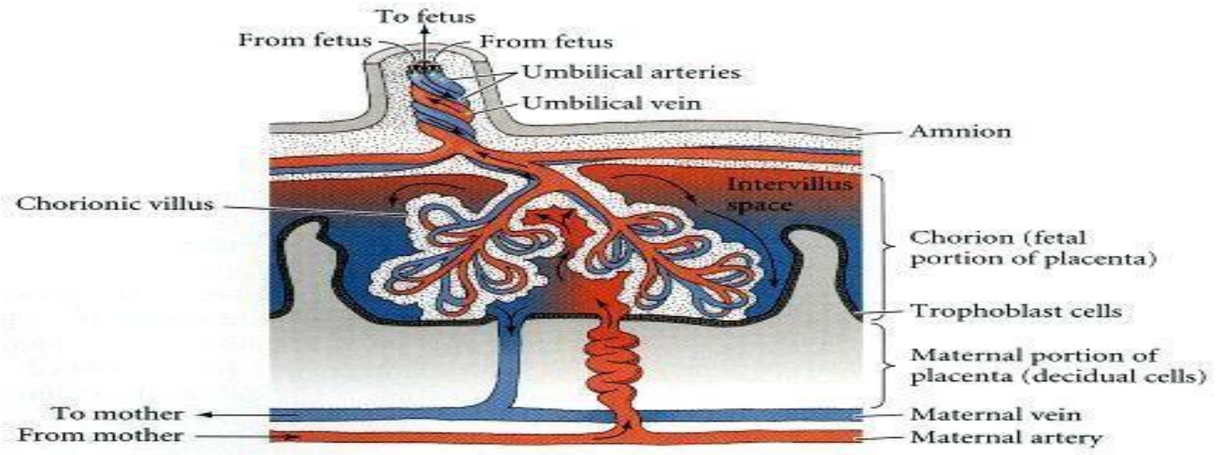
<u>Gastrulation in Mammals II</u> I

The original type of trophoblast cells constitute a layer called the **cytotrophoblast**, whereas the multinucleated type of cell forms the **syncytiotrophoblast**. The cytotrophoblast initially adheres to the endometrium through a series of adhesion molecules.

The extra-embryonic mesoderm joins the trophoblastic extensions and gives rise to the blood vessels that carry nutrients from the mother to the embryo. The narrow connecting stalk of extraembryonic mesoderm that links the embryo to the trophoblast eventually forms the vessels of the **umbilical cord**.

The fully developed extra-embryonic organ, consisting of trophoblast tissue and blood vesselcontaining mesoderm, is called the chorion, and it fuses with the uterine wall to create the placenta. Thus, the placenta has both a maternal portion (the uterine endometrium, which is modified during pregnancy) and a fetal component (the chorion).

Figure shows the relationships between the embryonic and extra-embryonic tissues of a 6-week human embryo. The embryo is seen encased in the amnion and is further shielded by the chorion.



The blood vessels extending to and from the chorion are readily observable, as are the villi that project from the outer surface of the chorion. These villi contain the blood vessels and allow the chorion to have a large area exposed to the maternal blood.Mother provides the fetus with nutrients and oxygen, and the fetus sends its waste products (mainly carbon dioxide and urea) into the maternal circulation.The maternal and fetal blood cells, however, usually do not mix.

TOPIC 101 TO 104 . Summary The Early Development of Vertebrates

Summary

The Early Development of Vertebrates

- Fishes, reptiles, and birds undergo discoidal meroblastic cleavage, wherein the early cell divisions do not cut through the yolk of the egg. These cells form a blastoderm.
- In fishes, the deep cells form between the yolk syncytial layer and the enveloping layer. These cells migrate over the top of the yolk, forming the hypoblast and epiblast layers.
- There appear to be two signaling centers supplying anterior-posterior information in fishes, one located at the border between the neural and surface ectoderm, the other in the lateral mesoderm.
- In chick embryos, early cleavage forms an area opaca and an area pellucida. The region between them is the marginal zone. Gastrulation begins at the posterior marginal zone, as the hypoblast and primitive streak both start there.
- The primitive streak is derived from anterior epiblast cells and the central cells of the posterior marginal zone. As the primitive streak extends rostrally, Hensen's node is formed. Cells migrating through Hensen's node become chordamesoderm (notochord) cells. These extend up to the presumptive midbrain, where they meet the prechordal plate.
- The **prechordal plate** induces the formation of the forebrain; the **chordamesoderm** induces the formation of the midbrain, hindbrain, and spinal cord. The first cells migrating laterally through the primitive streak become endoderm, displacing the hypoblast.
- In birds, gravity is critical in determining the anterior-posterior axis, while pH differences appear crucial for distinguishing dorsal from ventral. The left-right axis is formed by the expression of *nodal* on the left side of the embryo.
- Mammals undergo holoblastic rotational cleavage, characterized by a slow rate of division, a unique cleavage orientation, lack of divisional synchrony, and the formation of a blastocyst.
- The blastocyst forms after the blastomeres undergo compaction. It contains outer cells the trophoblast cells that become the chorion, and an inner cell mass that becomes the amnion and the embryo.
- The chorion forms the fetal portion of the placenta, which functions to provide oxygen and nutrition to the embryo, to provide hormones for the maintenance of pregnancy, and to provide barriers to the mother's immune system.
- Mammalian gastrulation is not unlike that of birds. There appear to be two signaling centers one in the node and one in the anterior visceral endoderm. The latter is critical for generating the forebrain, while the former is critical in inducing the axial structures caudally from the midbrain.
- Hox genes pattern the anterior-posterior axis and help to specify positions along that axis. If Hox genes are knocked out, segment-specific malformations can arise. Similarly, causing the ectopic expression of Hox genes can alter the body axis.
- The homology of gene structure and the similarity of expression patterns between *Drosophila* and mammalian **Hox genes** suggests that this patterning mechanism is extremely ancient.

