DEVELOPMENTAL BIOLOGY

M.Sc. ZOOLOGY

SEMESTER-I, PAPER-III

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DEVELOPMENTAL BIOLOGY

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FOREWORD

Since its establishment in 1976, Acharya Nagarjuna University has been forging ahead in the path of progress and dynamism, offering a variety of courses and research contributions. I am extremely happy that by gaining 'A+' grade from the NAAC in the year 2024, Acharya Nagarjuna University is offering educational opportunities at the UG, PG levels apart from research degrees to students from over 221 affiliated colleges spread over the two districts of Guntur and Prakasam.

The University has also started the Centre for Distance Education in 2003-04 with the aim of taking higher education to the door step of all the sectors of the society. The centre will be a great help to those who cannot join in colleges, those who cannot afford the exorbitant fees as regular students, and even to housewives desirous of pursuing higher studies. Acharya Nagarjuna University has started offering B.Sc., B.A., B.B.A., and B.Com courses at the Degree level and M.A., M.Com., M.Sc., M.B.A., and L.L.M., courses at the PG level from the academic year 2003-2004 onwards.

To facilitate easier understanding by students studying through the distance mode, these self-instruction materials have been prepared by eminent and experienced teachers. The lessons have been drafted with great care and expertise in the stipulated time by these teachers. Constructive ideas and scholarly suggestions are welcome from students and teachers involved respectively. Such ideas will be incorporated for the greater efficacy of this distance mode of education. For clarification of doubts and feedback, weekly classes and contact classes will be arranged at the UG and PG levels respectively.

It is my aim that students getting higher education through the Centre for Distance Education should improve their qualification, have better employment opportunities and in turn be part of country's progress. It is my fond desire that in the years to come, the Centre for Distance Education will go from strength to strength in the form of new courses and by catering to larger number of people. My congratulations to all the Directors, Academic Coordinators, Editors and Lesson-writers of the Centre who have helped in these endeavors.

Prof. K. Gangadhara Rao
M.Tech., Ph.D.,
Vice-Chancellor I/c
Acharya Nagarjuna University.

M.Sc. ZOOLOGY
SEMESTER - I
103ZO24 -DEVELOPMENTAL BIOLOGY

Course Objectives/Course Outcomes:

CO 1: The students can be able to remember the process of gametogenesis, including mitosis, meiosis and gamete formation in males and females.

CO2: Understanding the genetic and phenotypic variation that can arise from gamete formation, fertilization and the role of gametes in sexual reproduction and inheritance.

CO 3: By applying the differences between gametes and somatic cells in terms of chromosome number and DNA content.

CO 4: To analyze the evolutionary changes of gamete size , shape, factors that can influence gamete competition and mate choice .

CO 5: The reproductive strategies of different organisms including mogamy, promiscuity, asexuality and the ethical social implications of technologies related to gamete and embryo manipulations such as IVF, cloning and gene making,

UNIT - I:

Origin and migration of primordial germ cells (PGCs) to the genital ridges, differentiation of gonads in mammals. Spermatogenesis: Sperm - formation, structure and types; Leydig cells - endocrine regulation of spermatogenesis. Oogenesis: Formation and maturation of ovum, previtellogenesis, vitellogenesis, formation of yolk, functions of egg and types of eggs. Learning outcome: From the topic's gametogenesis the gonadial action with dual origin which helps in the maternal gene product with germ cell speciation in all invertebrates and vertebrates, which they confined with cytoplasmic bridges the during the yolk formation and function.

UNIT - II:

Fertilization: Cell surface molecules in sperm-egg recognition in animals, mechanism of fertilization, molecular events during fertilization and post fertilization. Early Development: Zygote formation, cleavage, blastulation, gastrulation and formation of germ layers in animals; Fate maps and cell lineage. Learning outcome: By learning the process of fertilization, the gametes play an important role in different mammals and insects with the formation (or) development during fertilization process in mammals and basic approach to life of gametes is the outcome work during fertilization process in animals

UNIT-III:

Cell aggregation and differentiation; ixes and pattern formation in Drosophila, amphibian and chick. Differentiation of neurons, post embryonic development. Larval formation, metamorphosis in insects and amphibians. Learning outcome: In cell aggregation and differentiation, the development of nervous system, embryos, larval development metamorphosis and the role of endocrine system play an important role regulation system in formation of Drosophil4 amphibians, chick and mammals in development biology.

UNIT - IV:

Programmed cell death: Incidence of apoptosis, apoptosis during animal development; apoptosis during limb development. Aging and senescence; Dietary restriction and anti-aging action; Age related diseases. Learning outcome: The detailed out come in this chapter with apoptosis in animal development and apoptosis role in development process with special reference to aging and senescence's with life expectancy disorders and to know the diseases in human related factors.

UNIT - V:

Potency, commitment, Specification, lnduction, Competence, Determination and differentiation. Hormonal regulation of Meta morphosis in insects and amphibians. Learning outcome: The detailed out come in this unit with potency and specification and hormonal regulation in insects and amphibians.

REFERENCE BOOKS:

l) Austen CR and Short RV. 1980. Reproduction in Mammals. Cambridge University Press.
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103ZO24

CENTRE FOR DISTANCE EDUCATION
ACHARYA NAGARJUNA UNIVERSITY
M.Sc. Degree Examination
M.Sc. ZOOLOGY, SEMESTER -I
Model Question Paper
Paper – III: DEVELOPMENTAL BIOLOGY

Time :3 Hours Max Marks :70

Answer ALL Questions
All question carries equal marks.

1. a) Give an account on various types of eggs and function of egg.

(or)

b) Write short notes on:
i. Leydig cells
ii. Vitellogenesis

2. a) What is Gastrulation and formation of germ layers

(or)

b) Write short notes on:
i. Determination and differentiation
ii. Molecular events during fertilization.

3. a) Describe the mechanism of Hormonal regulation in insects.

(or)

b) Describe the mechanism of Differentiation of Neurons.

4. a) What is apoptosis? Explain apoptosis in limb development.

(or)

b) Describe the Process of oogenesis in mammals.

5. a) Describe the Regulation Anti-aging action.

(or)

b) Describe the mechanism of Metamorphosis in insects and amphibians.

CONTENTS

LESSON - 1

PRIMODIAL GERM CELLS

AIMS AND OBJECTIVES:

1) Origin and migration of primordial germ cells
2) Understand the definition and significance of primordial germ cells in embryology and reproduction.
3) Learn about their origin and early development during embryogenesis.

STRUCTURE:

1.1 INTRODUCTION:

Primordial germ cells (PGCs) are specialized precursor cells that serve as the foundation for the formation of gametes-sperm in males and oocytes in females. These cells are essential for the perpetuation of genetic material across generations, making them critical to the process of reproduction. PGCs are among the first cell types to be specified during early embryonic development. Their unique properties set them apart from somatic cells, as they are specifically programmed to undergo processes that eventually lead to the formation of reproductive cells. These processes include migration to the developing gonads, extensive epigenetic reprogramming to reset genetic imprints, and differentiation into gametogenic cell lines.

1.2 ORIGIN OF PRIMODIAL GERM CELLS:

Mammalian germ cells are determined after PGC colonization of the nascent gonad "Mammalian primordial germ cells (PGCs) are induced in the embryonic epiblast, before migrating to the nascent gonads. In fish, frogs, and birds, the germline segregations even earlier, through the action of maternally inherited germ plasm. Early in development at the time of gastrulation a small group of cells are "put aside" to later form oocytes and spermatozoa, these cells described as the primordial germ cells (PGCs). The cells migrate initially through the primitive streak into the posterior Endoderm. that forms the hindgut and from there later into the genital ridge that will be the site of the developing gonad. The maintenance of pluripotency within this cell population may arise through epigenetic modifications that suppress somatic differentiation programs. These cells differentiate at different times in male testis and female ovary development. Recent molecular studies suggest that final determination occurs after PGCs colonize the developing gonad.

1.3 MIGRATION OF PRIMODIAL GERM CELLS:

Primordial Germ Cells (PGCs) are thought to be the first population of cells to migrate through the primitive streak in early gastrulation. Human embryonic disc showing the primitive streak region where gastrulation occurs, generation the trilaminar embryo. Arrows indicate direction of cell migration through the streak. This population of cells then lie at the hindgut and yolk sac junctional region and later migrate into the germinal ridge in early embryonic development. Sacrococcygeal teratomas - Remnant primitive streak cells (most common solid tumor in newborn infants) Germline teratoma - (Germinoma) abnormally differentiated/located PGCs fail to die.

The genetic sex of an embryo is determined at fertilization by the sperm that fertilizes the oocyte, but the gonads do not acquire male or female morphologic characteristics until week 7 of development. The early genital system is similar in both sexes, and in the beginning all human embryos are potentially bisexual. The period of early genital development is called the indifferent or primitive stage of the reproductive organs, The primordial germ cells are large, spherical primitive sex cells of about 25 to 30 mm, with a granular cytoplasm, rich in lipids, and containing a large attraction sphere or iodosome consisting of 2 centrioles surrounded by Golgi apparatus. The human primordial germ cells are discernible at about day 21 of embryonic life and are seen among the ectodermal cells in the wall of the yolk sac near the origin of the allantois. Thus, they are at first at some distance from their eventual definitive location in the genital or gonadal ridge.

Stages of primordial germ cell migration:

These primordial germ cells migrate to the developing gonads, which will form the ovaries in females and the testes in males. After a period of mitotic proliferation, the primordial germ cells undergo meiosis and differentiate into mature gametes-either eggs or sperm.

Germ cells differentiate to produce male and female gametes, sperm and unfertilized eggs (oocytes or ova), and undergo meiosis to produce a haploid set of chromosomes. Haploid gametes then unite to form a diploid zygote that develops into a new individual. The gametes possess a haploid genome of either parent and during fusion, these characters present in the genome pass on to the offsprings to form a diploid zygote. ... Meiosis, therefore, helps the germ cells make a single set of genes (haploid) from the normal two copies (diploid).

Germ cells produce gametes and are the only cells that can undergo meiosis as well as mitosis. These cells are sometimes said to be immortal because they are the link between generations. Somatic cells are all the other cells that form the building blocks of the body and they only divide by mitosis. When two germ cells combine, they will restore the normal number of chromosomes in the progeny, ensuring the stability of the DNA of the species. Such a mechanism of inheritance explains the results of the Mendel experiments, and is used by all sexually reproducing organisms.

There are three main categories of life cycles in eukaryotic organisms: diploid-dominant, haploid-dominant, and alternation of generations.

- Diploid-Dominant Life Cycle.
- Haploid-Dominant Life Cycle.
- Alternation of Generations.

A zygotic meiosis is a meiosis of a zygote immediately after karyogamy, which is the fusion of two cell nuclei. This way, the organism ends its diploid phase and produces several haploid

cells. The individuals or cells as a result of mitosis are haploids, hence this life cycle is also called haplontic life cycle.in humans, special cells called germ cells undergo meiosis and ultimately give rise to sperm or eggs. Germ cells contain a complete set of 46 chromosomes (23 maternal chromosomes and 23 paternal chromosomes) Each daughter cell is haploid, because it has half the number of chromosomes as the original parent cell.

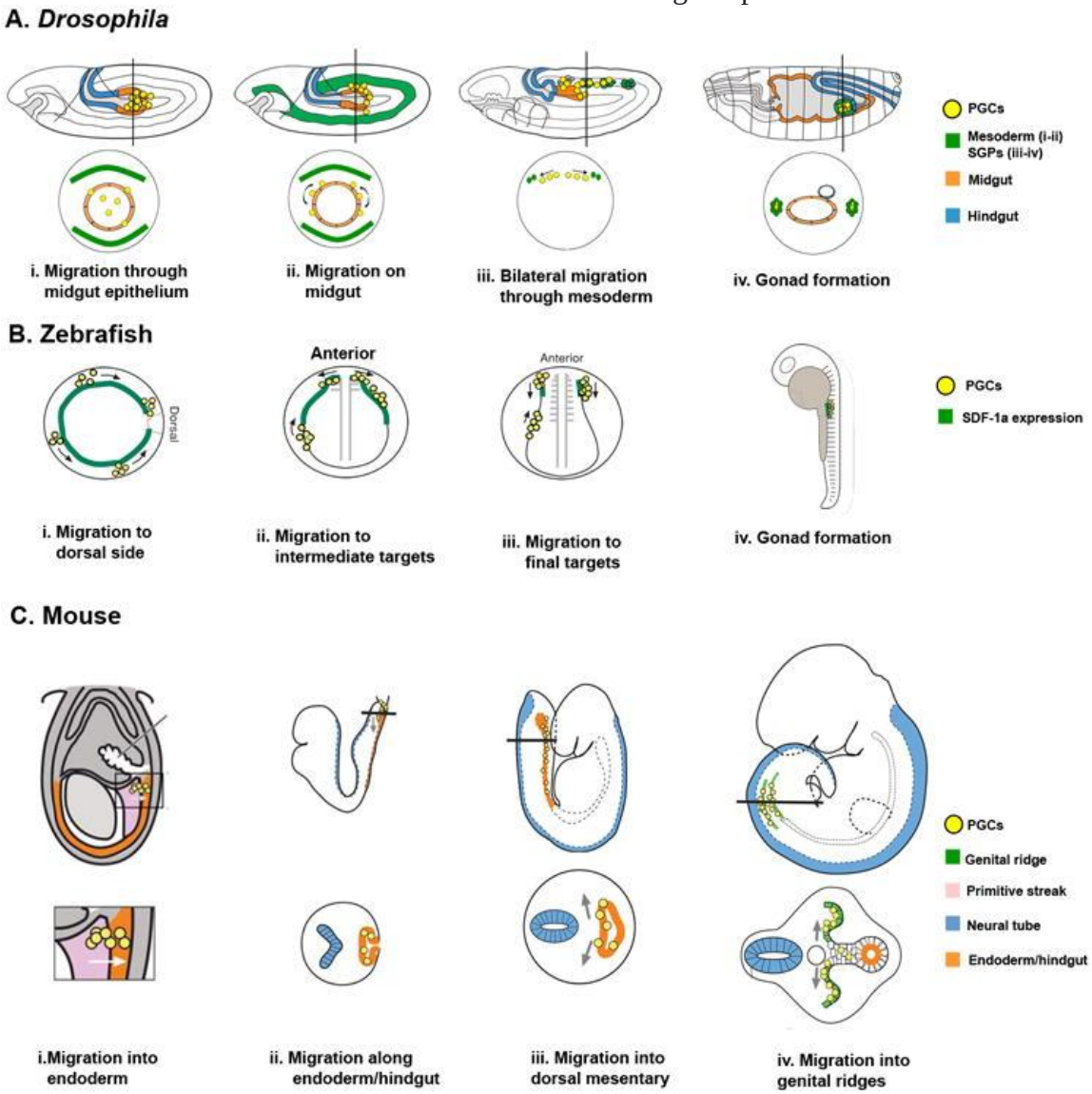

Figure 1: Species Comparison of Migration

Significant Features of Primordial gem cells:

- Primordial germ cell (PGC) migration provides a useful system for studying a group of individually migrating cells in vivo.
- PGC migration in all species follows similar steps: initiation of polarity and directed migration, regulated migration by attractive and repulsive cues, and termination of migration at the site of gonad formation.
- PGCs frequently utilize G protein-coupled receptor signalling to reach their targets tissues, a mechanism found in many types of migrating cells.
- Lipids have an essential role in regulating PGC migration and seem to work both directly as chemo attractants and by modifying and activating protein chemo attractants.

- Cell adhesion molecules, in particular cadherins, have important roles in several steps of PGC migration, such as initiation of migration, migrating through somatic tissues, and cessation of migration and gonad coalescence.
- The migration of PGCs is closely linked with their survival, and PGCs that do not properly migrate to the gonad are usually eliminated through cell death. However, mechanisms of PGC death might differ between species.

1.4 GONADAL RIDGE:

Embryology the gonadal ridge (or urogenital ridge) is the precursor to the gonads. The gonadal ridge initially consists mainly of mesenchyme and cells of underlying mesonephric origin. Once oogonia enter this area they attempt to associate with these somatic cells. Development proceeds and the oogonia become fully surrounded by a layer of cells (pre-granulosa cells).The gonadal ridge appears at approximately five weeks, and gives rise to the sexcords Gonadal (or Genital) Ridge Formation. The sexually undifferentiated gonadal ridge forms as a thickening of the coelomic or surface epithelium in an anterior/posterior direction on the ventral side of the mesonephros.

The gonadal primordia, called genital ridges, have the ability to develop into testes or ovaries depending on the genetic signals they receive. The primordial germ cells can go on to form pro spermatogonia and thereafter sperm, or oogonia and thereafter oocytes, irrespective of their genetic makeup- XX or XY. The gonads initially develop from the mesothelial layer of the peritoneum. The ovary is differentiated into a central part, the medulla, covered by a surface layer, the germinal epithelium. The immature ova originate from cells from the dorsal endoderm of the yolk sac.

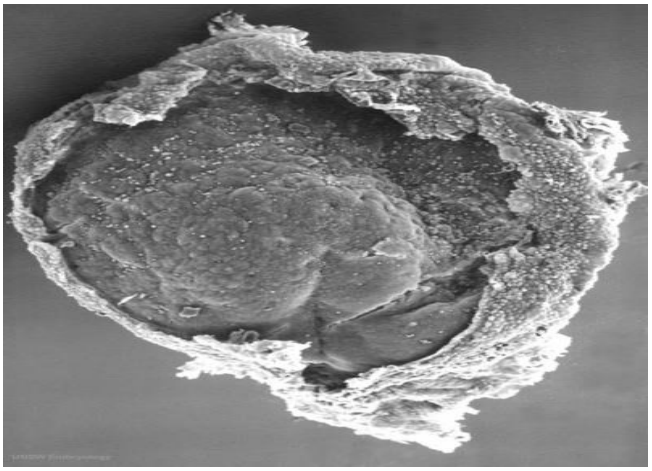

Figure 2: yolk sack

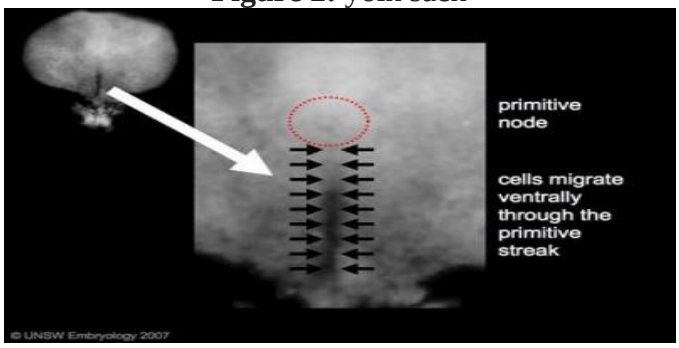

Figure 3: Gastrulation- Embryology

Gonadal Primordium (indifferent gonad):

The primordial germ cells migrate, by ameboid movement, along the dorsal mesentery of the hindgut during week 5 and reach the lumbar region of the developing embryo, the future gonadal ridge. The coelomic epithelium which lines the anterior internal side of the mesonephric (wolffian) body, thickens to form the genital or gonadal ridge and provides the nutrient supporting cells of the gonad The indifferent gonad now consists of an outer cortex and an inner medulla. In embryos with an XX sex chromosome complex, the cortex forms an ovary, and the medulla regresses; in one with an XY chromosome complex, the medulla differentiates into a testis, and the cortex regresses. The sex cords eventually become the seminiferous tubules in the male and the medullary cords in the female. The primary sex cords continue to proliferate actively, anastomose deep in the mesenchyme, and produce a complex network called the rete, which is seen as a bulge under the coelomic epithelium on the anterointernal side of the mesonephric (wolffian) body.

Urogenital Connections

During embryonic development, the rete (a network of tubules) connects with the nearby mesonephric proximal Convoluted tubules. This establishes the initial urogenital connections. Changes in the Mesonephric Body By the end of the second month, the mesonephric body (also known as the Wolffian body) starts to degenerate. The glomeruli (capillary clusters) disappear, leaving only the mesonephric tubules connected to the genital gland.

1.5 SUMMARY:

Primordial germ cells are essential components of reproduction and development. Their unique properties, such as pluripotency, epigenetic reprogramming, and migratory behavior, make them a critical area of study in genetics, epigenetics, and cell biology. Research into PGCs continues to reveal their pivotal role in life's continuity and their potential applications in biomedical science.

1.6 TECHINICAL TERMS:

Mammalian primordial germ cells, haploid cells, diploid chromosomes, wolffian body.

1.7 SELF ASSESSMENT QUESTIONS:

1) Explain Origin and migration of primordial germ cells (PGCs)
2) What Stages of primordial germ cell migration
3) Explain Gonadal ridge.

1.8 SUGGESTED READINGS:

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2. Gilbert SF. 2006. Developmental Biology, 8th Edition. Sinauer Associates Inc., Publishers, Sunderland, USA.
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Prof. M. Jagadish Naik

LESSON-2

SPERMATOGENESIS (OR) GAMATOGENESIS

AIMS AND OBJECTIVES

- Understand the stages of sperm production: mitosis, meiosis and spermiogenesis.
- Identify the role softesticular structures like seminiferoustubules, Sertolicells, and Leydigcells.
- Examine hormonal regulation by FSH, LH, and testosterone.
- Exploregeneticmechanismsensuringdiversityandstabilityduringmeiosis.
- Investigate germcell – Sertolicellinteractionsinsperm development.
- Analyse factorsaffectingspermatogenesisandtheirlinktomaleinfertility.
- Apply knowledge to develop treatments for infertility and advance reproductive medicine.

STRUCTURE:

2.1 INTRODUCTION

Mammalian spermatogenesis is a highly synchronized, regular, long and extremely complex process of cellular differentiation by which a spermatogonial “stem-cell” is gradually transformed into a highly differentiated haploid cell ‘Spermatozoon. “This differentiation involves three distinct classes of germinal cells-the spermatogonia, the spermatocytes, and the spermatids, which usually are arranged in concentric layers in the seminiferous tubules. In the adult mammal’s spermatogenesis is a continuous process, which can be divided into twodistinct phases and each characterized by specific morphological and biochemical changes of nuclear and cytoplasmic components.

The two phases include:

- formation of spermatids (mitosis and meiosis) and
- spermiogenesis.

2.2 FORMATION OF SPERMATIDS

This phase of spermatogenesis is further subdivided into three phases.

1. Multiplication phase
2. Growth phase
3. Maturation phase

1. Multiplication phase:

This phase is also known as proliferation and renewal of spermatogonia. During this phase the diploid spermatogonia which are situated at the periphery of the seminiferous tubule, multiply mitotically to form spermatocytes and also to give rise to new spermatogonia! stem cells and enter the phase of growth.

2. Growth phase:

During this phase, a limited growth of spermatogonia takes place; their volume becomes double and they are now called primary spermatocytes which are still diploid in number. Now these primary spermatocytes enter into the next phase namely, maturation phase.

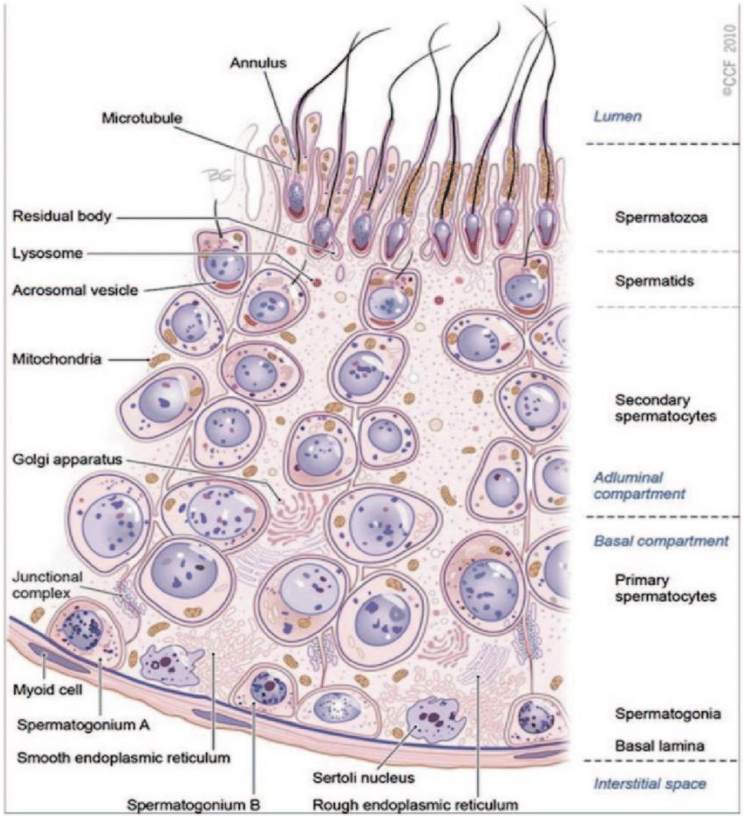

Figure 2: T.S of a part of a seminiferous tubules of human testis showing production of sperms

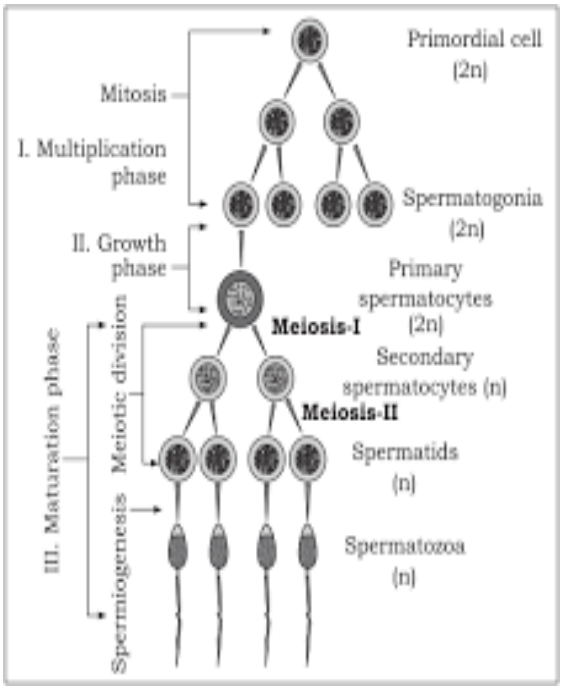

Figure 3: Gametogenesis in male

1. Maturation phase:

The primary spermatocyte enters into the prophase of meiotic or maturation division. Meiotic prophase is a very complex process characterized by an ordered series of chromosomal rearrangements which are accompanied by molecular changes. During meiosis, first nuclear DNA duplicates, each homologous chromosome starts pairing (synapsis) and longitudinally splits up into two chromatids, both of which remain joined by a common centromere.

By chiasma formation mutual exchange of some chromosome material between two non-sister chromatids of each homologous pair (tetrad) occurs (crossing over) to provide an almost indefinite variety of combinations of paternal and maternal genes in any gamete.

Lastly, two chromosomes of each homologous pair (tetrad) migrate towards opposite poles of the primary spermatocyte. Now each pole of primary spermatocyte has haploid set of chromosomes. Each set of chromosomes is surrounded by the nuclear membrane developed from the endoplasmic reticulum. The first meiotic division, as a rule, is followed by the division of cytoplasm (cytokinesis) which divides each primary spermatocyte into two haploid, secondary spermatocyte.Each secondary spermatocyte undergoes second meiotic or maturation division which is a simple mitosis and produces four haploid spermatids. These are non-functional male gametes. To become functional spermatozoa, they have to undergo a complex process of cytological and chemical transformations; a process usually referred to as spermiogenesis.

2.3 SPERMATOGENESIS:

The changes in the spermatids leading to the formation of spertmatozoa constitute the process of spermiogenesis. Because a spermatozoon is a very active and mobile cell, in order to provide real mobility to it, all the superfluous materials of the developing spermatozoa are to be discarded and a high degree of specialization takes place in the sperm cell through a

number of steps.During spermiogenesis two major parts of the sperm, the head and tail are formed by the following process.

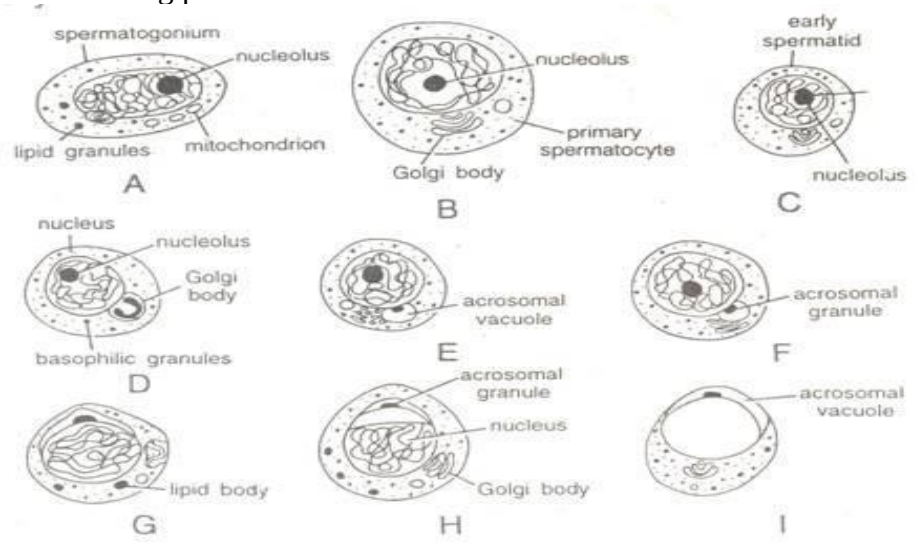

Fig. 4. Spermiogenesis (A->F) successive stages of maturing spermatid

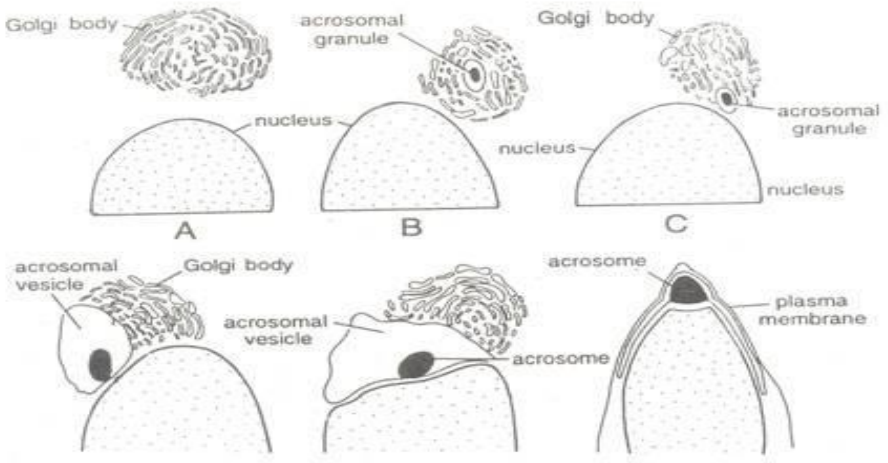

Figure4:stages in the formation of the Acrosome and head cap from the Golgi complex

1. FORMATION OF HEAD:

The two major parts of sperm head i.e. the nucleus and acrosome, undergo the following changes to form a sperm head.

a) Changes in the nucleus:

During spermiogenesis, the nucleus of spermatid shrinks by losing much of its water from the nuclear cap and the chromosomes become closely packed into a small volume. Whole of ribonucleic acid is eliminated, leaving only the genetic material, the deoxyribonucleic protein. Thus, the material, which is not directly concerned with the transforms of hereditary characters, is removed from the nucleus.

The spherical shape of the nucleus also becomes elongated and narrow. This shape is an obvious adaptation for the propulsion in any fluid medium, as well as penetrating the ovum. In different animals, it assumes different shapes which ultimately determine their prospective shapes.

b) Golgi phase:

The young spermatid is round with a spherical nucleus. The Golgi apparatus secretes glycoprotein rich granules which are stained with the periodic acid-Schiff technique. These granules referred to as proacrosomal granules, fuse to form a single large acrosomal granule attached to the nuclear membrane.

c) Cap phase:

The acrosomal granule flattens on the nucleus of the spermatid to form the head cap. The Golgi apparatus which secretes the acrosome separates from the head cap and move towards the opposite pole. The Centrioles which are close to the nucleus, on the side opposite the acrosome cap develop a flagellum.

d) **Acrosome phase:**

The definitive morphological contours of the acrosome become clearly defined. The remaining part of the Golgi apparatus is gradually reduced and ultimately discarded from the sperm as "Golgi -rest" along with some cytoplasm.

In the spermatozoon, the axial body or acrosomal core appears in between the acrosomal granule and nucleus, producing itself from behind into the acrosomal granule. This body develops during its approach to the egg. It contains a few enzymes which are used to dissolve the egg membranes during fertilization process.

2. FORMATION OF THE TAIL OF THE SPERMATOZOON:

The Centrosome of a spermatid after the second meiotic division consists of two Centrioles which have the structure of two cylindrical bodies, lying at right angle to each other. During early stages of sperm metamorphosis, the two Centrioles move to a position just behind the sperm, nucleus in the future neck region. A depression is formed in the posterior surface of the nucleus and one of the two Centrioles becomes placed in the depression with its axis approximately at right angles to the main axis of the spermatozoon.

This is the proximal Centriole and the other centriole i.e. the distal Centriole takes up a position behind the proximal one with its axis coinciding with the longitudinal axis of the spermatozoon. The distal Centriole now give rise to the axis filament of the flagellum of the spermatozoon for which it serves as basal granule.

Most of the mitochondria of spermatids concentrate around the distal Centriole and proximal (upper) part of the axial filament and form the neck and middle piece of the tail of spermatozoon. In the middle piece of the sperm the mitochondria lose their individuality by fusing to a greater or lesser extent. In mammals, the mitochondria join in one continuous body which becomes twisted spirally around the proximal part of the axial filament and the proximal Centriole.

In other animals, however, no spiral arrangement of mitochondria occurs, but instead, mitochondria fuse together to form massive clumps called mitochondrial bodies. The cytoplasm forms a condensed layer called sanchette around the periphery of the middle piece.

The manchette also surrounds the posterior part of the head of the spermatozoa, where it is not covered by the cap.

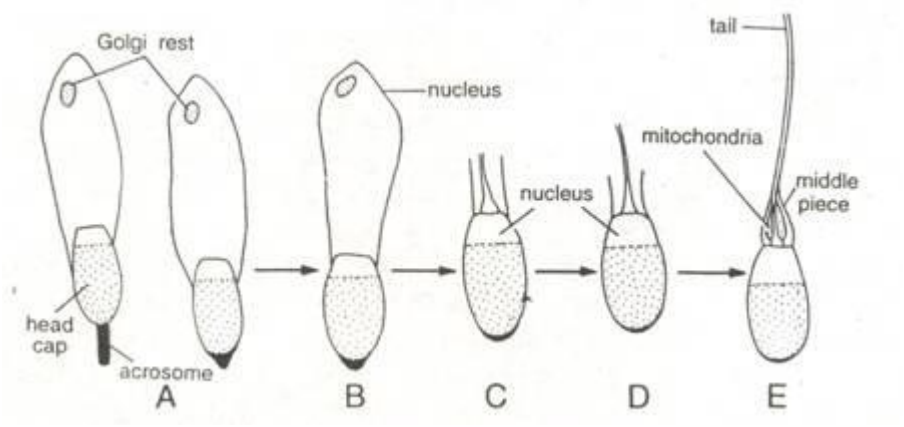

Figure 5:stages in the formation of the tail of spermatozoa

A dark ring called 'Ring Centriole' of unknown function is sometimes seen at the posterior end of the middle piece. It forms the boundary between the middle piece and the principal piece of sperm tail. In most animals, except mammals, the principal piece and tail piece of the sperm are composed of the axial filament only. In mammals, the axial filament of the principal piece is accompanied on the outer side by much thicker fibers which are wedge-shaped in cross section.