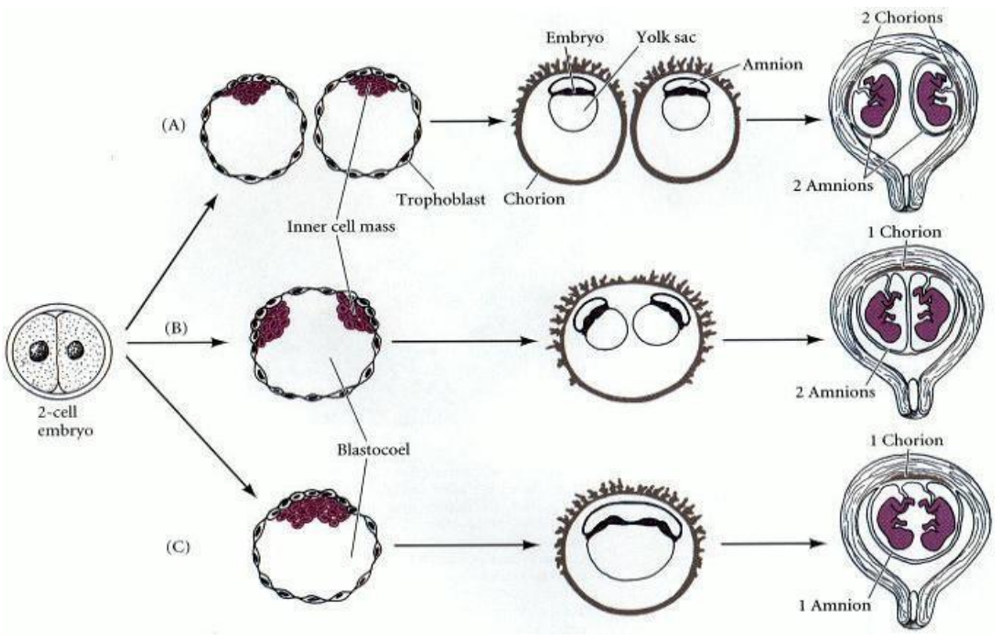
TOPIC 105.106. Development of Twins

Development of Twins

Each ICM (**Inner Cell Mass**) cell has the same potency (in this case, each cell can give rise to all the cell types of the embryo, but not to the trophoblast), and their fates will be determined by interactions among their descendants. The regulative capacity of the ICM blastomeres is also seen in humans. Human twins are classified into two major groups: **monozygotic** (one-egg, or identical) twins and **dizygotic** (two egg, or fraternal) twins.

Fraternal twins are the result of two separate fertilization events, whereas identical twins are formed from a single embryo whose cells somehow dissociated from one another.

Identical twins occur in roughly **0.25%** of human births. About **33%** of identical twins have two complete and separate chorions, indicating that separation occurred before the formation of the trophoblast tissue at **day 5.**

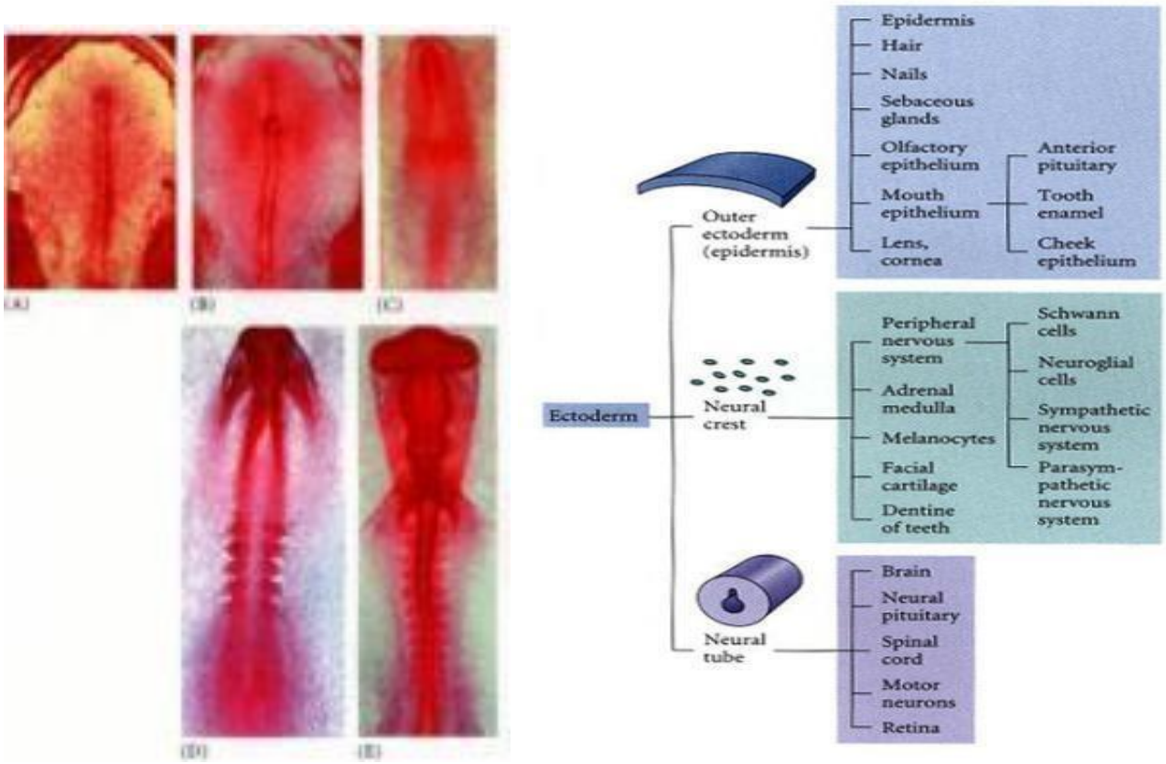


The remaining identical twins share a common chorion, suggesting that the split occurred within the inner cell mass after the trophoblast formed. By day 9, the human embryo has completed the construction of another extraembryonic layer, the **lining of the amnion.** This tissue forms the **amnionic sac** (or water sac), which surrounds the embryo with amnionic fluid and protects it from desiccation and abrupt movement. If the separation of the embryo were to come after the formation of the chorion on day 5 but before the formation of the amnion on day 9, then the resulting embryos should have one chorion and two amnions.

TOPIC 107.108. Development of central nervous system AND Development of central nervous system

Development of central nervous system

The fates of the vertebrate ectoderm is shown in figure.



A portion of the dorsal ectoderm is specified to become neural ectoderm, and its cells become distinguishable by their columnar appearance. This region of the embryo is called the **neural plate**. The process by which this tissue forms a **neural tube**.The rudiment of the central nervous system, is called **neurulation**, and an embryo undergoing such changes is called a **neurula**. The neural tube will form the brain anteriorly and the spinal cord.

Formation of the Neural Tube

There are two major ways of forming a neural tube

- **Primary neurulation**
- **Secondary neurulation**

  - In **primary neurulation**, the cells surrounding the neural plate direct the neural plate cells to proliferate, invaginate, and pinch off from the surface to form a hollow tube.
  - In **secondary neurulation**, the neural tube arises from a solid cord of cells that sinks into the embryo and subsequently hollows out to form a hollow tube. **Neurulation in fishes is exclusively secondary.**
  - In **amphibians**, such as ***Xenopus***, most of the tadpole neural tube is made by **primary neurulation**, but the tail neural tube is derived from **secondary neurulation**.
    - In **mice** (and probably **humans,** too), **secondary neurulation** begins at or around the level of **somite 35**.