These fibers start in the middle piece but do not reach up to the end piece of the spermatozoon tail. The fibers of axial filament of a mammalian sperm tail are also surrounded by flattened bands which occur as semi-circular ribs articulating with each other on the opposite sides of the sperm tail.The end piece has only axial filament which remains covered with cytoplasm and plasma membrane.

Biochemical Changes during Spermatogenesis:

A number of biochemical events occur during spermatogenesis. These are:

- The RNA synthesized during meiosis is eliminated from the nucleus during the two meiotic divisions and remains in the cytoplasm. The fully formed spermatozoon does not contain any detectable amounts of RNA. The meiotic RNA is probably associated with the synthesis of acrosomal proteins and flagellum.
- Nuclear protein synthesis is arrested in the middle of spermiogenesis.
- All of the non-histone proteins in the nucleus are eliminated during spermiogenesis.
- All the synthetic events occurring during spermiogenesis are probably regulated by stable RNA produced during the meiotic stages.
- The suppression of genetic activity in spermatids and spermatozoa may depend on a regulatory mechanism which causes disappearance of activating proteins and RNA from the chromosomes.
- The histone molecules associated with DNA may play a protective role by establishing the DNA against changes occurring in their transport through the male and female reproductive tracts.

Control of Spermatogenesis:

Spermatogenesis is either controlled environmentally or physiologically. Temperature, light, hormones and psychological state play an important role depending upon the organism. Pituitary gland plays an important role in regulating spermatogenesis by secreting certain gonadotrophin hormones. But the pituitary itself and the gonadal activities of birds, rodents, and many other vertebrates are affected by the temperature, light and the length of the day.

2.4 TYPES OF SPERM:

There are different types of sperm based on their characteristics, role, or morphology. Below is a detailed explanation of the various types:

1. Based on Fertility and Function:

- **Fertilizing Sperm**: These are healthy, motile sperm capable of fertilizing an egg. They have normal morphology and proper motility.
- **Non-Fertilizing Sperm**: These sperm are either immotile, have abnormal shapes, or are incapable of fertilizing the egg due to defects in structure or function.

2. Based on Morphology:

- **Normal Sperm**: Sperm with a standard head, midpiece, and tail. The head contains the genetic material, the midpiece supplies energy, and the tail ensures motility.
- **Abnormal Sperm**: Sperm with defects in the head (e.g., double head, giant head), midpiece (e.g., bent or irregular), or tail (e.g., coiled or absent). These abnormalities can impair fertility.

3. Based on Motility (World Health Organization categories):

- **Progressive Motile Sperm**: Sperm that swim actively and straight toward the egg.
- **Non-Progressive Motile Sperm**: Sperm that move but do not travel in a straight line.
- **Immotile Sperm**: Sperm that show no movement.

4. Based on Genetic Content:

- **X-Sperm**: Carry the X chromosome and result in female offspring (XX) when they fertilize an egg.
- **Y-Sperm**: Carry the Y chromosome and result in male offspring (XY) when they fertilize an egg. Y-sperm tend to swim faster but are less resilient compared to X-sperm.

4. Based on Role in Reproduction (Polyspermy Species):

- **Eusperm**: Functional sperm capable of fertilizing an egg.
- **Parasperm**: Sperm that do not fertilize the egg but play supportive roles, such as blocking rival sperm or aiding eusperm.

6. Based on Source:

- **Epididymal Sperm**: Immature sperm stored and matured in the epididymis.
- **Ejaculated Sperm**: Fully mature sperm released during ejaculation.
- **Testicular Sperm**: Sperm retrieved directly from the testes; often immature and used in assisted reproductive techniques.

7. Cryopreserved Sperm:

Sperm stored in liquid nitrogen for future use in artificial insemination or IVF.

2.5 LEYDIG CELLS, TESTOSTERONE, SETORI CELLS

1) Leydig cells

Leydig cells are embedded in groups that surround the connective tissue between seminiferous tubules in the testicle. These endocrine cells are the principal source of testosterone, the production of which is stimulated by LH.
In the testes, testosterone levels are highest at the basement membrane of the seminiferous tubules.

2)Testosterone

Testosterone, the major male androgen in circulation and in the Leydig cells, is responsible for primary and secondary sex characteristics.
Primary sex characteristics are structures responsible for promoting the development, preservation, and delivery of sperm cells while secondary sex characteristics are structures and behavioral features that externally differentiate men from women.

3) Sertoli Cells

Sertoli cells, also known as nurse cells, are highly specialized cells that regulate the development of spermatogonia into spermatozoa.
They originate from the tubular basement membrane and extend up toward the lumen of the seminiferous tubules.

Functions of the Leydig cells	Functions of Sertoli cells
Initiation and maintenance of spermatogenesis Activation of the hypothalamus-pituitary-gonadal axis Production of testosterone-manifestation of male secondary sex characteristics Differentiation of male genital organs Masculinization of the brain and sexual behaviour	Maintains the integrity of seminiferous tubules epithelium Secretion of hormones-inhibin and androgen-binding protein (ABP) Secretes tubular fluid into the tubular lumen for transport of sperm within the duct Delivery of nutrients to germ cells Steroidogenesis and steroid metabolism Aids in process of phagocytosis and elimination of cytoplasm Regulates the spermatogenic cycle Acts as a hormonal target for LH, FSH, and testosterone

2.6 SUMMARY

Spermatogenesis is the process of sperm cell production in the seminiferous tubules of the testes, starting at puberty and regulated by hormones like testosterone, FSH, and LH. It begins with the mitotic division of **spermatogonia** (stem cells), some of which differentiate into primary spermatocytes. These cells undergo two rounds of meiosis to form haploid spermatids, which then transform into mature spermatozoa through **spermiogenesis**, involving the development of the acrosome, flagellum, and nuclear condensation. Mature sperm are released into the tubule lumen and further matured in the epididymis, with the entire process taking approximately 64-72 days.

2.7 TECHINICAL TERMS:

Spermatogonia, Primary Spermatocytes, Secondary Spermatocytes, Spermatids, Spermatozoa, SertoliCells, Leydig Cells.

2.8 SELF-ASSESSMENT QUESTIONS:

1) Explain Formation of spermatids
2) What is spermatogenesis
3) Write short notes on:

a) Leydig cells
b) testosterone, satori cells.

2.9 FURTHER READINGS:

1. Austen CR and Short RV. 1980. Reproduction in Mammals. Cambridge University Press.
2. Gilbert SF. 2006. Developmental Biology, 8th Edition. Sinauer Associates Inc., Publishers, Sunderland, USA.
3. Schatten H and Schatten G. 1989. Molecular Biology of Fertilization. Academic Press, New York.

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LESSON - 3

OOGENESIS (OR) OOCYTE FORMATION

AIMS AND OBJECTIVES

- Understand the stages of oogenesis: formation, growth, and maturation of oocytes.
- Identify the roles of hormones such as FSH, LH, and estrogen in regulating oogenesis.
- Explain the cellular processes of mitosis and meiosis in oocyte development.
- Differentiate oogenesis from spermatogenesis in terms of timing, output, and regulation.
- Analyse the clinical significance of oogenesis and its role in fertility.

STRUCTURE:

3.1 INTRODUCTION:

It occurs in the ovary of female animals. It is comparable to spermatogenesis so far as nuclear changes are concerned. But the cytoplasmic specialization in oogenesis is different from spermatogenesis.

3.2 FORMATION OF OVUM:

Oogenesis is the process of developing a mature ovum (egg cell), which begins during the embryonic stage of a female's life and continues until menopause.

It is divisible into following three phases:

1) Multiplication phase
2) Growth phase
3) Maturation phase

1) Multiplication phase:

The primary germinal cells of the ovary with diploid number of chromosomes (2n) divide several times mitotically so as to form a large number of daughter cells known as oogonia.

- In the embryonic ovaries, primordial germ cells (PGCs) proliferate by mitosis to form **oogonia** (diploid cells).
- Oogonia are the precursors to primary oocytes.
- By the time of birth, all oogonia develop into **primary oocytes**, which are arrested in **prophase I** of meiosis. No new oocytes are formed after birth.

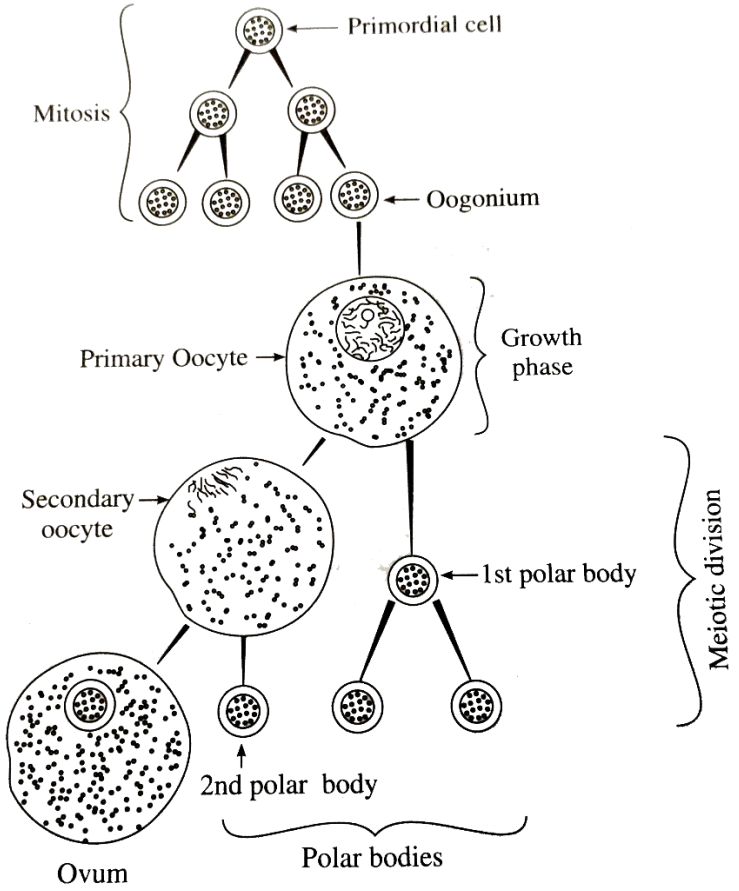

Figure 1: Oogenesis

2) Growth phase:

The oogonium does not divide but increases in size enormously to form a primary oocyte. The growth is associated with both nuclear and cytoplasmic growth. The nuclear growth is due to accumulation of large amount of nuclear sap and is termed as germinal vesicle. The cytoplasmic growth is associated with increase in number of mitochondria, endoplasmic reticulum and Golgi complex and accumulation of reserve food material called yolk or vitellin.

- The primary oocyte remains arrested in prophase I during childhood. During puberty, hormonal signals initiate the maturation of a selected number of primary oocytes in each menstrual cycle.
- The oocyte enlarges significantly due to the synthesis and accumulation of cytoplasmic organelles, RNA, proteins, and nutrients.
- Follicular cells surrounding the oocyte form structures like primary follicles, secondary follicles, and finally the Graafian follicle.

3) Maturation phase:

The primary oocyte undergoes two successive divisions by meiosis. The first division is meiosis-I and two unequal daughter cells are produced. The large cell is called secondary oocyte containing haploid (n) set of chromosomes (due to reductional or dis junctional division) and entire amount of cytoplasm. The smaller cell is called first polar body or polocyte containing 'n' number of chromosomes and practically no cytoplasm.

The secondary oocyte and first polar body then undergo second maturation division by meiosis-II which is an equational division. As a result of this division one large ovum is formed containing entire amount of cytoplasm and 'n' number of chromosomes and a second polar body like the first polar body.

Simultaneously, the first polar body may divide into two polar bodies or may not divide at all. Thus, only one functional ovum is formed and the two or three polar bodies soon degenerate. In vertebrates the first polar body is formed after the primary oocyte is released from ovary and has entered into the oviduct. The second polar body is formed only when the sperm enters into ovum during fertilization.

3.3 MATURATION OF OVUM:

Oogenesis is followed by the formation of protective coverings called egg membranes. Primary membrane is formed surrounding the plasma membrane of ovum and is secreted by the ovum itself. It is called vitelline membrane in frog and zona pellucida in rabbit. The secondary membrane called chorion is formed from ovarian follicle cells. The tertiary membranes are secreted in oviduct when the ovum passes from ovary to outside. The egg white (albumin), calcareous shell etc. come under this category.

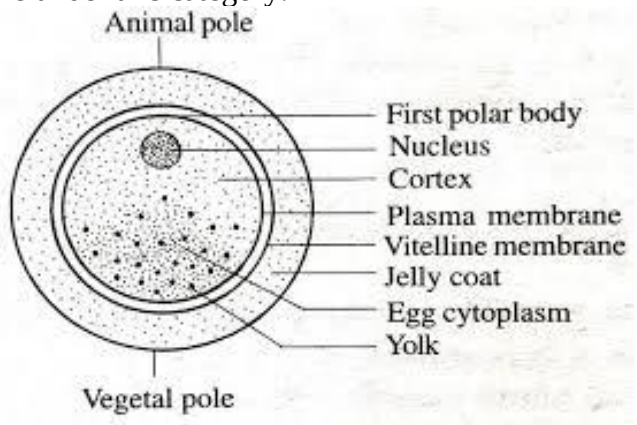

Figure 2: ovum

The ripe ovum is spherical or oval and non-motile. Depending upon the amount of yolk, it may be as small as 0.15 mm as in mammals (microlecithal); it may be 2 mm as in frog (mesolecithal) or it may be as large as 30 mm as in hen (megalecithal).

In a ripe ovum, the polarity is fixed. The top-most point is animal pole and the bottom point is vegetal pole. The density of yolky cytoplasm increases from the animal pole towards the vegetal pole. In frog, the animal hemisphere is highly pigmented and appears black while the vegetal hemisphere is highly pigmented and appears white.

Maturation of ovum involves 3 **stages**:

1. Nuclear Maturation:

- The egg undergoes meiotic division to achieve the haploid chromosome number.
- This ensures proper genetic material exchange during fertilization.

2. Cytoplasmic Maturation:

The cytoplasm grows and accumulates critical materials, including:

1) **mRNAs** for protein synthesis after fertilization.
2) **Proteins** required for cell division.
3) **Mitochondria** for energy production during early development.
4) Other organelles and signalling molecules.

3. Formation of Protective Layers:

1) The zona pellucida, a glycoprotein-rich protective layer, is formed around the oocyte. It regulates sperm binding and prevents polyspermy.
2) Follicular cells form a layer called the corona radiata, which provides nutrients and structural support.

Significance:

1. The process leads to formation of germ cells or gametes.
2. The normal body cells known as somatic cells are diploid (2n) whereas the germ cells are haploid (n).
3. During fertilization one haploid sperm unites with one haploid ovum to form a normal diploid somatic cell thus keeping the chromosome number constant generation after generation.
4. During first maturation division, the reshuffling of paternal and maternal genes take place resulting in variation.

3.4 PREVITELLOGENESIS:

Previtellogenesis refers to the stage in oogenesis that occurs before yolk deposition begins. It is characterized by:

- Active growth and metabolism within the oocyte.
- Accumulation of RNA, ribosomes, and proteins necessary for further development.
- Multiplication of organelles, including mitochondria, to prepare the oocyte for subsequent yolk synthesis and fertilization.
- The nucleus, called the germinalvesicle, enlarges and becomes metabolically active.
- This phase lays the groundwork for the later stages of oocyte growth and differentiation.

3.5 VITELLOGENESIS:

Vitellogenesis is the phase where yolk is synthesized and deposited into the growing oocyte. It is a critical step in ensuring that the egg has enough nutrients to sustain the embryo during early development. This process has two main components:

1. Endogenous Vitellogenesis:

- Yolk precursors are synthesized within the oocyte cytoplasm itself.
- Lipid droplets and granules are formed, contributing to yolk accumulation.

2. Exogenous Vitellogenesis:

- Yolk precursors like vitellogenin are synthesized in the liver (or similar organ) and transported to the oocyte through the bloodstream.
- The oocyte takes up these precursors via receptor-mediated endocytosis and converts them into yolk platelets.
- Yolk platelets consist of proteins, lipids, and carbohydrates, which provide nourishment for the embryo.

3.6 FORMATION OF YOLK:

Yolk formation is an essential process during oocyte development, where nutrient-rich materials are stored in the cytoplasm to support embryonic growth and development. It occurs primarily during the vitellogenesis phase of oogenesis. The yolk serves as a reservoir of vital nutrients, as early-stage embryos depend entirely on these stored resources for their metabolic and developmental needs before external nutrition or placental support becomes available.

The yolk is composed of proteins, lipids, and carbohydrates, each serving a distinct function. Proteins act as the primary building blocks for the growing embryo, facilitating the formation of cellular structures and enzymes. Lipids serve as a rich energy source, providing sustained energy reserves necessary for the prolonged developmental stages. Carbohydrates, though present in smaller quantities, also contribute additional energy to meet the metabolic demands of the

embryo.Within the oocyte cytoplasm, the yolk is stored in specialized structures known as yolk granules or yolk platelets. Yolk granules are small, spherical bodies containing a blend of proteins, lipids, and carbohydrates, while yolk platelets are larger, flattened structures predominantly composed of proteins and phospholipids. These storage structures organize and stabilize the yolk to ensure its availability for utilization during embryonic development.The process of yolk formation begins with the synthesis of yolk precursors, such as vitellogenin, in the liver or equivalent organs. These precursors are transported via the bloodstream to the developing oocyte, where they are absorbed through receptor-mediated endocytosis. Inside the oocyte, the precursors are processed into functional components and stored in granules or platelets. This process is often regulated by hormones like estrogens, which stimulate the synthesis and uptake of yolk precursors.The distribution and amount of yolk in the oocyte vary across species, reflecting differences in reproductive strategies and modes of development. For instance, human oocytes contain very little yolk, as embryonic nourishment is primarily provided by the placenta after implantation. In contrast, species like birds and reptiles, which lay eggs, have large amounts of yolk to fully sustain the embryo until hatching. This variation influences embryonic cleavage patterns and developmental pathways, with species showing distinct adaptations based on their ecological and reproductive needs.

3.7 FUNCTIONS OF EGG:

The egg is a crucial structure in reproduction and early development, performing several important functions that ensure the creation and growth of new life. One of its primary roles is contributing half of the genetic material required to form the zygote. This genetic information, combined with that of the sperm, results in the formation of a unique individual with a complete set of chromosomes. In addition to its genetic contribution, the egg provides a rich supply of cytoplasm filled with essential organelles, such as mitochondria, and other factors needed for the early stages of embryonic development. These components drive the processes of cell division and differentiation until the embryo becomes capable of functioning independently.

The egg also serves as a source of nutrition, especially in species where the embryo must develop outside the mother’s body. The yolk inside the egg provides the energy and nutrients required for the developing embryo to grow and mature. In mammals like humans, where yolk content is minimal, the egg supports the embryo until the placenta forms and takes over the role of providing nutrients. Protection is another vital function of the egg. Surrounding structures, such as the zona pellucida, protect it from physical damage and help prevent polyspermy, ensuring that only a single sperm fertilizes the egg. These protective measures safeguard the integrity of the egg and promote healthy development.

The egg also plays a critical role in establishing polarity, which is essential for the proper organization of the embryo. The distribution of substances in the egg determines its animal-vegetal axis, which later influences the formation of the body axis in the developing organism. This polarity helps guide how cells differentiate and organize during development, shaping the structure and orientation of the embryo. In all these ways, the egg is a remarkable and essential component of reproduction, laying the foundation for the continuation of life.

3.8 TYPES OF EGG:

Types of Egg

1. Based on the amount of yolk:

1) Microlecithal Eggs (Low yolk content):
- Found in mammals (including humans) where the embryo is nourished by the placenta after implantation.

2) Mesolecithal Eggs (Moderate yolk content):
- Found in amphibians where yolk supports early embryonic development until the larva can feed independently.

3) Macrolecithal Eggs (High yolk content):
- Found in birds and reptiles where yolk provides all nutrients until hatching.

Based on the distribution of yolk:

1) Solecithal Eggs:
- Yolk is evenly distributed throughout the cytoplasm.
- Found in species like humans and other mammals.

2) Telolecithal Eggs:
- Yolk is concentrated at one pole (vegetal pole), leaving the other pole (animal pole) relatively yolk-free.
- Found in birds, reptiles, and amphibians.

3) Centrolecithal Eggs:
- Yolk is concentrated in the centre of the egg.
- Found in insects.

- **Based on Fertilization:**

1. Fertilized Eggs:
Eggs that have undergone fertilization (e.g., most animals).

2. Unfertilized Eggs:
Eggs that can develop without fertilization in some species (e.g., parthenogenesis in bees).

- **Based on Development:**

1) **Oviparous Eggs**:
Eggs are laid outside the female body, and development occurs externally (e.g., birds, frogs).

2) **Viviparous Eggs**:
Eggs develop within the female body and are nourished directly by the mother (e.g., most mammals).

3) Ovoviviparous Eggs:

Eggs develop inside the female body but rely on yolk for nourishment (e.g., some sharks, reptiles).

3.9 SUMMARY:

Oogenesis is the process of egg formation in females, occurring in the ovaries. It begins before birth when oogonia (germ cells) multiply and form primary oocytes, which enter meiosis I but pause in prophase I. At puberty, hormonal cycles resume meiosis, and one primary oocyte completes meiosis I each month, forming a secondary oocyte and a polar body. The secondary oocyte begins meiosis II but halts at metaphase II, being released during ovulation. Meiosis II is completed only if fertilization occurs, resulting in a mature ovum and another polar body.

3.10 TECHINICAL TERMS:

Primordial Follicle, Polar Bodies, Ovulation, Corpus Luteum, oogonia.

3.11 SELF-ASSESMENT QUESTIONS:

1. Describe the process of ovum formation and maturation
2. What are previtellogenesis and vitellogenesis? Explain their significance in preparing the oocyte for fertilization.
3. Discuss the process of yolk formation and its importance in embryonic development
4. What are the different types of egg.

3.12 SUGGESTED READINGS:

1. AustenCRandShortRV.1980.ReproductioninMammals.CambridgeUniversityPress.
2. GilbertSF.2006. Developmental Biology, 8th Edition. Sinauer Associates Inc., Publishers, Sunderland, USA.
3. Schatten H and Schatten G. 1989. Molecular Biology of Fertilization. Academic Press, New York.

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LESSON - 4

ENDOCRINE REGULATION OF REPRODUCTION

AIMS AND OBJECTIVES:

- To understand the role of endocrine hormones in regulating spermatogenesis.
- To examine the functions of key hormones such as GnRH, FSH, LH, testosterone, and inhibin in male reproductive physiology.
- To explore the feedback mechanisms involved in hormone regulation during spermatogenesis.

STRUCTURE:

4.1 INTRODUCTION:

The endocrine regulation of spermatogenesis is a finely tuned physiological process essential for male fertility. It involves the interaction of hormones produced by the hypothalamus, pituitary gland, and testes. The hypothalamus secretes gonadotropin-releasing hormone (GnRH), which stimulates the anterior pituitary to release follicle-stimulating hormone (FSH) and luteinizing hormone (LH). These gonadotropins, in turn, regulate the production of testosterone in the testes and facilitate the development of sperm. Feedback loops, primarily involving testosterone and inhibin, help maintain hormonal balance. The combined action of these hormones ensures the proper development and maturation of sperm cells within the seminiferous tubules of the testes.

4.2 HORMONES ON REPRODUCTION:

I. Gonadotropin Releasing Hormone (GnRH)

GnRH is a neuropeptide (a decapeptide) that is produced in the hypothalamic surge and tonic centers. In the male and the female, the target tissue is the anterior pituitary gland, specifically Gonadotroph cells. In males and females, secretion of GnRH results in the release of Follicle Stimulating Hormone (FSH) and LuteinizingHormone (LH) from the anterior pituitary gland. GnRH-producing neurons are stimulated into production in response to spontaneous rhythms and by sensory impulses from sensory inputs derived from the external environment. Alterations in the internal conditions of the body can also result in

altered GnRH production. For example, in some species such as the sheep, there is seasonal sexual activity and the cerebral cortex, hypothalamus, pituitary and testes interact to regulate functions further along further the single chain.

In females when the estrogen concentration prior to ovulation reaches a certain threshold, large quantities of GnRH are released in the form of a surge. This results in a corresponding peak in LH that stimulates ovulation. In females this surge center is often called the preovulatorycenter Therefore another hormone produced from the developing ovarian follicle in the female and Sertori cells in the male acts as a negative feedback mechanism for FSH. Sex hormones also alter the level of production of GnRH from the hypothalamus via a negative feedback system. High concentrations of progesterone or testosterone will reduce the secretion of GnRH and also therefore the secretion of LH and FSH.

II. Luteinizing Hormone (LH)

LH is a type of glycoprotein that is produced in the anterior pituitary via gonadotroph cells and serves to regulate the function of the gonads. In males LH stimulates the production and secretion of testosterone from the testes via Leydig cells. In females LH stimulates the production of estrogens and progesterone from the ovary via theca internal cells and luteal cells. Concentrations of LH increase during ovulation and with the formation of the corpora lutea with progesterone secretion. The secretion of LH is regulated via the secretion of GnRH (see earlier section).

III. Follicle Stimulating Hormone (FSH)

FSH is a type of glycoprotein that is produced in the anterior pituitary via gonadotroph cells. FSH secretion is regulated by GnRH from the hypothalamus. The target tissue of FSH in males are the Sertori cells within the testes and in the female the granulosa cells of the ovary. FSH stimulates the maturation of germ cells within the testes and ovaries. In the female it also stimulates follicular Development and estradiol synthesis.

IV. Prolactin (PRL)

Prolactin is a protein that is produced from by the anterior pituitary via lactotroph cells. This hormone exerts a stimulatory effect on milk synthesis within the mammary glands. It has also been shown to have some degree of gonadal function in some domestic species and rodents. In birds increased concentrations of prolactin have been linked with brooding behaviors and the associated metabolic changes that birds undergo during brooding. Prolactin secretion is regulated by the hypothalamus which produces several neurohormones that affect prolactin concentrations. The most important within this is dopamine (or prolactin inhibitory hormone, PRL-IH) which exerts a totally dominant inhibitory action on prolactin synthesis. The hypothalamic regulation of prolactin secretion is via signals from the central nervous system. Prolactin synthesis is increased when the mother is suckling via a reflex stimulation of the teats. This stimulation reflex reduces the secretion of dopamine and increases the hormone prolactin releasing hormone (PRL-RH). Estradiol can also have an effect on the prolactin producing cells within the anterior pituitary and is responsible for increased concentrations of prolactin in females undergoing puberty and may also contribute to the increased concentrations during late pregnancy.

V. Oxytocin (OT)

OT is a neuropeptide (an octapeptide) which is synthesized in the hypothalamus and stored in the posterior pituitary. OT is primarily involved in upregulating the activity of smooth muscle cells in the uterus and the smooth muscles surrounding the alveoli ducts of the mammary glands. At parturition, OT causes strong contractions from the myometrium. OT is also essential for 'milk let-down'inmostdomesticspecies.

VI. Estradiol (E_2)

Estradiol (E_2) is a steroid hormone and is part of the estrogens group of hormones and is the principal estrogen in females. Estrone and estriol are chemically similar to estradiol but are found in lower concentrations and have a lower estrogenic activity. Production of estrogen's occurs in the ovary via granulosa cells, the placenta and the Zona reticularis of the adrenal cortex. In males in it is produced in Sertori cells found in the testes. Estradiol is synthesized from cholesterol.

VII. Progesterone (P_4)

Progesterone is a steroid hormone that along with estrogen is based on a cholesterol molecule produced by the corpus luteum and the placenta using cholesterol as the base molecule. Progesterone is produced by the corpus luteum as well as by the fetal-placental unit and in the zona reticularis of the adrenal cortex (to a lesser extent). More detailed information regarding corpus luteum formation and regression please use the links. Progesterone prepares the uterus for reception of fertilized oocytes and is transported via the blood bound to plasma proteins. Progesterone also prepares the mammary tissues for milk production as well as inhibiting female reproductivebehaviorsassociatedwithestrous.

VIII. Progesterone During Pregnancy

During pregnancy the plasma concentration of progesterone is maintained at an elevated level. Progesterone also inhibits secretion of FSH and LH (negative feedback at hypothalamic level by inhibiting GnRH) and thus also prevents the ovulation of follicles during the luteal phase and during pregnancy. In most domestic species the corpus luteum persists for the entire length of gestation. The exception to this rule is the mare in which the progesterone concentration falls during the later stages of pregnancy. This is due to the regression of the corpus luteum around day180ofthe330-340daygestationperiod. It is possible to use the relative concentration of progesterone as an aid to pregnancy diagnosis, for example in cattle. However, for a definitive diagnosis a high level of progesterone is required on two separate samples due to the overlap between the luteal phase and pregnancy.

IX. Testosterone (T)

The male sex hormone is called testosterone and this hormone is required for spermatogenesis. Testosterone is a steroid hormone that is produced in the Leydig cells within the testes. A relatively high concentration of testosterone is maintained within the testicular tissue and testosterone is circulated around the body by diffusion of the hormone from the spermatic cord into the testicular veins and arteries. The primary action of testosterone is anabolic growth, spermatogenesis promotion and promotion of secretion from the accessory sex glands.

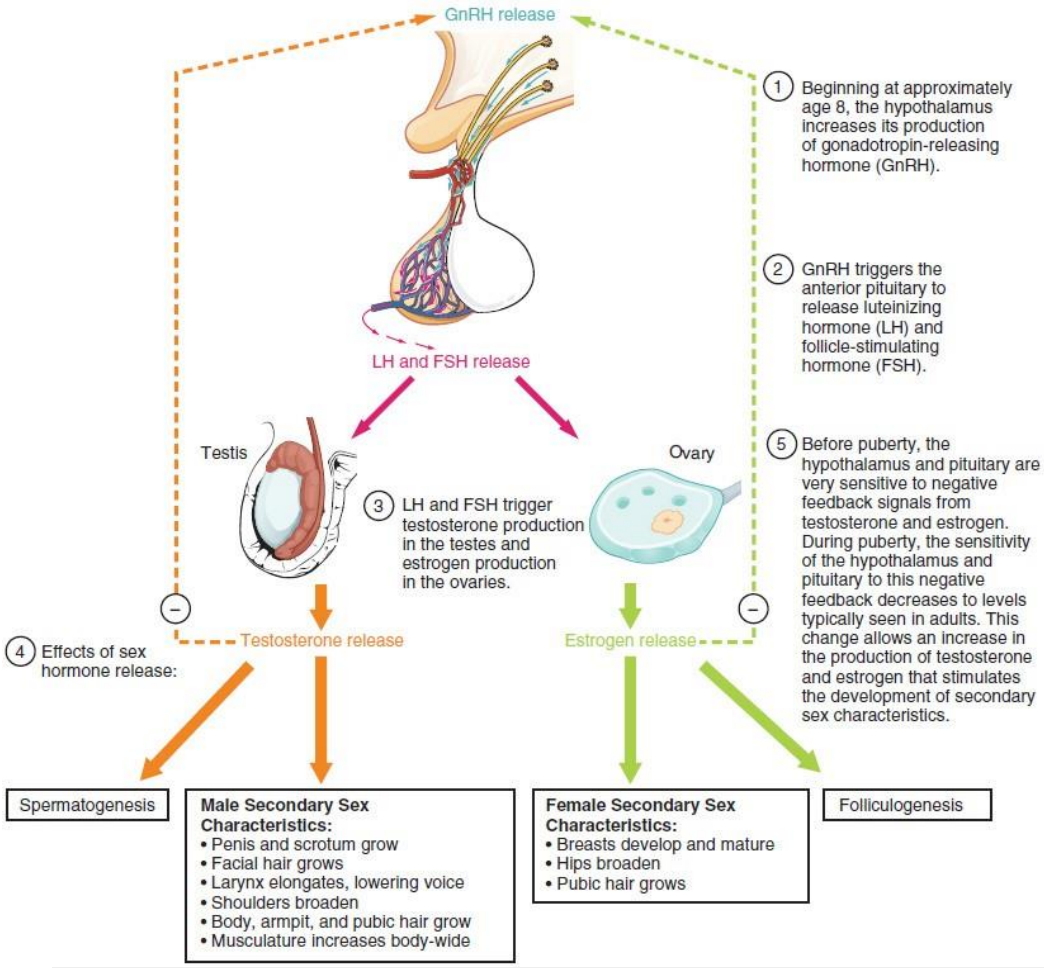

Figure 1: During puberty, the release of LH and FSH from the anterior pituitary stimulates the gonads to produce sex hormones in both male and female adolescents.

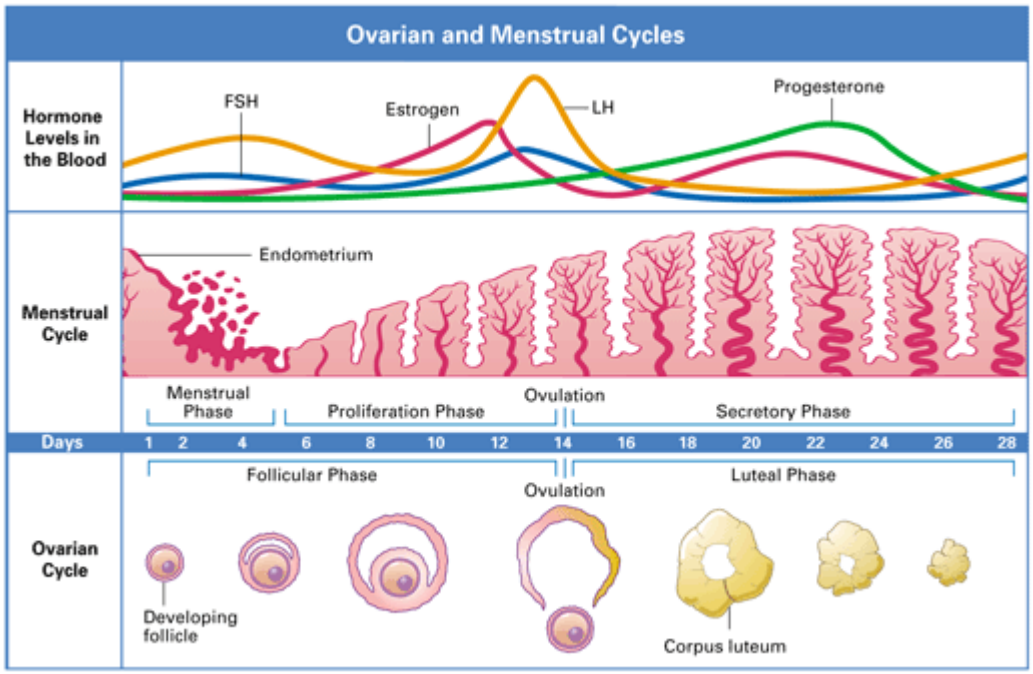

Figure 2: ovarian and mensural cycle

4.3 EFFECTS OF MALE SEX HARMONE:

I. TESTOSTERONE

Testosterone plays a crucial role in the development of male sex organs during fetal growth where increased production of testosterone causes penis growth and development of accessory sex glands during puberty. Testosterone also affects a number of other characteristics of the male, often called the "secondary sex characteristics". Testosterone is able to bind to receptors in the cytosol of cells in the same manner as other steroid hormones and these hormone-receptor complexes are then able to bind to DNA in the nucleus resulting in alterations in the level of transcription

II. Inhibin

Inhibin is a type of glycoprotein that is synthesized within the granulosa cells of ovarian follicles in females and in Sertori cells located in the seminiferous tubules within the testes in the male. In both males and females, the target organ for inhibin is the adenohypophysis, specifically the gonadotrophic cells (basophilic cells). In the male inhibin production is stimulated via androgens. Inhibin inhibits FSH secretion, which together with decreased concentrations of LH and testosterone results in decreased spermatogenesis and therefore decreased sperm output and quality.