TOPIC 109. Events in Primary Neurulation

Primary neurulation

During primary neurulation, the original ectoderm is divided into three sets of cells.

- The internally positioned neural tube, which will form the brain and spinal cord.
- The externally positioned epidermis of the skin.

- The neural crest cells. The neural crest cells form in the region that connects the neural tube and epidermis, but then migrate elsewhere; they will generate the peripheral neurons and glia, the pigment cells of the skin, and several other cell types.

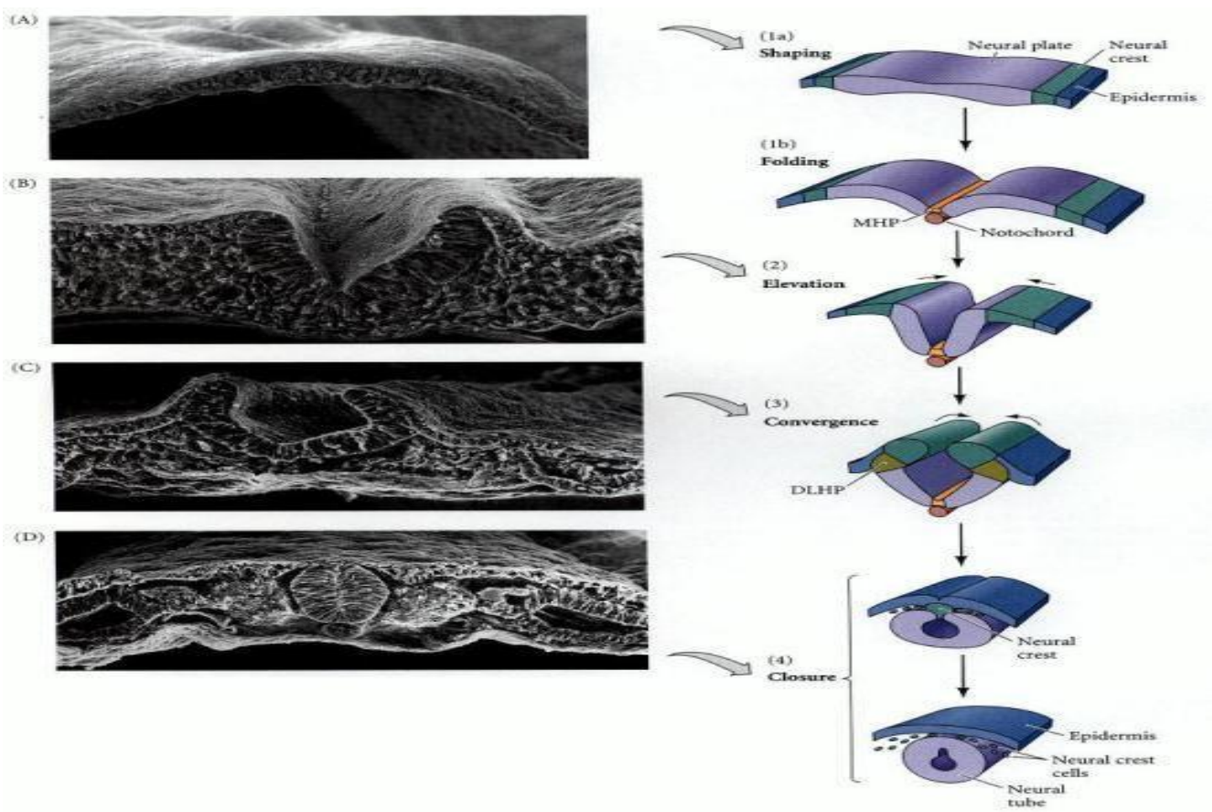
TOPIC 110. Formation of neural groove during Primary Neurulation

Shortly after the neural plate has formed, its edges thicken and move upward to form the **neural folds**, while a U-shaped **neural groove** appears in the center of the plate, dividing the future right and left sides of the embryo.

- The neural folds migrate toward the midline of the embryo, eventually fusing to form the neural tube beneath the overlying ectoderm. The cells at the dorsalmost portion of the neural tube become the **neural crest** cells.

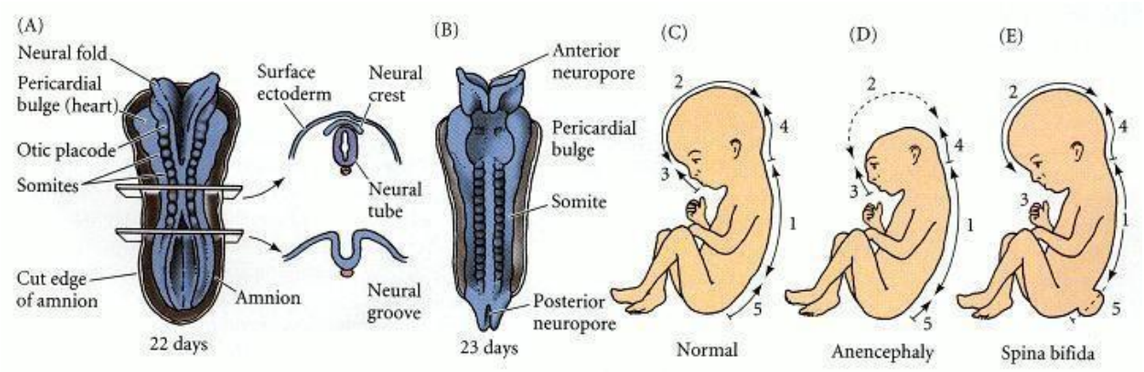
TOPIC 111.Primary Neurulation in chick and Mammals

- The events of primary neurulation in the chick are illustrated in Figure



In birds and mammals, the cells at the midline of the neural plate are called the **medial hinge point** (**MHP**) **cells**. They are derived from the portion of the neural plate just anterior to Hensen's node and from the anterior midline of Hensen's node.

Different **neural tube defects** are caused when various parts of the neural tube fail to close.