In females some studies have suggested that inhibin may also be produced by the placenta. In females' inhibin inhibits FSH secretion. It does however not have any effect on the secretion of LH. When inhibin is secreted, a relatively higher concentration of LH is secreted from the anterior pituitary gland than FSH. Therefore, during follicle development, the increased LH concentration causes cessation of the recruitment of further follicles under the effect of FSH. The hormonal changes resulting from the production of inhibin cause some of the previously recruited follicles to and estrogen. Inhibin in the female can also be diminished by GnRH and enhanced by insulin-like growth factor-1 (IGF-1).

III. Prostaglandin $F_{2\alpha}$

Prostaglandin is a C_2O fatty acid and is produced within the uterine endometrium and vesicular glands. Estradiol stimulates prostaglandin synthesis while progesterone inhibits it. The target tissue in the female is the corpus luteum, uterine myometrium and ovulatory follicles. In the female $PGF_{2\alpha}$ cause luteolytic and can also cause the induction of tone and contractions within the uterus.It plays an important role in parturition in ruminants. If a pregnancy is to remain viable then luteolytic needs to be avoided and this is achieved where concentrations of $PGF_{2\alpha}$ remain below a threshold level allowing the corpus luteumto continue to secrete progesterone and thus maintain pregnancy. There are two main factors involved in the regulation of uterine secretions of $PGF_{2\alpha}$; oxytocin secretions from thecorpus luteum and molecules secreted by the developing embryo that facilitate the maternal recognition of pregnancy.

IV. Prostaglandin (PGE_2)

PGE_2 is another form of prostaglandin that is produced by the ovary, uterus and embryonic membranes. This form of prostaglandin also has other important roles including vasodilation,

smooth muscle relaxation, and inhibition of the release of noradrenaline from sympathetic nerve terminals.

In females its target tissue is the cervix (it is a potent cervical dilator), corpus luteum and the oviduct where it helps induce ovulation and the secretion of progesterone from the corpus luteum. PGE_2 also plays an important role during labor where it aids the softening of the cervix in animals with a soft-type cervix(equine and human) and aids stimulation of uterine contractions. It can thus be used to prepare the tract for parturition.

V. Human Chorionic Gonadotrophin (hCG)

hCG is a form of glycoprotein that is synthesized within the trophoblast cells of a blastocyst. hCG is particularly important in primate reproduction where it has a similar effect to LH in stimulating the continued production of progesterone and estrogen. This represents part of the system involved in fetal-maternal communication and pregnancy recognition. Primate blastocysts therefore produce hCG in relatively high concentrations during the first 3 months of pregnancy. hCG has also been suggested to play a role in defense of the embryo from the maternal immune system during the initial stages of pregnancy. In males hCG increases the growth of thefetaltestes. As hCG is only produced by embryonic cells, the presence of this hormone within maternal blood can be used for pregnancy confirmation.

VI. Placental Lactogen (PL)

Placental lactogen is a form of protein that is produced by the placenta and is chemically close in composition to growth hormone. The primary target tissue of PL are the mammary glands wherethey stimulate the growth of alveoliduring pregnancy. PL is also referred to as Chorionic Somatomammotropin (CS).

VII. Relaxin

Relaxin is produced mainly by the corpus luteum in most species and in the placenta(main contributor in the equine) and ovaries throughout pregnancy. During pregnancy relaxin prevents the initiation of uterine contractions, together with progesterone. Relaxin accumulates trough out pregnancy and is released in large amounts a few days before parturition. Its target organs are the cervix, vagina, pubic symphysis and related structures. Relaxin is responsible for the softening and relaxation of connective tissues in the cervix, muscles and ligaments in the pelvis prior to parturition. Estradiol priming is required for this. This relaxation of tissues via relaxin is performed in conjunction with prostaglandin.

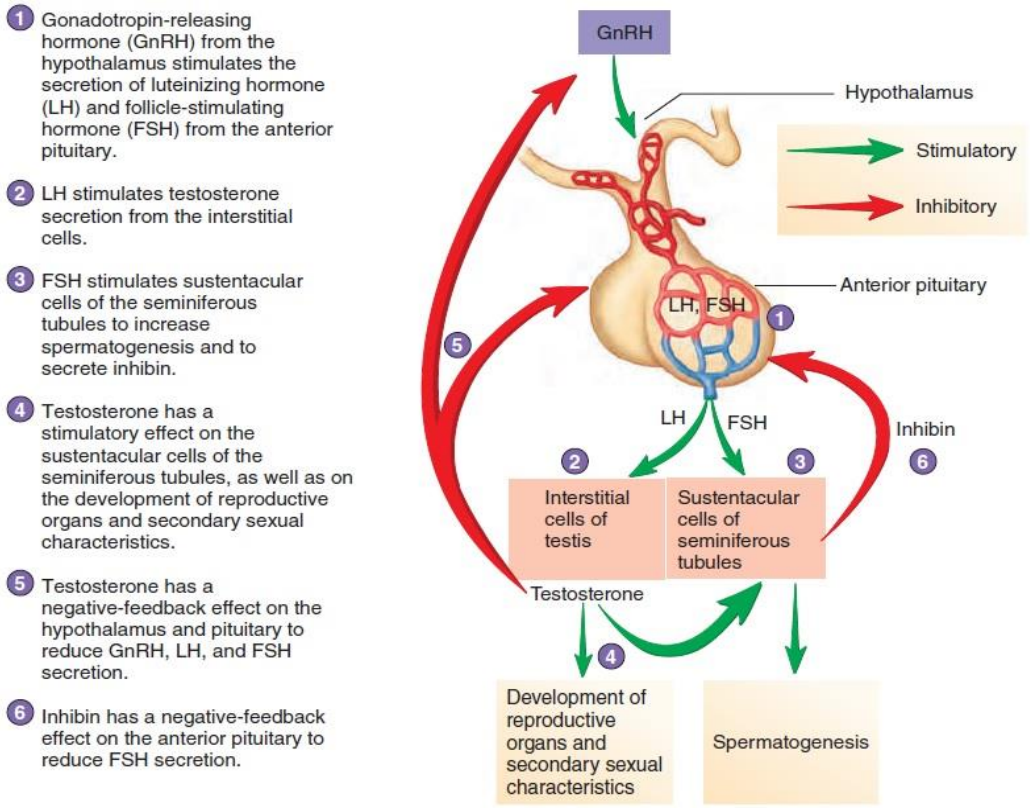

Figure 3: Regulation of reproductive hormone Secretion in male

4.4 SUMMARY:

Spermatogenesis is a complex process where primordial germ cells mature into functional spermatozoa in the testes. This process is tightly regulated by a hormonal axis involving GnRH, FSH, LH, and testosterone. GnRH from the hypothalamus triggers the release of FSH and LH from the anterior pituitary. FSH promotes the activity of Sertoli cells, which support germ cell development, while LH stimulates Leydig cells to produce testosterone, which is essential for spermatogenesis and secondary male characteristics. Inhibin, produced by Sertoli cells, provides feedback to suppress FSH secretion. This integrated hormonal network ensures optimal sperm production and male reproductive function.

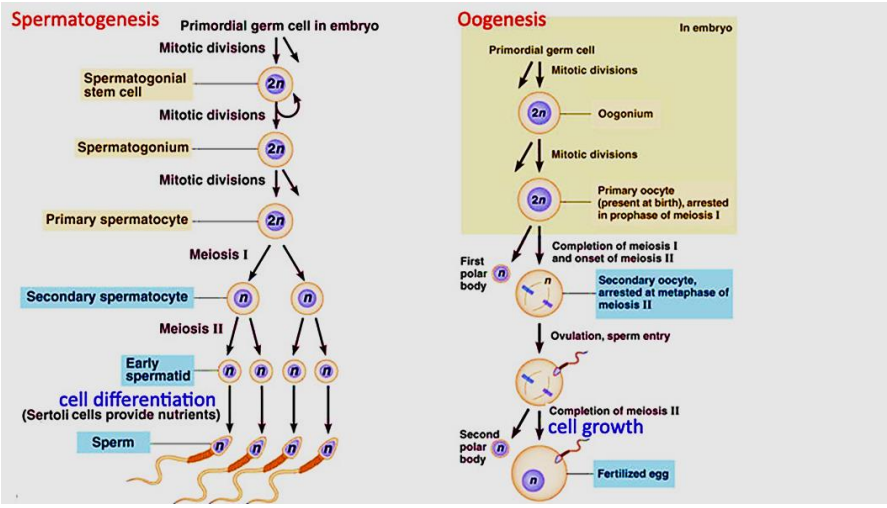

Figure 4: Stages of Gametogenesis

4.5 TECHINICAL TERMS:

Gonadotropin-Releasing Hormone (GnRH), Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LH), Prolactin (PRL), Oxytocin (OT), Corpus Luteum.

4.6 SELF-ASSESSMENT QUESTIONS:

1. What role do gonadotropins (FSH and LH) play in the regulation of spermatogenesis?
2. What are the effects of male sex hormone?

4.7 SUGGESTED READINGS:

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Prof. M. Jagadish Naik

LESSON-5

FERTILIZATION

AIMS AND OBJECTIVES

1. To understand the role of specific cell surface molecules in mediating sperm-egg recognition.
2. To identify the molecular interactions that ensure species-specific fertilization.
3. To explore how these molecules contribute to binding and fusion processes.
4. To understand the sequential events in the fertilization process.
5. To examine the mechanisms of sperm entry, egg activation, and zygote formation.
6. To analyze how fertilization restores diploidy and initiates embryogenesis.

STRUCTURE:

5.1 INTRODUCTION:

Sperm-egg recognition is the initial and highly specific step in fertilization. This interaction is mediated by specialized cell surface molecules present on both gametes. These molecules play a pivotal role in ensuring species specificity, as they recognize and bind to complementary molecules on the opposing gamete. This specificity prevents cross-species fertilization and ensures successful reproduction within a species. In animals like sea urchins, **bindin** on the sperm and its receptor on the egg mediate this recognition. In mammals, molecules such as **ZP glycoproteins** (e.g., ZP3 in the zona pellucida) and **Izumo1** on sperm are critical for recognition and fusion.

Fertilization is the union of haploid sperm and egg to form a diploid zygote, marking the beginning of a new organism. The process involves several mechanisms: sperm-egg recognition, binding, membrane fusion, egg activation, and pronuclear fusion. These events are tightly regulated and involve cellular and molecular changes that ensure genetic material is correctly combined and development is initiated.

5.2 CELL SURFACE MOLECULES IN SPERM-EGG RECOGNITION:

Fertilization is a complex process involving specific molecular interactions between sperm and egg cells. These interactions are mediated by surface molecules on both the sperm and

the egg, ensuring successful recognition, adhesion, and fusion. In mammals, the process of sperm-egg recognition is highly coordinated and relies on specific molecules present on the surfaces of both sperm and egg cells.

Sperm surface molecules:

On the sperm's surface, proteins like **Izumo1** play a crucial role in fusion by binding to **Juno**, a receptor on the egg. Other important molecules include **Fertilization Antigen (FA-1)**, which helps the sperm attach to the zona pellucida, and **SPAM1**, which facilitates the sperm's penetration through this protective layer by breaking it down with enzymes. Proteins from the **ADAM family** (like ADAM1, ADAM2, and ADAM3) aid in adhesion and help the sperm navigate through the female reproductive tract. Additionally, **CRISP proteins** contribute to adhesion and the regulation of the acrosome reaction, a critical step where enzymes like hyaluronidase and acrosin are released to help the sperm penetrate the zona pellucida.

Egg surface molecules:

The egg's surface also has key players in this process. The **zona pellucida**, a glycoprotein matrix surrounding the egg, consists of proteins such as **ZP1, ZP2, ZP3, and ZP4**, which are essential for sperm recognition and attachment. Among these, **ZP3** is particularly important as it binds to sperm and triggers the acrosome reaction. After the sperm penetrates the zona pellucida, **ZP2** facilitates further binding, directing the sperm toward the egg's plasma membrane. At this point, **Juno**, a receptor on the egg surface, binds to **Izumo1** on the sperm, ensuring proper recognition and attachment. The final step is the fusion of the sperm and egg membranes, which is supported by the tetraspanin protein **CD9**, allowing the sperm's nucleus to enter the egg.

Other surface molecules:

Integrins and tetraspanins (apart from CD9) may assist in the fusion process, though their roles are less well-defined.

5.3 MOLECULAR STEPS OF SPERM-EGG INTRACTIONS IN ANIMALS

1. Initial Contact and Binding

- Sperm binds to the zona pellucida using surface proteins like **SPAM1**.
- Zona pellucida proteins (primarily **ZP3**) act as receptors for sperm binding.
- This interaction is species-specific.

2. Acrosome Reaction

- Triggered by the binding of sperm to **ZP3**.
- The acrosome, a cap-like structure on the sperm head, releases enzymes such as hyaluronidase and acrosin.
- These enzymes digest the zona pellucida, enabling the sperm to penetrate it.

3. Sperm Penetration Through the Zona Pellucida

- Sperm progresses through the zona pellucida, assisted by acrosomal enzymes and motility.
- Secondary binding between sperm and **ZP2** ensures progression.

4. Binding to the Egg Plasma Membrane

- After penetrating the zona pellucida, sperm reaches the egg plasma membrane.
- **Izumo1** on the sperm binds to **Juno** on the egg, forming a tight interaction.

5. Membrane Fusion

- Fusion of the sperm and egg membranes occurs, facilitated by **CD9** and associated proteins.
- Sperm contents, including the nucleus, are released into the egg cytoplasm.

Importance of These Molecules

Ensure **species-specific fertilization**, preventing cross-species fertilization.Facilitate a highly regulated sequence of events critical for successful fertilization.Disruption of these molecules (e.g., through genetic mutations) can lead to infertility.

Molecule	Source	Function
Izumo1	Sperm	Sperm-egg membrane fusion
Juno	Egg	Receptor for Izumo1
ZP3	Egg	Triggers acrosome reaction
ZP2	Egg	Secondary sperm binding
CD9	Egg	Facilitates membrane fusion
SPAM1	Sperm	Zona pellucida binding
ADAM Proteins	Sperm	Adhesion to the zona pellucida
CRISP Proteins	Sperm	Adhesion and acrosome reaction

These molecules work in a coordinated manner to ensure successful fertilization in mammals.

1.4 MECHANISM OF FERTILIZATION:

Special features of the gametes for fertilization: Both egg and sperm acquire structural specializations for fertilization. Eggs are non-motile, surrounded by protective egg coverings. These serve to recognize the sperm specifically and prevent fertilization by more than one sperm (polyspermy). The mammalian eggs zona pellucida layer around the plasma membrane beneath which cortical granules are present. The zona pellucida layer makes the egg impenetrable to more than one sperm. Sperms are highly motile cells consisting of nucleus, mitochondria to provide energy source and a flagellum for movement. The anterior end of the sperm is highly specialized which aims in penetration of the egg. Sperms are typically designed to activate the egg and to deliver their nuclei into the egg cytoplasm.Basic requirements of fertilization: Fertilization requires a fluid medium in most animals. It maybe seawater in marine forms, fresh water in fresh water forms and body fluid in viviparous animals.To increase the probability of fertilization, the number of sperms must exceed the number of eggs.Moreover, the lifespan of gametes is limited; therefore, fertilization must take place within a shortduration of time. Eggs that are shed in water like that of most invertebrates, fishes and amphibians,have shorter life span while those fertilized within the body of female, generally have longer life span.

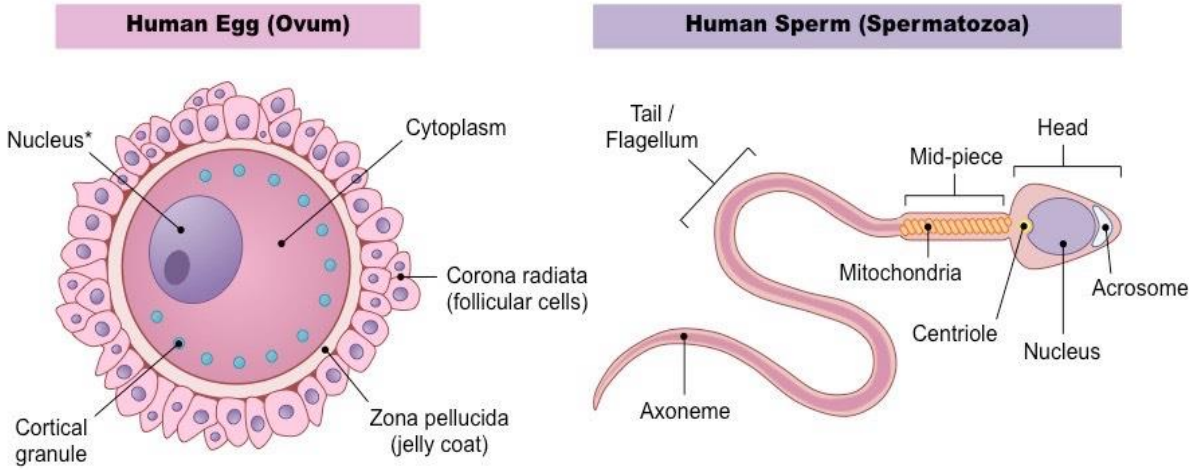

Figure 1: Ovum and Sperm

The site of fertilization:

Fertilization may be either **external** or **internal**. In **external fertilization** thegametes are discharged in the aquatic medium and the fertilization occurs outside the body of bothmale and female parents, as in most invertebrates and some vertebrates (fish and frog). The aquaticmedium for external fertilization may be either seawater or fresh water. When fertilization occursinside the body of female parent, it is **internal fertilization**, as in Drosophila, birds and mammals.

The process of fertilization has been mostly studied in invertebratessuch as sea urchins and in vertebrates like amphibians and mammals. Fertilization begins with theapproach of the sperm to the egg and ends up with the formation of diploid zygote. The process offertilization requires five general events:

1. Recognition of egg and sperm (approach of spermatozoan to the egg, attachment and binding)
2. Acrosome reaction and penetration
3. Fusion of plasma membranes of egg and sperm.
4. Activation of egg
5. Fusion of egg and sperm pronuclei

1. Recognition of egg and sperm (approach of spermatozoan to the egg, attachment and binding)

During internal fertilization, such as in mammals, the gametes of both sexes are deposited in thefemale reproductive tract. The fluid movements within the reproductive tract, assist in transportingthe gametes to the site of fertilization.Sperm attraction: In many animals, sperms are attracted towards eggs of their species by“chemotaxis” i.e. following a gradient of a chemical secreted by the egg. Chemotaxis has beendemonstrated in cnidarians, molluscs, echinoderm and urochordates (Miller, 1985; Yoshida et al,1993). Similarly in the egg jelly of the sea urchin, chemotactic factors are present for spermattraction. A chemotactic factor called resacet, a 14-amino acid peptide, has been isolated fromthe egg jelly of sea urchin Arbacia punctulataThey diffuse readily into seawater and are species specific.

Fertilizin and Anti fertilizin interactions:

Factors that mediate sperm– egg interactions evenbefore they make contact were identified by F.R. Lillie (1912). He proposed the first theory ofphysiology of fertilization called fertilization theory. He observed that the egg water (seawater surrounding unfertilized sea urchin eggs), agglutinated the sperm and activated their motility. Thereaction was species specific. This factor called fertilizin came from the egg jelly coat. It slowlydissolved as in sea water. Fertilizin was later shown to be the constituent of both jelly coat and eggmembrane such as vitelline membrane and plasma membrane. Fertilizin is a proteoglycan. Both theamino acids and monosaccharides of fertilizin vary from one species to another so that each speciespossesses its specific type of fertilizin. Each molecule of fertilizin has more than one ‘active group’s so that one fertilizin particle may attach to two or more sperms and bind them together.

- **Acrosome reaction and penetration:**

The acrosomal reaction in sea urchinOnce the sperm makes contact with the egg, then it has to penetrate surface coats that surroundthe egg. The penetration is facilitated by the acrosome reaction in which the membrane enclosingthe acrosome is shed, releasing the contents of acrosome. The acrosomal reaction involves twoprocesses:

- exocytosis of acrosomal vesicle and
- extension of acrosomal process

Exocytosis of acrosomal vesicle:

Contact of the sperm with the egg jelly coat component, a fucose containing polysaccharide triggers the acrosome reaction and causes influx of calcium into the sperm head. This initiates fusion of the outer acrosomal membrane with sperm plasma membrane and ultimate breakdown of acrosomal vesicle. Hydrolytic enzymes called lysins presenting the acrosomal vesicle, are released. Lysins digest the egg envelope locally and clear the path for spermatozoa to reach the egg surface (vitelline membrane). The exocytosis of acrosomal vesicle is thus caused by calcium – mediated fusion of acrosomal membrane with the adjacent sperm plasma membrane. The egg jelly factors that stimulate the acrosome reaction are highly species specific.

Extension of acrosomal process:

The extension of the acrosomal process is a crucial event during fertilization, particularly in species like sea urchins and some mammals. It begins with the acrosome reaction, triggered when sperm encounters the egg's outer layer, such as the jelly coat or zona pellucida. This reaction involves the release of hydrolytic enzymes from the acrosome, a cap-like structure on the sperm head, to digest the egg's protective layers. Following this, actin filaments within the sperm head polymerize, forming the acrosomal process, a filamentous structure that extends outward. The tip of this process carries binding proteins, such as bindin in sea urchins, which recognize and attach to specific receptors on the egg's surface, ensuring species-specific interaction. This extension facilitates the fusion of the sperm and egg membranes, allowing the sperm nucleus and other cellular components to enter the egg,

ultimately leading to fertilization. The acrosomal process is essential for penetrating the egg's barriers and enabling successful reproduction.

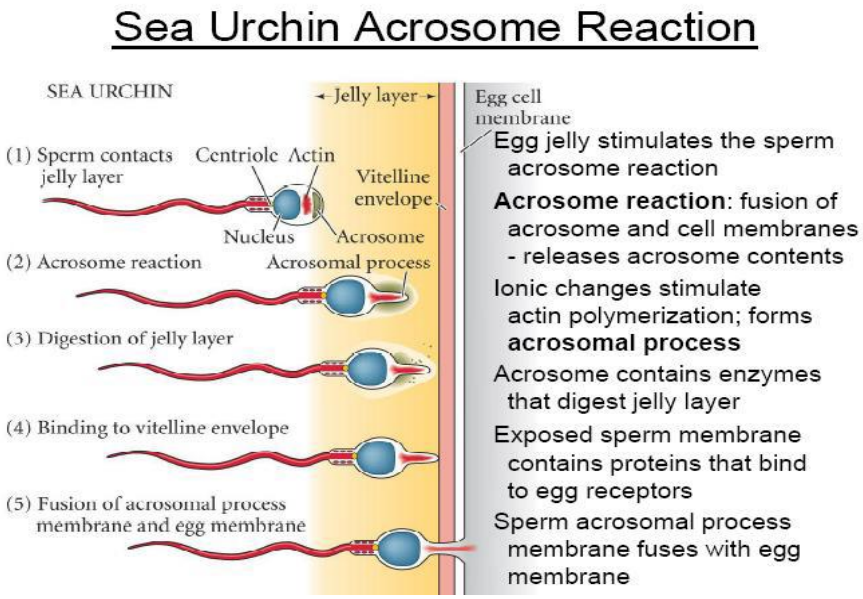

Figure 2: Sea urchin acrosome reaction

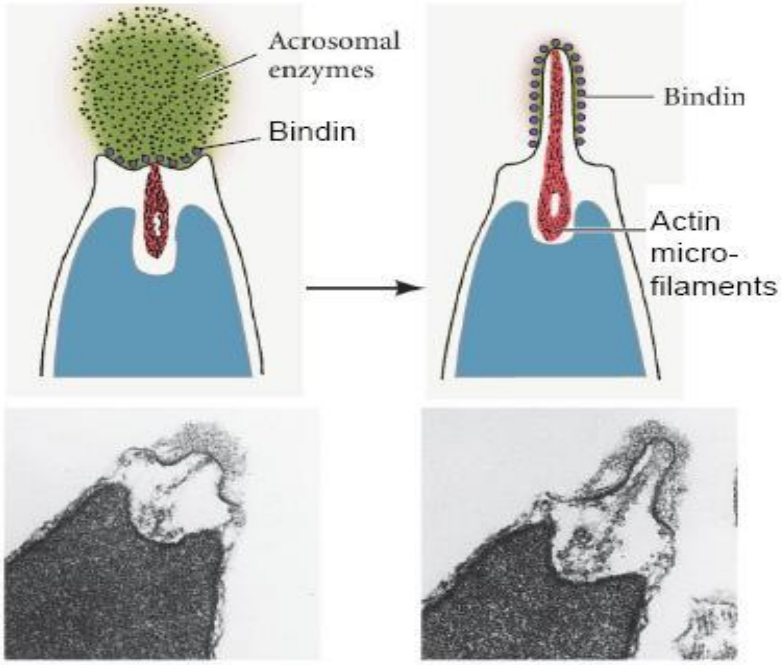

Figure 3: Acrosome reaction

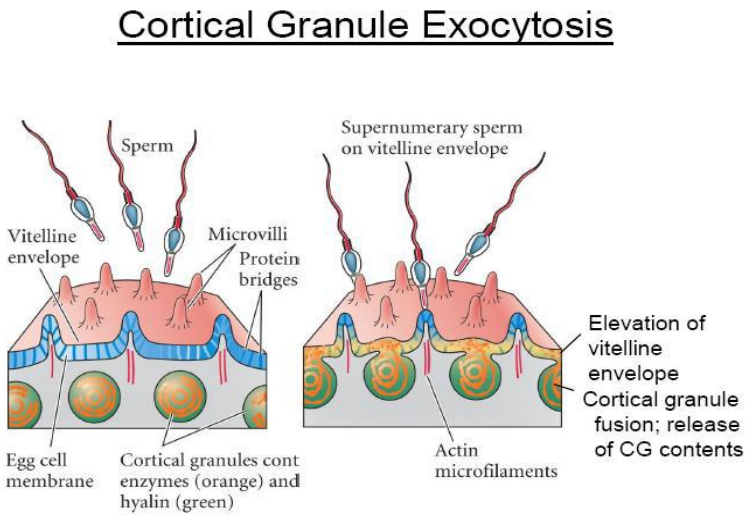

Figure 4: Cortical granule reaction

Gamete binding and recognition in mammals:

A. Capacitation

In mammals' fertilization is internal. The reproductive tract plays a very active role in fertilization.The differentiated sperms are unable to undergo the acrosome reaction without residing for sometime in the female reproductive tract where they undergo physiological changes. The change in themammalian spermatozoan, which makes it capable of fertilizing the egg, is called capacitation.There are four sets of molecular changes, which take place during capacitation:

- Albumin proteins, present in the female reproductive tract, remove the cholesterol there by altering the fluidity of sperm plasma membrane.
- Certain proteins or carbohydrates on the sperm surface are lost during capacitation.
- Membrane potential of the sperm becomes more negative, as potassium ions leave the sperm.

This change in membrane potential opens up the calcium channels and allows calcium to enter the sperm facilitating the process of membrane fusion during acrosomal reaction. Protein phosphorylation occurs. However, it not known whether these events are independent of one another and to what extent each one of them causes sperm capacitation's) Gamete binding. The mammalian egg is surrounded by extracellular envelope called zona pellucida. Around zona pellucida is a layer of cumulus cells (corona radiate) embedded in a cementing substance, hyaluronic acid. Hyaluronidase activity on the surface of the sperm head helps it to penetrate this layer. Next, sperm must bind to zona pellucida before they make contact with the surface of egg itself. The zona pellucida in mammals plays a role analogous to that of vitelline envelope in lower vertebrates and invertebrates. The zona pellucida is a glycoprotein matrix synthesized and secreted by the growing oocyte. It plays two important roles in fertilization. It binds the sperm and initiates acrosome reaction.

Acrosome reaction in mammals

Binding of the spermatozoa triggers the acrosome reaction, which allows the sperm to penetratethe zona. Acrosome reaction in mammals involves the fusion of the outer membrane of theacrosome with the sperm plasma membrane. After the fusion, the acrosomal membrane

vesiculates which results in the release of acrosomal contents. Subsequently, the outer portion of the acrosomal membrane disappears and only the inner portion adjacent to the nucleus remains intact. When the acrosomal contents are exocytosis, several enzymes are released. These enzymes allow the spermto approach the egg plasma membrane. The mammalian acrosome reaction differs fromthat of sea urchin in that no acrosomal process is formed.

Gamete fusion & Prevention of polyspermy

Sperm & egg plasma membrane fusion. After penetration of the extracellular layers by sperm, there occurs the fusion of sperm plasmamembrane with that of the egg.In sea urchin, all regions of the egg plasma membrane are capable of fusing with sperm Thecytoplasm of the egg bulges forward at the point of contact producing a process of hyalinectoplasm called the fertilization cone. Sperm egg fusion appears to cause the polymerization ofactin into microfilaments and extension of several microvilli to form the fertilization cone.Fertilization cone and microfilaments facilitate sperm entry. The sperm and egg membrane join together forming cytoplasmic bridge. The sperm nucleus and tail pass through the cytoplasmic bridge, which is widened by the actin polymerization. The prevention of polyspermy. The entry of the sperm into the egg activates the egg.

- **The fast and temporary block to polyspermy**

The fast block is a temporary measure, which is mediated by a transient depolarization of the eggplasma membrane, caused by sperm-egg fusion. Within 1-3 seconds after entry of the first spermthe electrical membrane potential across the egg plasmamembrane shifts from–70 mvto +20 mv.This change is caused by a small influx of sodium ions into the egg & lasts for about 60 seconds afterwhich the membrane potential returns to its original level. Some acrosomal proteins of sperm openthe sodium channel in the egg that causes influx of sodium ions into the egg & depolarizes the eggmembrane. This results in the fast block to polyspermy.

The slow & permanent block to polyspermy.

The fast block to polyspermy is for a very short duration (about a minute only). This brief period is not sufficient to prevent polyspermy. Therefore, the fast block to polyspermy is supplemented by asecond mechanism, known as cortical reaction. It is a slower mechanical block to polyspermy.Sperm entry into the sea urchin egg results in the release of intracellular calcium ions that arestored in the endoplasmic reticulum in egg cortex. The calcium ions are first released at the site ofspermentry and within a minute, a wave of calcium ions traverses the entire egg. This wave ofreleased calcium ions initiates cortical reaction. The cortical reaction consists of a wave ofexocytosis of cortical granules, which are present just beneath the plasma membrane in the mature egg. The cortical granules fuse with the egg plasma membrane and release their contents into the perivitellinespace. This space lies between the plasma membrane and the vitelline envelope.

Several proteins are released by this cortical granule exocytosis, which are as follows:

- Proteolytic enzymes (proteases) released, break the bonds that bind the vitelline envelope totheegg plasmamembrane. This creates a perivitelline space. These enzymes also clip off the bindinreceptors and any spermattached to it.mechanisms the fast block, which is initiated by sodium influx into the cell, and the slow blockinitiated by the intracellular release of calcium ions. Within one second, the membrane potential ofegg

rises and sperm-egg fusion takes place within 6 seconds followed by cortical vesicleexocytosis within 15- 60 seconds.

- Ionic changes: certain intracellular changes occur in the concentration of cations such as sodium,potassium and calcium. There is increase in pH (remains high). The change in calcium ionconcentration has great significance in the metabolic activation of the egg.
- Activation of protein and DNA synthesis: there is increase in the rate of protein synthesis by utilizing mRNA already present in the oocyte cytoplasm.

- After 5 minutes of fertilization, the rate of protein synthesis increases three to twelve folds. About 20 minutes after fertilization DNA synthesis is initiated.
- Resumption of meiosis: in most animal's meiosis is arrested at a particular stage and resumes onlyafter fertilization. The time of fertilization varies from species to species. It has been found thatspermatozoan may enter the egg at different stages of maturation in different animals.
- Initiation of mitosis: the initiation of mitosis occurs because (a) the rate of DNA synthesis increases after fertilization and (b) by the contribution of centriole by sperm to the egg, which is needed for proper mitosis.

Fusion of plasma membranes of egg and sperm.

In sea urchins, the sperm nucleus enters the egg perpendicular to the egg surface. After entry, thesperm nucleus and its centriole separate from the mitochondria and flagellum. The mitochondriaand the flagellum disintegrate inside the egg. Second meiotic division is completed after the entry ofthe sperm and the resulting haploid egg nucleus is known as female pronucleus. The sperm nucleusonce inside the egg, undergoes several changes and becomes male pronucleus. Its nuclear envelopebecomes vesicular. De-condensation of chromatin and the formation of pronuclear envelope takeplace. The male pronucleus inside the egg rotates at 180 degrees so that the sperm centriole comesto lie between male pronucleus and the female pronucleus. The female pronucleus is located in thecentre of the egg and male pronucleus is in the cortical region of egg at the site of sperm entry. Forfusion the male pronucleus has to travel a considerable distance through the egg cytoplasm to re female pronucleus. The sperm asters mediate this movement. The sperm aster is a complex of longmicrotubules that radiate from the sperm centriole. The sperm centriole acts as a micro tubule organizing centre for sperm aster. The fusion of male and female pronuclei is called amphimixis.

In mammals the entry of sperm is tangential to the eggs surface. Once inside the egg, the sperm nucleus becomes male pronucleus. The sperm centrosome produces asters and contacts the female pronucleus. Male & female pronuclei migrate towards each other, become apposed but do not fuse. They remain adjacent to each other; their nuclear envelopes break down but instead of forming a zygote nucleus the chromatin condenses into chromosomes orienting them on a common mitotic spindle. Thus, only after completion of the first division of fertilized egg, the paternal and maternal chromosomes become enclosed by a common nuclear membrane to form the nuclei of twoblastomeres.