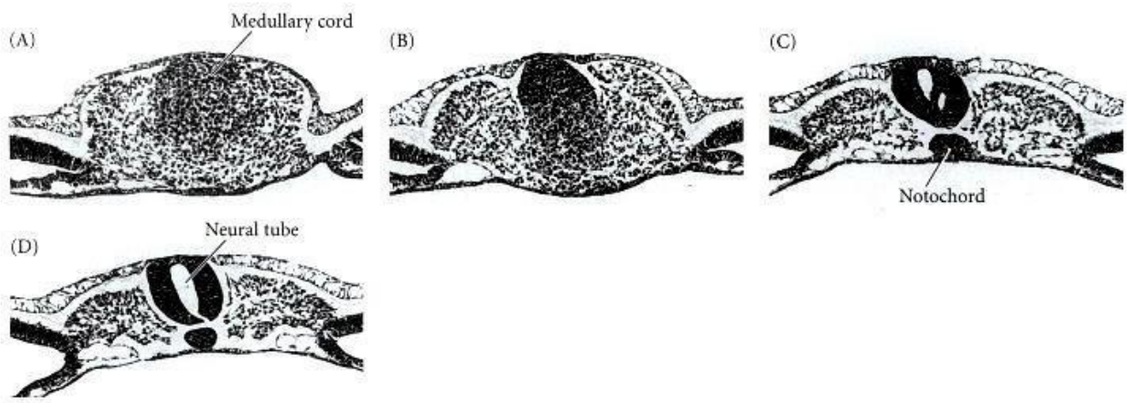
TOPIC 112.Abnormalities in Primary neurulation

Failure to close the human *posterior* neural tube regions at **day 27** (or the subsequent rupture of the posterior neuropore shortly thereafter) results in a condition called **spina bifida**, the severity of which depends on how much of the spinal cord remains exposed. Failure to close the *anterior* neural tube regions results in a lethal condition, **anencephaly**. Here, the forebrain remains in contact with the amniotic fluid and subsequently degenerates. Fetal forebrain development ceases, and the vault of the skull fails to form. The failure of the entire neural tube to close over the entire body axis is called **Craniorachischisis**. Collectively, neural tube defects are not rare in humans, as they are seen in about 1 in every 500 live births.

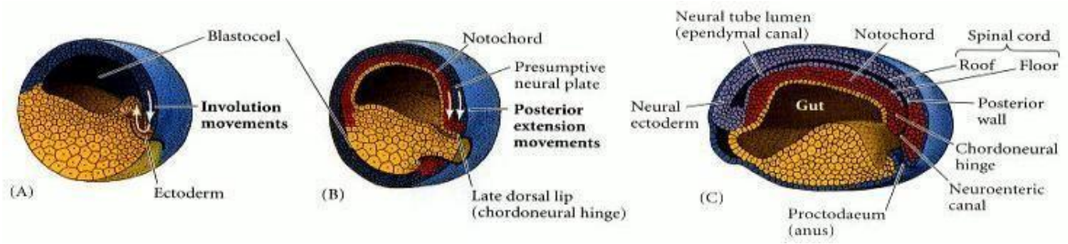
TOPIC 113.114. Secondary neurulation

Secondary neurulation

Secondary neurulation involves the making of a **medullary cord** and its subsequent hollowing into a neural tube.



In frogs and chicks, secondary neurulation is usually seen in the neural tube of the lumbar (abdominal) and tail vertebrae. In both cases, it can be seen as a continuation of gastrulation. The growing region at the tip of the lip is called the **chordoneural hinge**, and it contains precursors for both the posteriormost portion of the neural plate and the posterior portion of the notochord. The tip of the tail is the direct descendant of the dorsal blastopore lip, and the cells lining the blastopore form the **neurenteric canal**.



The proximal part of the neurenteric canal fuses with the anus, while the distal portion becomes the **ependymal canal.**

TOPIC 115. TO.118. Differentiation of the Neural Tube

Differentiation of the Neural Tube

The anterior-posterior axis

In this region, the neural tube balloons into three primary vesicles.

- Forebrain (**prosencephalon**), o Midbrain (**mesencephalon**),
- Hindbrain (**rhombencephalon**).
- The **Prosencephalon** becomes subdivided into the anterior **telencephalon** and the more caudal **diencephalon**.

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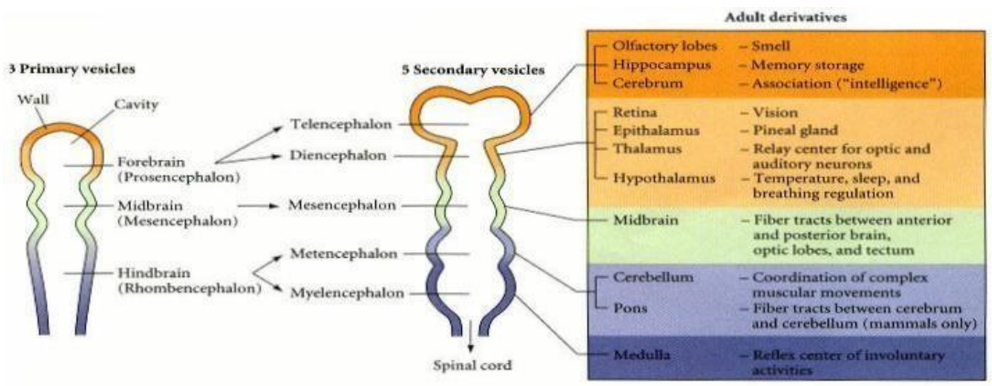


The telencephalon will eventually form the **cerebral hemispheres**, and the diencephalon will form the **thalamic and hypothalamic brain regions** that receive neural input from the retina.

The Rhombencephalon becomes subdivided into a posterior **Myelencephalon** and a more anterior **Metencephalon**. The Myelencephalon eventually becomes the **Medulla oblongata**, whose neurons generate the nerves that regulate respiratory, gastrointestinal, and cardiovascular movements

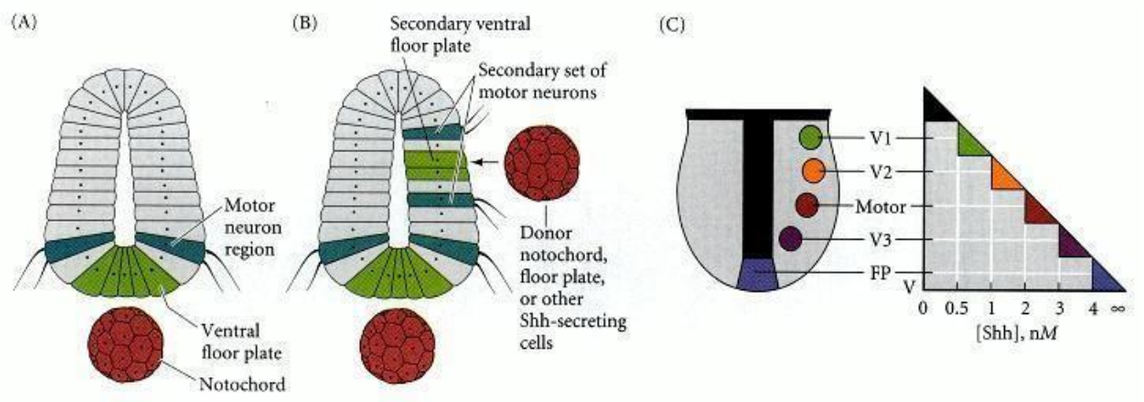
The **Metencephalon** gives rise to the cerebellum, the part of the brain responsible for coordinating movements, posture, and balance.

The dorsal-ventral axis

The neural tube is polarized along its dorsal-ventral axis. In the spinal cord, for instance, the *dorsal* region is the place where the spinal neurons receive input from sensory neurons, while the *ventral* region is where the motor neurons reside. In the middle are numerous interneurons that relay information between them. The polarity of the neural tube is induced

by signals coming from its immediate environment. The dorsal pattern is imposed by the epidermis, while the ventral pattern is induced by the notochord.

The specification of the ventral neural tube appears to be mediated by external tissues.One agent of ventral specification is the **Sonic hedgehog** protein, probably originating from the notochord. Another agent specifying the ventral neural cell types is **retinoic acid**, which probably comes from the adjacent somites.

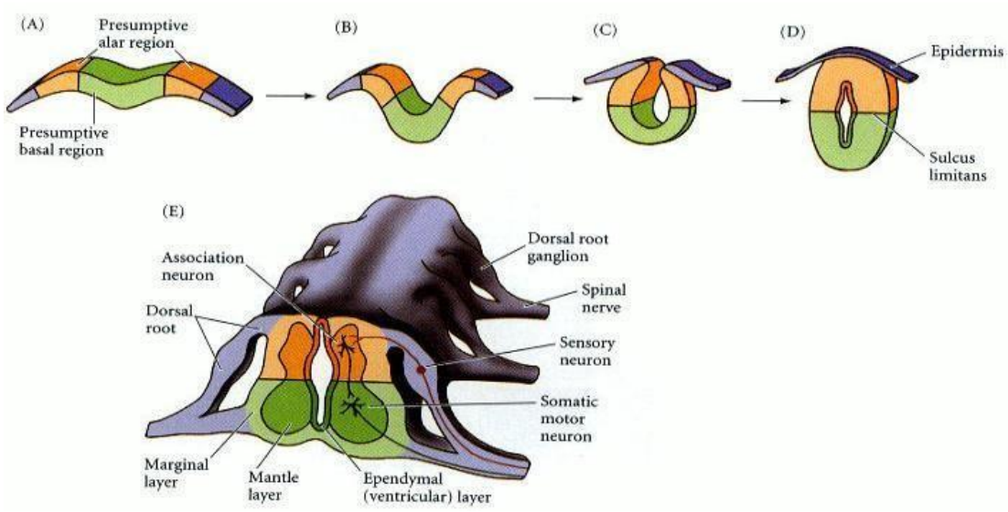


The secreted Sonic hedgehog induces the medial hinge cells to become the **floor plate** of the neural tube. These floor plate cells also secrete Sonic hedgehog, which forms a gradient highest at the most ventral portion of the neural tube. Those cells adjacent to the floor plate that receive high concentrations of Sonic hedgehog become the **ventral (V3) neurons**, while the next group of cells, exposed to slightly less Sonic hedgehog, become **motor neuron**

TOPIC 119.120.Spinal chord and medulla organization

Spinal chord and medulla organization

As the cells adjacent to the lumen continue to divide, the migrating cells form a second layer around the original neural tube. This layer becomes progressively thicker as more cells are added to it from the germinal neuroepithelium. This new layer is called the **mantle** (or **intermediate**) **zone**, and the germinal epithelium is now called the **ventricular zone** (and, later, the **ependyma**).



The mantle zone cells differentiate into both neurons and glia. The neurons make connections among themselves and send forth axons away from the lumen, thereby creating a cell-poor **marginal zone**. Eventually, glial cells cover many of the axons in the marginal zone in myelin sheaths, giving them a whitish appearance. Hence, the mantle zone, containing the neuronal cell bodies, is often referred to as the **Gray matter**; the axonal, marginal layer is often called the **White matter**. In the spinal cord and medulla, this basic three-zone pattern of ependymal, mantle, and marginal layers is retained throughout development. The gray matter (mantle) gradually becomes a butterfly-shaped structure surrounded by white matter; and both become encased in connective tissue.

As the neural tube matures, a longitudinal groove the **sulcus limitans** divides it into dorsal and ventral halves. The dorsal portion receives input from sensory neurons, whereas the ventral portion is involved in effecting various motor functions.

TOPIC 121.122. Cerebellar organization

Cerebellar organization

In the cerebellum, some neuronal precursors enter the marginal zone to form clusters of neurons called **nuclei**. Each nucleus works as a functional unit, serving as a relay station between the outer layers of the cerebellum and other parts of the brain.

In the cerebellum, some neuronal precursors can also migrate away from the germinal epithelium. These precursor cells, called **neuroblasts**, migrate to the outer surface of the developing cerebellum and form a new germinal zone, the **external granule layer**, near the outer boundary of the neural tube.

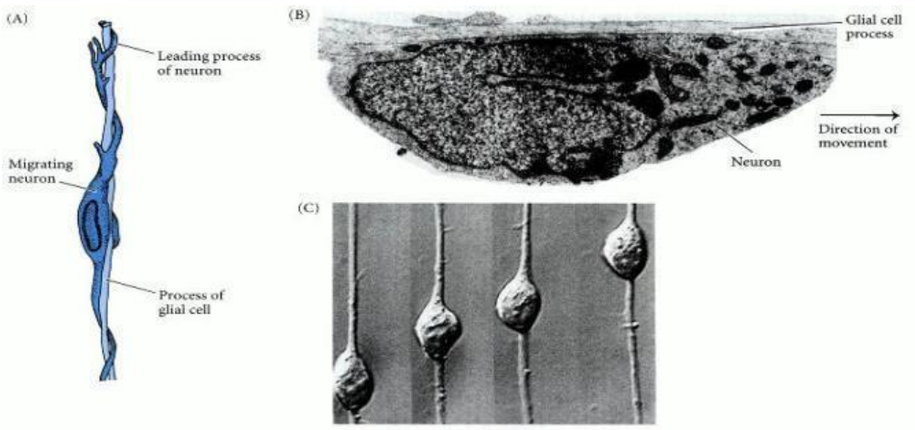
The inner compartment of the external granule layer contains postmitotic neuroblasts that are the precursors of the major neurons of the cerebellar cortex, the **granule neurons**.

These granule neurons migrate back into the developing cerebellar white matter to produce a region called the **internal granule layer**.

Meanwhile, the original ependymal layer of the cerebellum generates a wide variety of neurons and glial cells, including the distinctive and large **Purkinje neurons**.