Rearrangement of egg cytoplasm:

One of the consequences of egg activation during fertilization is reorganization of egg cytoplasm. Inmammals or sea urchin eggs, the cytoplasmic movements are not obvious but in

several other animals these cytoplasmic displacements are crucial for the determining cell fate during development. Most spectacular cytoplasmic movements have been observed in ascidian, Stylea partita and in frog. In both these animals, a bilateral symmetry is established in the cytoplasm of fertilized egg. Preparation for cleavage:Before fertilization, the egg, which has been under metabolic arrest, is released from this arrest on the entry of the sperm. This initiates the process of development by active protein and DNAsynthesis in the egg leading to the beginning of cleavage. The first cleavage is not random but tends to be specified by the point of sperm entry and the subsequent rotation of the egg cytoplasm.

Activation of egg:

Activation of the Egg is a complex and highly regulated process that prepares the egg for the next stages of development after fertilization. This process begins when the sperm and egg fuse, triggering a cascade of intracellular signalling events, most notably a sharp and sustained rise in calcium ion concentration within the egg. This calcium release originates from the endoplasmic reticulum and spreads as a wave across the egg's cytoplasm, a phenomenon essential for activating various biochemical pathways. One of the first consequences of this calcium surge is the **cortical reaction**, where cortical granules located beneath the egg's plasma membrane undergo exocytosis. The contents of these granules modify the extracellular matrix surrounding the egg, such as the zona pellucida in mammals or the vitelline envelope in invertebrates. This modification prevents additional sperm from entering the egg, thereby establishing a block to polyspermy and ensuring that only one sperm contributes its genetic material.

Simultaneously, calcium signals induce the resumption and completion of meiosis, a process that had previously been arrested at a specific stage, such as metaphase II in most mammals. The egg progresses through the remaining steps of meiosis, resulting in the formation of a haploid female pronucleus and the extrusion of a polar body, which eliminates excess genetic material. Alongside these changes, the metabolic activity of the egg increases dramatically. Maternal mRNAs stored within the egg are translated, producing proteins necessary for early embryonic development. Other events, such as cytoskeletal rearrangements, occur to prepare the egg for pronuclear migration. Microtubules and microfilaments play critical roles in positioning cellular components and supporting the structural reorganization required for the later fusion of pronuclei. These activation events collectively ensure that the egg is metabolically and structurally prepared for its transition into a zygote.

Fusion of Egg and Sperm Pronuclei:

Fusion of Egg and Sperm Pronuclei is the culmination of the fertilization process and is essential for the formation of a genetically complete zygote. After the sperm penetrates the egg, its tightly packed chromatin begins to decondense, and the sperm nuclear membrane disassembles. Concurrently, the egg completes meiosis and forms its haploid pronucleus. The sperm contributes a centrosome, which organizes microtubules into an aster that guides both the male and female pronuclei toward each other. These microtubules form a dynamic network that ensures the precise alignment and migration of the pronuclei to the centre of the egg.

When the male and female pronuclei meet, their nuclear envelopes dissolve, allowing the chromosomes from both gametes to intermix. During this process, the chromatin of the sperm, initially packaged with protamine's for tight condensation, is restructured using

histones provided by the egg. This repackaging ensures that the sperm's DNA is compatible with the egg's cellular machinery. Before the pronuclei fuse, their DNA is replicated to prepare for the first mitotic division. Following fusion, the resulting diploid nucleus organizes on a shared mitotic spindle, marking the zygote's transition to the first cleavage division.

The fusion of pronuclei restores the diploid chromosome number, ensuring genetic continuity between generations and combining genetic material from both parents. This process is also the starting point of the zygote's journey through mitotic divisions, leading to the formation of a multicellular organism. The coordination of events during egg activation and pronuclear fusion is critical for successful fertilization, ensuring the egg transitions into a zygote with the potential for normal embryonic development.

5.5 SUMMARY:

Sperm-egg interactions follow a series of molecular steps, from binding to the egg's surface, triggering the acrosome reaction, penetrating the protective layers, and fusing membranes. These steps involve precise molecular coordination to ensure successful fertilization.
The mechanism of fertilization involves sperm-egg recognition, penetration, and fusion, followed by egg activation and zygote formation. This process restores the diploid chromosome number and initiates embryogenesis, ensuring the continuity of life.

5.6 TECHINICAL TERMS:

Zona Pellucida, Bindin, Izumo1 and Juno, Species Specificity, Polyspermy,

5.7 SELF-ASSESSMENT QUESTIONS:

1. Describe various steps of fertilization in mammals?
2. Define the acrosome reaction and its significance in fertilization?
3. Describe the processes of egg activation and their importance in fertilization?

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Prof. K. Sunitha

LESSON - 6

MOLECULAR EVENTS DURING FERTILIZATION

AIMS AND OBJECTIVES

1. Understand the molecular events that occur during fertilization and their significance.
2. Explore the metabolic activation of the egg and its role in initiating development.
3. Analyze different theories of egg activation.
4. Learn about the components of spermatozoon entering the egg and their functions.
5. Comprehend the processes of pronuclei migration, amphimixis, and post-fertilization changes.

STRUCTURE

6.1 INTRODUCTION

Fertilization is a fundamental biological process that marks the beginning of a new life. It involves the union of sperm and egg, triggering a cascade of molecular events critical for development. This process initiates significant metabolic, ionic, and structural changes within the egg. The interaction between sperm and egg not only activates the egg but also facilitates the fusion of their genetic material. This document explores the intricate molecular mechanisms, theories of activation, and post-fertilization changes that collectively shape early embryonic development.

6.2 METABOLIC ACTIVATION

After the sperm penetrates the unfertilized egg a series of diverse cytoplasmic reactions are initiated. Following metabolic changes occur in the egg at fertilization.

a) Changes in plasma membrane

The permeability of plasma membrane increases for the molecules of water and certain other chemicals like ethylene glycol, phosphate, K^+etc. At fertilization, the electrical potentiality of plasma membrane becomes more positive and it gradually becomes more negative. The change in the potentiality of the membrane is governed by the unequal distribution of

chloride ions.Besides this a plasma membrane enzyme, adenyl cyclase becomes activated at the time offertilization and it starts the formation of a chemical molecule $3^1 - 5^1$ cyclic AMP, which is supposed to activate most of the metabolic reactions in a fertilized egg.

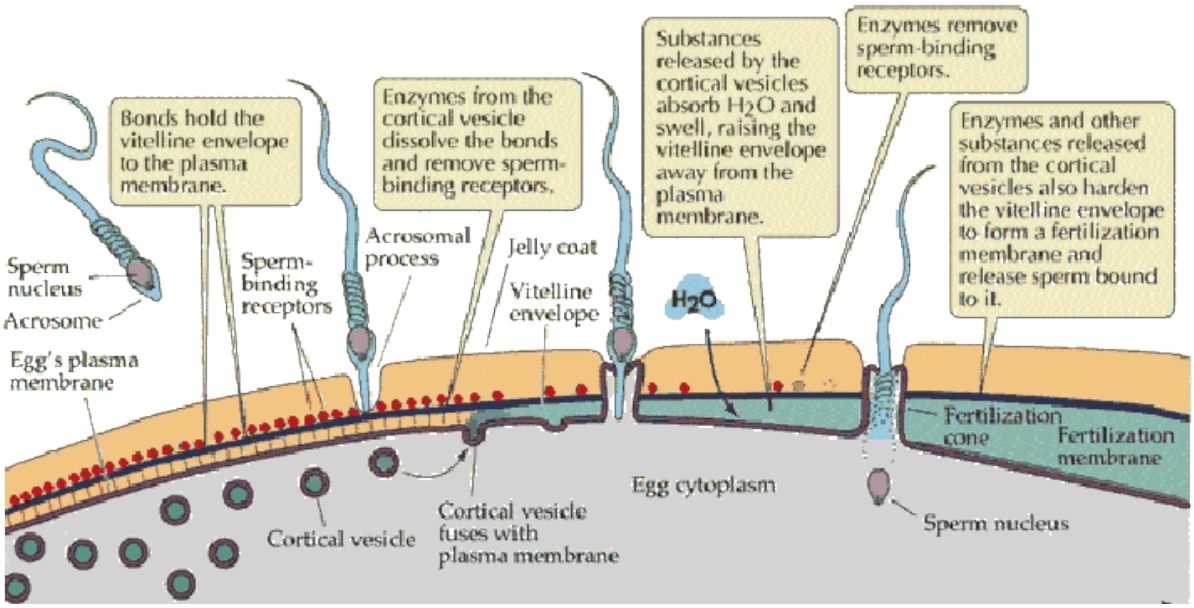

Figure 1:

b) Ionic changes:

Certain intracellular changes occur in the concentration of cat ions, especially those of sodium, potassium and calcium. The change in calcium ion concentration in a fertilized egg has great significance in the metabolic activation of the egg.

c) Changes in the rate of respiration

In a fertilized egg, the rate of respiration either increases (e.g. sea urchins) or decreases (e.g. Chaetopterus and Molluscs, Cumingia) or remains tactic (e.g. Bufo and Fundulus). There appears to be a relation between the post-fertilization oxygen consumption and the stage of maturation of the egg at fertilization.The increased oxygen consumption is related with the oxidation of glycogen and other food stuffs of the egg and synthesis of numerous ATP molecules.

d) Co-enzyme changes

The primary action of the spermatozoon consists of the release or activation of the oxidative enzymes of the egg and that the ensuing increase in oxidation provides the energy necessary for the performance of other changes in the egg and for the development of the egg in general.In a fertilized egg inter conversion of pyridine coenzyme, NAD into another co-enzyme NADP and also NADPH due to phosphorylation of the NAD in the presence of a enzyme NAD Kinase takes place.

NAD + ATP → NAD Kinase NADP + ADP

There are ample evidences that NAD kinase enzyme, though present in the unfertilized egg, it exists in an activated state. It is activated only at the time of fertilization. The increased NADP and NADPH contents may initiate many synthetic pathways of fertilized egg.

e) Changes in the rate of protein synthesis:

The cytoplasm of a mature unfertilized egg, though contains complete machinery for protein synthesis, such as DNA molecules, tRNA, mRNA, ribosomes and proteolytic enzymes required during protein synthesis, none or very little protein synthesis occurs because the mRNA of unfertilized egg remains “masked”.There are evidences that during later phases of oogenesis some inhibitor or repressor proteins are manufactured in sea urchins’ egg which inactivate chromosomal genes, mRNA molecules, ribosomes etc.

During fertilization there is an increase in proteolytic activity of the egg immediately following the penetration of spermatozoon which removes these inhibitor proteins from them and unmasks the mRNA and active protein synthesis is started. In the egg of frog, however, the rate of protein synthesis is increased quite early at the stage of ovulation itself.

f) Initiation of mitosis:

The initiation of mitosis for cleavage is the most significant aspect of egg activation. The initiation of mitosis occurs because (i) The rate of DNA synthesis increases with great pace immediately after fertilization; (ii) the unfertilized egg cytoplasm although possesses a Centriole, yet this Centriole is incapable of division and also to form a mitotic spindle. Thus sperm stimulates the first mitotic division (cleavage) of fertilized egg by contributing its Centriole to the egg.

6.3 THEORIES OF ACTIVATION

The process of activation of egg by sperm during fertilization is still not very clear. However, various theories have been put forward by different embryologists, to explain the mode of activation of egg. Some of the important theories concerning the activation of egg by sperm are as follows:

1) **Bataillon's theory:**

Bataillon (1910,)suggested that the exudation or the excretion of substances into the perivitelline space and elevation of the fertilization membrane activate the egg. He believed that the unfertilized egg was inhibited because of accumulation of metabolic products and the activation or fertilization led to release of these substances to the exterior of the egg.

2) **Sensitization to calcium theory:**

The importance of calcium in egg activation has been emphasized in two independently developed theories, namely "sensitization to calcium" theory of Dlacq, Pasteels and Brachet (1936) and the "Calcium release-protoplasmic clotting" theory of Heilbrunn (1930-1940).
These theories revealed that (i) isotonic calcium chloride solutions or calcium rich solutions are effective activators in many species of animals; (ii) activation of other chemical agents is dependent upon the presence of calcium ions; and (iii) depriving the eggs of calcium will sensitize them to subsequent action of calcium solution.

3) **Loeb's theory:**

Loeb (1964) believed that in normal fertilization the sperm brings in a lytic principle which brings about cortical cytolysis and a second substance called "corrective factor" which regulates oxidation. Lovtrup (1974) has supported Loeb's theory of activation.
According to him there may be some cytolytic agents which may function as activators by causing a partial dissolution and perforation of plasma membrane opening of cortical granules and subsequent swelling of perivitelline space and further activation of fertilized egg.

4) **Lillie's theory:**

R.S. Lillie (1941) suggested that the cortical changes are the main aspects of activation of egg. The decrease in viscosity permits the interaction of various cytoplasmic substances which normally are kept separated in the unactivated egg. The activating substance 'X', formed in the egg is comparable to the growth hormone. This substance is formed by the

union of two substances ‘Y’ and ‘Z’ that may be initially present in low concentration in the egg.

Substance ‘Y’ is considered to be a product of hydrolytic process in the egg and its formation to be stimulated by the action of such agents as heat and acids, which can act under anaerobic conditions. Substance ‘Z’ is considered to be formed by synthetic processes that may be stimulated by agents like hypertonicity acting only in aerobic conditions. Since some substance of each type is present, the threshold concentration of ‘X’ can be reached by increase in either ‘Y’ or ‘Z’.

6.4 COMPONENTS OF SPERMATOZOON IN EGG INTERIOR:

Many variations have been observed in different group of animals, as to how much part of the spermatozoon is engulfed into the interior of egg, during fertilization. In most cases, the sperm nucleus, periacrosomal material, proximal Centriole and mitochondria make their entry as a rule. The plasma membrane of the sperm becomes one of the entriesof plasma membrane of the egg. In mammals, complete structure of spermatozoon (i.e. head, middle piece and tail) penetrates into the egg cytoplasm.In vertebrates, as a rule the egg completes its first meiotic division in the ovary and reaches the metaphase stage of the second meiotic division. At this stage all further progress is arrested, ovulation takes place and the egg may become fertilized. The second polar body gets extruded only if the egg is fertilized by a spermatozoon or activated in some other way. In ascidians, the egg reaches only the metaphase of the first meiotic division when it becomes ripe and if fertilized, only then the egg completes the first reduction division and carries out the second meiotic division.

6.5 MIGRATION OF PRONUCLEI AND AMPHIMIXIS:

At the time of penetration of spermatozoon inside the egg cytoplasm, the sperm nucleus remains compact and its mitochondria and Centriole remain situated behind it. To perform the act of amphimixis, the sperm nucleus has to undergo two activities (i) it has to become pronucleus, and (ii) it has to migrate from the site of amphimixis.

As the sperm nucleus moves inwards from the site of fertilization cone, it soon rotates through an angle of 180°C, so that its mitochondria and Centriole assume the leading position. Besides this rotation, the sperm nucleus starts swelling and its chromatin, which is very closely packed, becomes finely granular. It ultimately becomes vesicular and has an appearance like the interphase nucleus and is called male pronucleus.

At the same time, the sperm aster forms around the proximal Centriole of the sperm in the egg cytoplasm. As the male pronucleus develops and migrates towards the site of amphimixis, the sperm aster seems to lead it.

The sites of amphimixis lies either near the center of microlecithal and mesolecithal eggs or in the center of the active cytoplasm at the animal pole of macrolecithal and Teleolecithal eggs. As the sperm pronucleus and Centriole move inward, it may be accompanied by some cortical and subcortical cytoplasm.If the latter is heavily pigmented, as in amphibian eggs, the trajectory of the sperm pronucleus may be marked by pigmented granules trailing along its path. This is called penetration path.This movement of the sperm appears to be directed and some investigators feel that it is due to a chemo toxic effect of chemicals liberated by the female pronucleus. During this movement toward the female pronucleus, the sperm may have

to deviate from its penetration path. If it does, the new pathway is taken. This is referred to as copulation pathway.

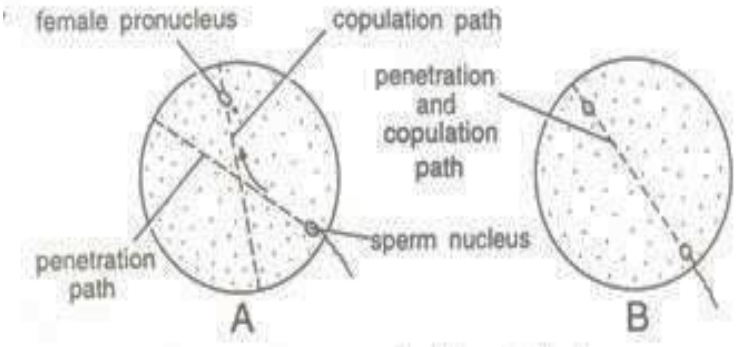

Figure 2: Possible sperm parts during fertilization

Amphimixis:

The fusion of male and female pronuclei is called as amphimixis. The actual fusion of pronuclei may differ in different animal.

- In sea urchins and vertebrates, the nuclear membranes of both pronuclei are broken down at the point of contact and their content unite in one mass surrounded by a common nuclear membrane. At the approach of first cleavage of fertilized egg, the nuclear membrane dissolves, chromosomes of maternal and paternal origin become arranged on the equator of the achromatic spindle.
- In Ascaris, some molluscs and annelids, the male and female pronuclei don't fuse but the nuclear membranes in both dissolve and the chromosomes become released. In the meantime, the Centrosome of the spermatozoon has divided into two and the Centrosome derived from male and female pronuclei become attached to the spindle.

Only after the completion of the first division of the fertilized egg, the paternal and maternal chromosomes become enclosed by common nuclear membrane to form the nuclei of two blastomeres into which the egg has been divided.