They secretes Sonic hedgehog, which sustains the division of granule neuron precursors in the external granule layer

One mechanism thought to be important for positioning young neurons within the developing mammalian brain is **glial guidance.**



TOPIC I23. Neuronal Types

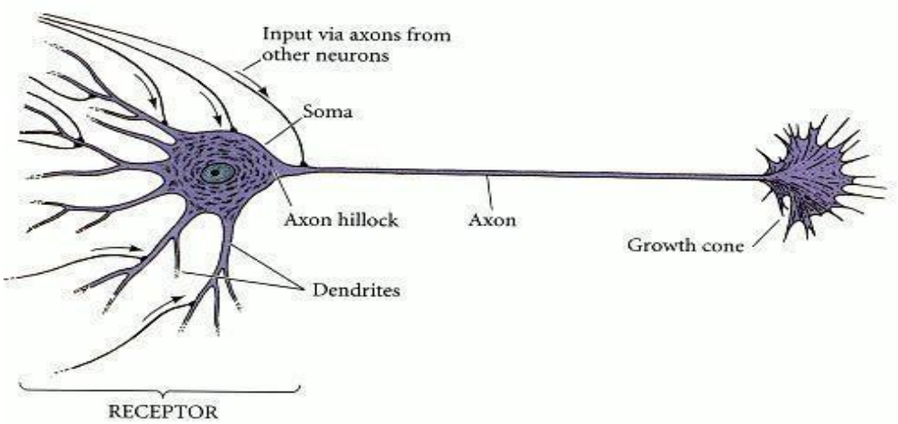
The human brain consists of over 10^{11} neurons associated with over 10^{12} glial cells.

Those cells that remain integral components of the neural tube lining become ependymal cells. These cells can give rise to the precursors of neurons and glial cells.

The fine extensions of the neuron that are used to pick up electrical impulses from other cells are called dendrites. Some neurons develop only a few dendrites, whereas other cells (such as the Purkinje neurons) develop extensive dendritic trees.

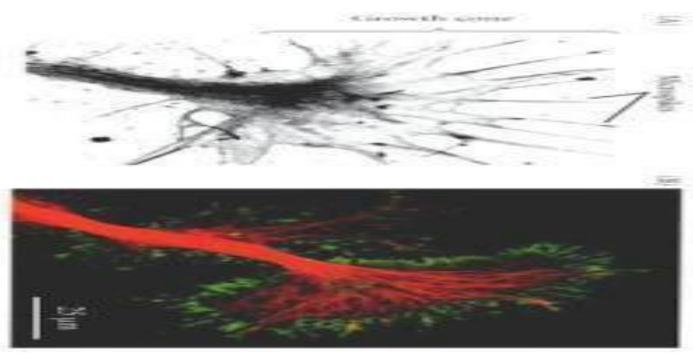
TOPIC 124. Development of Neurons

Another important feature of a developing neuron is its axon (sometimes called a neurite).



Dendrites are often numerous and do not extend far from the neuronal cell body, or soma, axons may extend for several feet.The Nerve outgrowth is led by the tip of the axon, called the

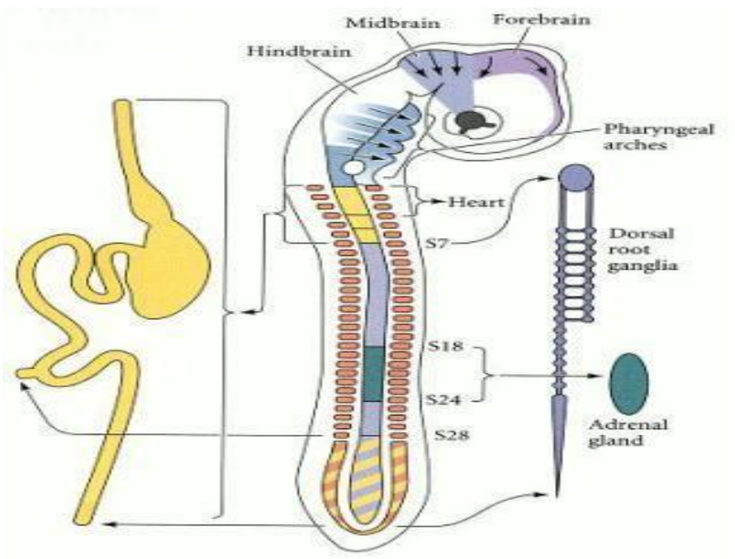
growth cone. The growth cone moves by the elongation and contraction of pointed filopodia called Microspikes. These microspikes contain microfilaments, which are oriented parallel to the long axis of the axon.



TOPIC 125.126. The Neural Crest AND 1Major pathways in differentiation of neural crest

The Neural Crest

A group of ectodermal cells that gives rise to the spinal ganglia and various structures of the autonomic nervous system.The neural crest cells originate at the dorsal most region of the neural tube.The neural crest can be divided into four main functional (but overlapping) domains.



The cranial (cephalic) neural crest, whose cells migrate dorsolaterally to produce the craniofacial mesenchyme that differentiates into the cartilage, bone, cranial neurons, glia,and connective tissues of the face.

Major pathways in differentiation of neural crest

The trunk neural crest, whose cells take one of two major pathways.

Neural crest cells that become the pigment-synthesizing melanocytes migrate dorsolaterally into the ectoderm and continue on their way toward the ventral midline of the belly.

The second migratory pathway takes the trunk neural crest cells ventrolaterally through the anterior half of each sclerotome.

Sclerotomes are blocks of mesodermal cells, derived from somites, that will differentiate into the vertebral cartilage of the spine.

The vagal and sacral neural crest, whose cells generate the parasympathetic (enteric) ganglia of the gut.

The vagal (neck) neural crest lies opposite chick somites 1-7, while the sacral neural crest lies posterior to somite 28.

The cardiac neural crest is located between the cranial and trunk neural crests.

The cardiac neural crest cells can develop into melanocytes, neurons, cartilage, and connective tissue (of the third**,** fourth, and sixth pharyngeal arches).

TOPIC 127 TO 130. Summary Central Nervous System and Epidermis

Summary

Central Nervous System and Epidermis

- The neural tube forms from the shaping and folding of the neural plate. In primary neurulation, the surface ectoderm folds into a tube that separates from the surface. In secondary neurulation, the ectoderm forms a cord and then forms a cavity within it.
- Primary neurulation is regulated by both intrinsic and extrinsic forces. Intrinsic wedging occurs within cells of the hinge regions to bend the neural plate. Extrinsic forces include the migration of the surface ectoderm towards the center of the embryo.
- Neural tube closure is also a mixture of extrinsic and intrinsic forces. In humans, if the neural tube fails to close various diseases can result.
- The neural crest cells arise at the lateral borders of the neural tube and surface ectoderm.
- There is a gradient of maturity in many embryos, especially those of amniotes. The anterior develops earlier than the posterior.
- The dorsal-ventral patterning of the neural tube is accomplished by proteins of the TGF-β family secreted from the surface ectoderm and roof of the neural tube.
- The brain forms three primary vesicles: prosencephalon (forebrain), mesencephalon (midbrain), and rhombencephalon (hindbrain).
- The brain expands through fluid secretion putting positive pressure on the vesicles.
- The neurons of the brain are organized into cortices (layers) and nuclei (clusters).
- New neurons are formed by mitosis in the neural tube. The germinal zone at the lumen of the neural tube is called the ventricular zone.
- The cerebral cortex in humans has six layers, and the mantle zone is called the Neocortex. Cell fates are often fixed as they undergo their last division. Neurons derived from the same stem cell may end up in different functional regions of the brain.
- Dendrites receive signals from other neurons, while axons transmit them. The place where the signaling takes place (through the release of neurotransmitters) is called a **synapse**.
- Axons grow from the nerve cell body, or soma. They are led by the growth cone.
- The retina forms from the optic vesicle that extends from the brain. **Pax6** plays a major role in eye formation, and the down regulation of Pax6 by Sonic hedgehog in the center of the brain splits the eye-forming region of the brain in half.

- The photoreceptor cells gather the light and transmit the impulse through interneurons to the retinal ganglion cells. The axons of the retinal ganglion cells form the optic nerve.
- The basal layer of the surface ectoderm becomes the stratum germinativum, or germinal layer of the skin. These cells divide to produce a stem cell and a cell committed to become an epidermal cell (keratinocyte). Stem cells appear to be able to make hair.
- Paracrine factors such as **TGF- and FGF7** are important in normal skin development.
- **Cutaneous appendages hair, feathers, and scales** are formed by **epithelialmesenchymal interactions** between the epidermis and the dermal mesoderm.

TOPIC 131.132. Myogenesis:The Development of Muscle

Myogenesis:
The Development of Muscle

Paracrine factors instruct the **Myotome cells** to become muscles by inducing them to synthesize the **MyoD protein.**

Factors from the surrounding environment induce the **Pax3** transcription factor.

In the absence of other inhibitory transcription factors (such as those found in the sclerotome cells), Pax3 then activates the genes encoding two muscle-specific transcription factors, **Myf5** and **MyoD**.

MyoD and Myf5 belong to a family of transcription factors called the **myogenic bHLH** (basic helix-loop-helix) **proteins** (sometimes also referred to as the MyoD family).

The proteins of this family all bind to similar sites on the DNA and activate muscle-specific genes.MyoD also directly activates its own gene. While Pax3 is found in several other cell types, the **myogenic bHLH proteins** are specific for muscle cells. Any cell making a myogenic bHLH transcription factor such as MyoD or Myf5 is committed to becoming a muscle cell.

Transfection of genes encoding any of these myogenic proteins into a wide range of cultured cells converts those cells into muscles.

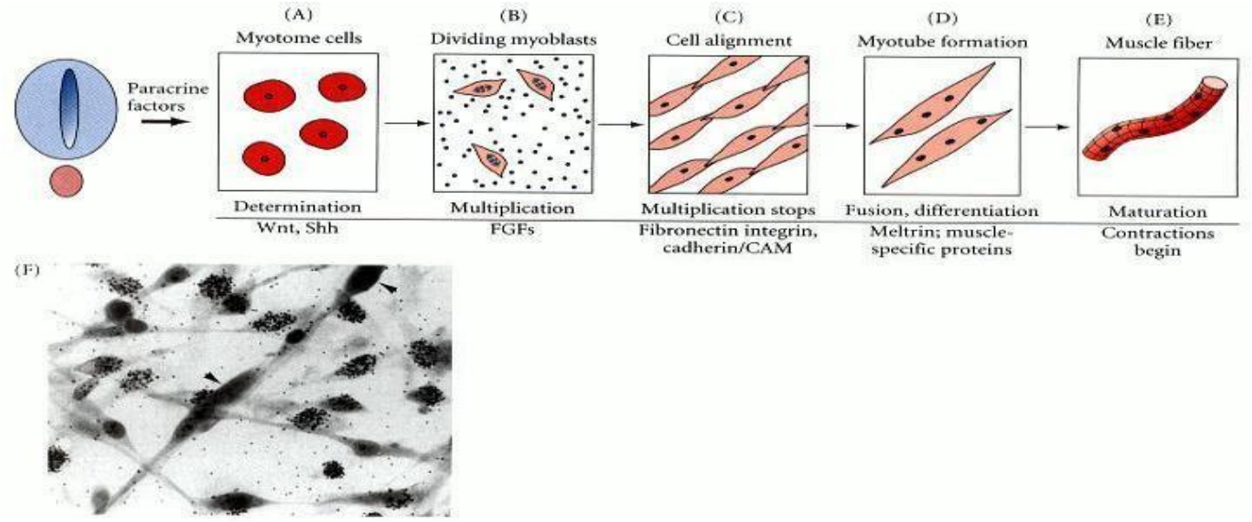
TOPIC 133.134. Muscle cell fusion AND Steps in Muscle cell fusion

Muscle cell fusion

The myotome cells producing the myogenic bHLH proteins are the myoblasts- committed muscle cell precursors. These cells align together and fuse to form the multinucleated **myotubes, a** characteristic of muscle tissue.Muscle cell fusion begins when the myoblasts leave the cell cycle. As long as particular growth factors (particularly fibroblast growth factors) are present, the myoblasts will proliferate without differentiating.

Steps in Muscle cell fusion

When these factors are depleted, the myoblasts stop dividing, secrete **fibronectin** onto their extracellular matrix, and bind to it through **5β1 integrin**, their major fibronectin receptor. The second step is the alignment of the myoblasts together into chains.



The third step is the cell fusion event itself. As in most membrane fusions, calcium ions are critical, and fusion can be activated by **calcium ionophores, such as A23187**, that carry calcium ions across cell membranes.

Fusion appears to be mediated by a set of metalloproteinases called **meltrins**. These proteins were discovered during a search for myoblast proteins that would be homologous to **fertilin**, a protein implicated in sperm-egg membrane fusion.

TOPIC 135 .136. Osteogenesis: The Development of Bones

Osteogenesis:
The Development of Bones

Some of the most obvious structures derived from the paraxial mesoderm are bones.

There are three distinct lineages that generate the skeleton.

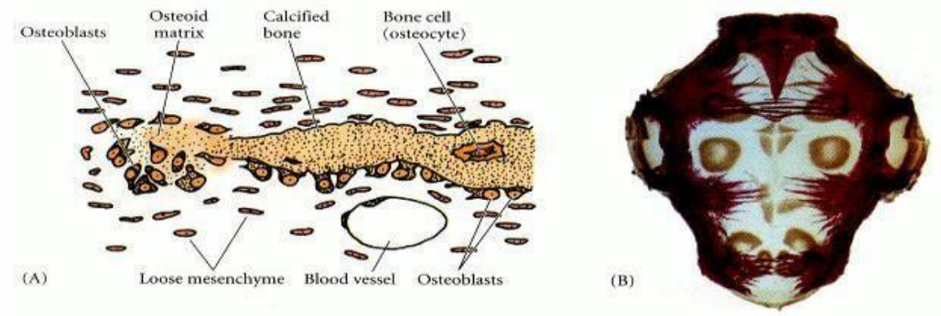
- The somites generate the axial skeleton.
- The lateral plate mesoderm generates the limb skeleton.
- The cranial neural crest gives rise to the branchial arch and craniofacial bones and cartilage.
- There are two major modes of bone formation, or **osteogenesis**, and both involve the transformation of a preexisting mesenchymal tissue into bone tissue.
- The direct conversion of mesenchymal tissue into bone is called **intramembranous ossification**. This process occurs primarily in the bones of the skull.
- In other cases, the mesenchymal cells differentiate into cartilage, and this cartilage is later replaced by bone. The process by which a cartilage intermediate is formed and replaced by bone cells is called **endochondral ossification**.

TOPIC 137.138. Intramembranous ossification

Intramembranous ossification

Intramembranous ossification is the characteristic way in which the flat bones of the skull and the turtle shell are formed. During intramembranous ossification in the skull, neural crest derived mesenchymal cells proliferate and condense into compact nodules.

Some of these cells develop into capillaries; others change their shape to become **osteoblasts**, committed bone precursor cells.



The osteoblasts secrete a **collagen-proteoglycan matrix** that is able to bind calcium salts. Through this binding, the prebone (**osteoid**) matrix becomes calcified.

In most cases, osteoblasts are separated from the region of calcification by a layer of the osteoid matrix they secrete.

Occasionally, though, osteoblasts become trapped in the calcified matrix and become **osteocytes** bone cells. As calcification proceeds, bony spicules radiate out from the region where ossification began.

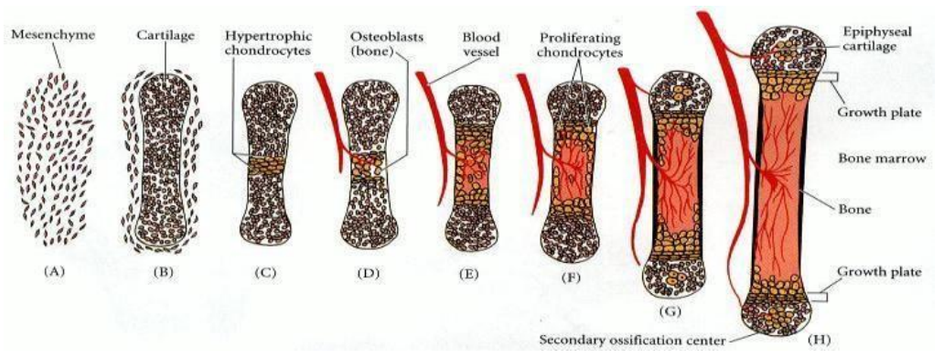
Furthermore, the entire region of calcified spicules becomes surrounded by compact mesenchymal cells that form the **Periosteum** (a membrane that surrounds the bone).

TOPIC 139. TO 142 Endochondral ossification

Endochondral ossification

Endochondral ossification involves the formation of **cartilage** tissue from aggregated mesenchymal cells, and the subsequent replacement of cartilage tissue by bone.

The process of Endochondral ossification can be divided into five stages.



First, the mesenchymal cells are committed to become cartilage cells. This commitment is caused by paracrine factors that induce the nearby mesodermal cells to express two transcription factors, Pax1 and **Scleraxis**.

Pax1 and Scleraxis are the transcription factors are thought to activate cartilage-specific genes.

Thus, **Scleraxis** is expressed in the mesenchyme from the sclerotome, in the facial mesenchyme that forms cartilaginous precursors to bone, and in the limb mesenchyme

During the second phase of endochondral ossification, the committed mesenchyme cells condense into compact nodules and differentiate into **chondrocytes**, the cartilage cells. Ncadherin appears to be important in the initiation of these condensations.

Endochondral ossification II

During the second phase of endochondral ossification, the committed mesenchyme cells condense into compact nodules and differentiate into **chondrocytes**, the cartilage cells.

In humans, the ***SOX9* gene**, which encodes a DNA-binding protein, is expressed in the precartilaginous condensations. Mutations of the *SOX9* gene cause **camptomelic dysplasia**, a rare disorder of skeletal development that results in deformities of most of the bones of the body.

During the third phase of endochondral ossification, the chondrocytes proliferate rapidly to form the model for the bone. As they divide, the chondrocytes secrete a cartilage-specific extracellular matrix.

In the fourth phase, the chondrocytes stop dividing and increase their volume dramatically, becoming **hypertrophic chondrocytes**. These large chondrocytes alter the matrix they produce (by adding collagen X and more fibronectin) to enable it to become mineralized by calcium carbonate.

The fifth phase involves the invasion of the cartilage model by blood vessels.

The hypertrophic chondrocytes die by apoptosis. This space will become **bone marrow**. As the cartilage cells die, a group of cells that have surrounded the cartilage model differentiate into osteoblasts.

The osteoblasts begin forming bone matrix on the partially degraded cartilage. Eventually, all the cartilage is replaced by bone. Thus, the cartilage tissue serves as a model for the bone that follows.

TOPIC 143.144 Osteoclast

Osteoclasts

Osteoclasts are probably derived from the same precursors as macrophage blood cells, and they dissolve both the inorganic and the protein portions of the bone matrix.

Each osteoclast extends numerous cellular processes into the matrix and pumps out hydrogen ions onto the surrounding material, thereby acidifying and solubilizing it.

The blood vessels also import the blood-forming cells that will reside in the marrow for the duration of the organism's life. The number and activity of osteoclasts must be tightly regulated.

If there are too many active osteoclasts, too much bone will be dissolved, and **osteoporosis** will result. Conversely, if not enough osteoblasts are produced, the bones are not hollowed out for the marrow, and **osteopetrosis** results.

Osteopetrosis or Marble bone disease

Osteopetrosis is a bone disease that makes bones abnormally dense and prone to breakage (fracture).

This disorder is characterized by increased bone density and abnormal bone growth.