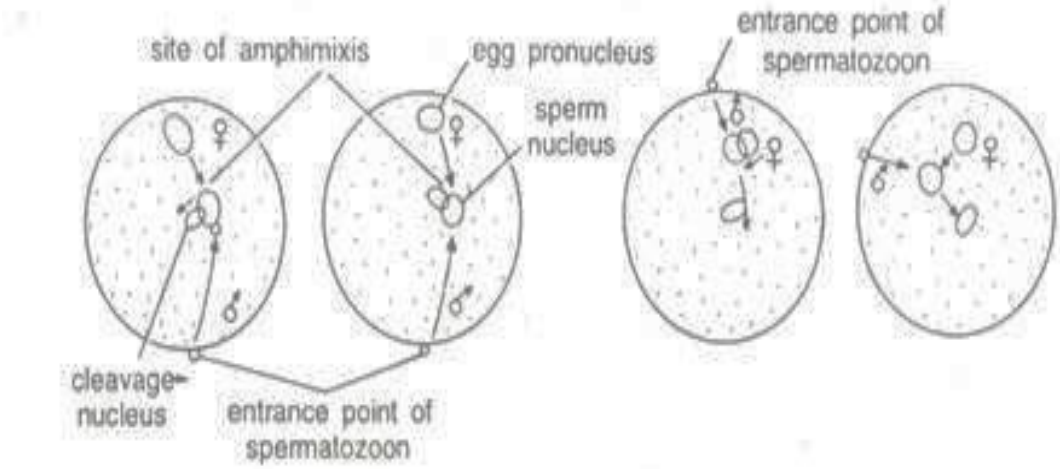

Figure 3: Amphimixis in sea-urchin, tracks of pronuclei in their movements towards each other and the site of the cleavage nucleus, with sperm entering at opposite side from the site of egg nucleus and from the same side, respectively

Post-fertilization changes in the egg cytoplasm:

The penetration of spermatozoa into the egg causes far-reaching displacements of the cytoplasmic constituents. As a result, the distribution of various cytoplasmic substances and inclusions in a fertilized egg may be very considerably different from that in the unfertilized egg and even quantitatively new areas may appear.

As a result of the extrusion of cortical granules, a large part of the original outer egg cell surface is replaced by the inner surface which surrounds the cortical granules and now are averted on the exterior. Most spectacular post-fertilization displacements in the egg cytoplasm have been observed in Ascidian, Styela partita and in frog. In both these animals, there establishes a bilateral symmetry in the cytoplasm of fertilized eggs.

6.6 SUMMARY:

Fertilization initiates metabolic activation in the egg, driving essential processes like ionic regulation, respiration, protein synthesis, and mitosis. Key molecular changes include altered plasma membrane permeability, increased calcium ion concentration, and cyclic AMP formation. Pronuclei migration and amphimixis unite paternal and maternal genetic material, preparing the egg for the first mitotic division. Several theories explain egg activation, including calcium sensitization and cortical changes. Post-fertilization cytoplasmic rearrangements establish polarity and symmetry in the egg, facilitating early developmental stages. These coordinated events ensure the successful onset of embryogenesis.

6.7 TECHINICAL TERMS:

Cyclic AMP, pronucleus, NAD kinase, cortical granules, amphimixis, adenyl cyclase.

6.8 SELF-ASSESSMENT QUESTIONS:

1. How do ionic changes influence egg activation?
2. What are the different theories proposed for egg activation?
3. How do post-fertilization changes in the cytoplasm impact egg development?
4. What are the primary changes in the egg's plasma membrane during fertilization?

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Prof. K. Sunitha

LESSON – 7

EARLY DEVELOPMENT

AIMS AND OBJECTIVES

- Understand the process of zygote formation and its significance in embryonic development.
- Explain the patterns of cleavage and how they influence embryonic development.
- Explore the properties of cell division during cleavage.
- Analyze the planes of cell division during cleavage and their orientation.
- Study the patterns of cleavage determined by the orientation and fate of blastomeres.
- Understand the process of gastrulation and its role in forming the three germ layers.

STRUCTURE

7.1 INTRODUCTION

Zygote formation marks the beginning of life after fertilization. Cleavage is the process of rapid cell division of the zygote without significant growth, forming blastomeres. The cleavage patterns depend on yolk distribution, orientation of blastomeres, and the fate of these cells. Gastrulation is the process where the blastula reorganizes into a gastrula, forming the ectoderm, mesoderm, and endoderm, which are essential for tissue and organ development.

7.2 ZYGOTE FORMATION & PATTERNS OF CLEVAGE

Between fertilization and organogenesis, two steps of development are critical; one is to generate many cells from the zygote and another is to reorganize them into a definite pattern. The first step is cleavage and the second is gastrulation. In fact, when fertilization is the first step of life of a sexually reproducing organism, cleavage is the first step of development of a multicellular individual. It is obvious that metabolically inactive state of an egg is transformed into a very active state by fertilization, the immediate consequence is cleavage.

Cleavage actually involves a series of rapid cell division (Fig.1). Waves of mitotic divisions follow one another, almost without any pause. As a result, unusually large zygote produces smaller cells, progressively nearer to normal dimensions. The cells resulted from cleavage are called blastomeres**.** Initially, a few successive divisions are synchronous in most animals, so that number of cells doubles with each division cycle. At this stage, the embryo seems like a mulberry and called morula(Fig. 1). Subsequent divisions become asynchronous, which varies with species and largely depends on the amount of yolk present. Towards the end of cleavage, the embryo appears like a hollo ball, called blastula(Fig. 1). The cavity within blastula is known as blastocoeland the cellular layer surrounding blastocoel is called blastoderm**.**

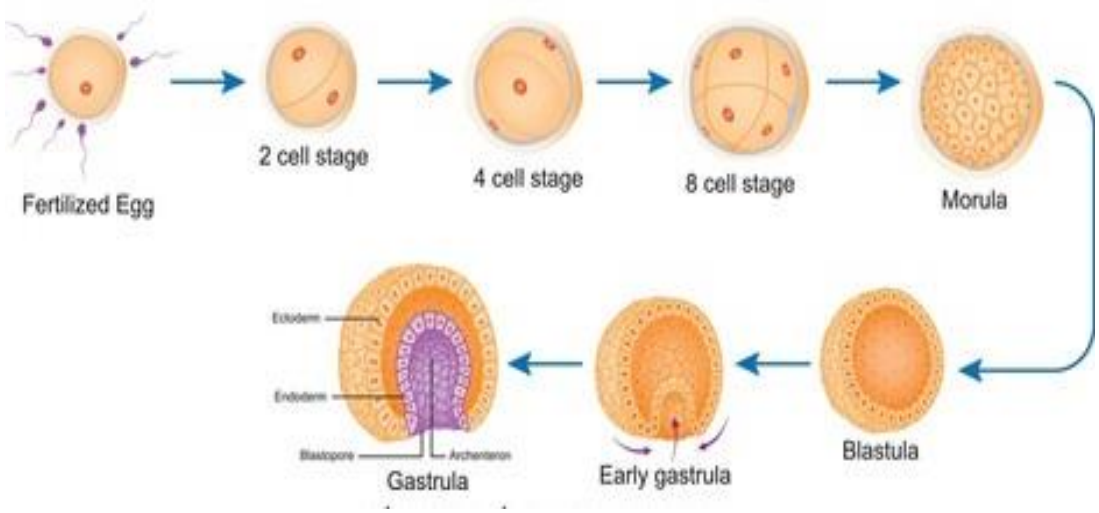

Figure 1:cleavage involves a series of rapid cell division producing morula and blastula stage.

7.3 PROPERTIESOF CELL DIVISION IN CLEAVAGE:

1. Cell division in cleavage is typically mitotic; each blastomere receives complete copies of zygotic genome.

2. Cell division is rapid without any intervening gap phase between DNA replication and mitosis. Thus, successive blastomeres become half of previous generations and there is no net growth of embryo. In absence of growth, number of cells in the embryo increases at the cost of decrease of cell size.

3. Initial few divisions are synchronous (exception in mammals). So, cell population doubles with each division. Zygote produces two blastomeres, which produce four and so on. However, later divisions become asynchronous depending on various influencing factors including amount of yolk.

4. Rate of cell division during cleavage is extremely rapid, which is not seen in individuals again, In *Drosophila*, cell division occurs in every 10 minutes for over 2 hours and within 12 hours, about 50,000 cells are produced. A frog zygote produces about 37,000 cells in just 43 hours. Rapid division during cleavage is adaptive to the defenseless phase of development. Contrarily, mammalian embryos are protected within maternal womb and cleavage in them occurs at a leisurely pace.

5. One great achievement of division without growth is the increase of nucleocytoplasmicratio**.** This ratio is a critical cellular parameter and defined as the ratio of nuclear volume to cytoplasmic volume of a cell. To sustain itself, a cell must constantly synthesize the most vital but short-lived molecules like RNA and protein. A cell with smaller nucleocytoplasmic ratio is unable to replace the RNA and proteins losing from its large volume of cytoplasm. Nucleocytoplasmic ratio in a sea urchin zygote is about 1:550. During cleavage, it becomes 1:18 at 4-celled stage and 1:6 at 64 celled stages.

6. During initial synchronous divisions, zygote genome is transcriptionally inactive. So, cleavage is controlled by maternal gene products stored in the zygote. These maternal gene products determine the rate of cleavage, spatial arrangements and relative size of the blastomeres. Because transcription of zygote genome does not occur during initial divisions of cleavage, transcription-inhibiting drugs fail to stop cleavage during these stages. However, in later stage of cleavage transcription of zygote genome begins and takes the control of development. This special point is known as mid-blastulatransition.

7. Activation of cleavage following fertilization is caused by **mitosis-promoting factor** (MPF). MPF is responsible for resumption of meiotic division of oocyte. But after fertilization, MPF continues to play a regulatory role in cell division during early cleavage. During this time, two steps of cell cycle occur – M and S depending upon the cyclical availability and degradation of MPF; the cyclical activity is controlled by subunit of itself-the cyclin B.

7.4 PLANE OF CELL DIVISION DURING CLEAVAGE:

The general rule is that the plane of cell division always follows the right angle to the long axis of the mitotic spindle. But during cleavage, various forces determine the position of mitotic spindle of dividing blastomeres. As the position of spindle alters, the plane of cleavage also changes. Four types of planes of cell division, hence cleavages are recognized during cleavage.

1. **Meridional cleavage:** When the furrow of cytokinesis passes through the middle of animal pole-vegetal pole axis, the plane of cleavage is called meridional (Fig.2). Such a plane bisects both the poles; resultant daughter blastomeres are equal to each other and remain in a single tier. Example-1st and 2nd cleavage of Amphioxus, amphibian and chick.

2. **Vertical cleavage:** The cleavage furrow passes through animal pole to vegetal pole, but not through the mid –axis, rather parallel to the mid-axis; such cleavage plane is known as vertical (Fig.2). Resultant daughter blastomeres are unequal in size but remain in same tier. Example: 3rd cleavage of chick.

3. **Equatorial cleavage:** The cleavage plane passes through the equator and bisects the mid-axis of egg/blastomere at right angle, so the animal pole and vegetal pole remain on either side of the cleavage plane (Fig. 2). Resultant daughter blastomeres are equal to each other but remain in two tiers. Example: 1st cleavage of higher mammals.

4. **Latitudinal/horizontal cleavage:** The cleavage furrow bisects the mid-axis of egg/blastomere at right angle but does not pass through the equator or dividing cell, rather parallel to the equator (Fig. 2). Resultant daughter blastomeres are unequal in size and remain in two tiers. Example: 3rd cleavage of Amphioxus and amphibians.

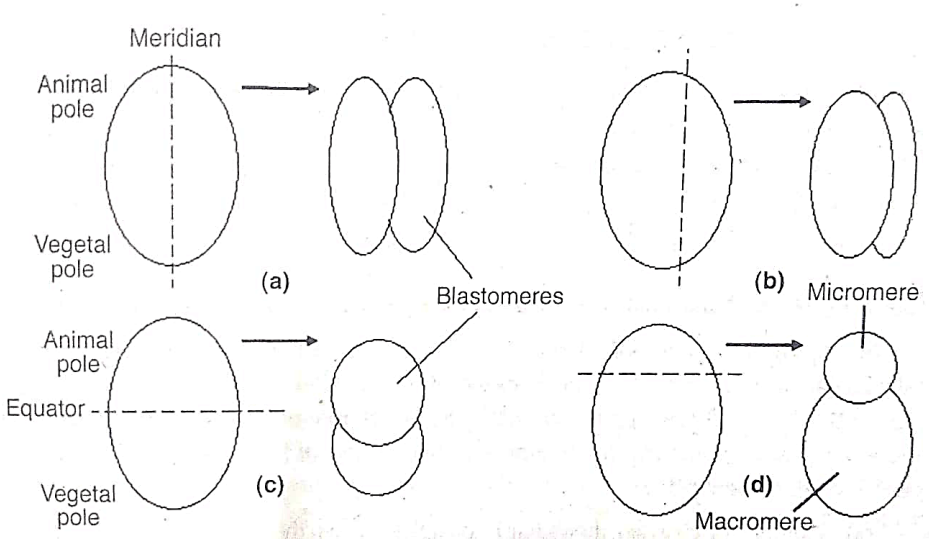

Figure 2: Plane of cell division during cleavage-(a) meridional (b) vertical (c) equatorial and (d) latitudinal.

Essentially all planes of cleavage are of two broad classes – vertical and horizontal. When vertical plane passes through the mid-axis, it is meridional. When horizontal plane passes through equator, it is equatorial. Division in vertical plane increases the number of blastomeres in as single tier, while division in horizontal plane increases the number of tiers of blastomeres. Again, when vertical and horizontal planes of cleavage produce unequal size of blastomeres, the smaller ones are called micromeres and larger ones are called macromeres (Fig. 2).

7.5 PATTERNS OF CLEVAGE:

Cleavage is not simple cell division where nucleus and cytoplasm divide into equal parts. In fact, pattern of cleavage is distinctly different in different organisms. In general, cleavage pattern is determined by two major factors:

- The amount and distribution of yolk in the egg cytoplasm, and
- Factors in the egg cytoplasm that influence the position and orientation of mitotic spindle.

Patterns of cleavage along with the plane of cleavage determine the relative size of the blastomeres, as well as allow differential segregation of cytoplasmic components in blastomeres. These are very important to ensure the distinct fate of each blastomere.

7.5.1 Patterns of cleavage determined by amount and distribution of yolk:

In majority eggs one pole is relatively yolk-rich than the other pole. The former is called vegetal pole and other one is called animal pole. The nucleus of the zygote is frequently displaced towards the animal pole, where rate of cell division is faster than the vegetal pole. According to amount and distribution of yolk, two broad patterns of cleavage are recognized:

(a) **Holoblastic cleavage** [holos (Gr.) =complete]: when the cleavage furrow completely bisects the egg into two daughter cells, it is called holoblastic cleavage. In oligolecithal (little yolk) and isolecithal eggs, cleavage results two equal or roughly equal blastomeres, and known as **equal holoblastic cleavage** (Fig.3), as found in echinoderms and *Amphioxus.*

In Mesolecithal (moderate yolk) eggs, resultant blastomeres are complete but unequal in size, the cleavage is called **unequal holoblastic** (Fig.3). In such cleavage the smaller blastomeres or micromeres are formed at animal pole and larger macromeres are formed at vegetal pole. Unequal holoblastic cleavage is found in amphibians.

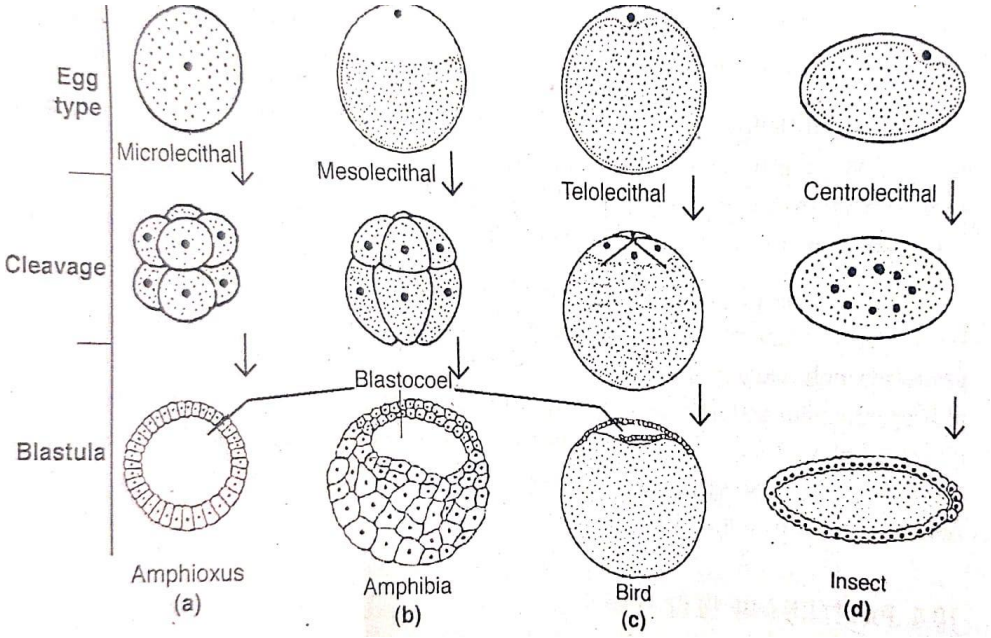

Figure 3: Types of cleavage is largely determined by amount and distribution of yolk in egg - (a) Equal Holoblastic, (b) Unequal Holoblastic, (c) Discoidal Meroblastic and (d) Superficial Meroblastic.

Meroblastic cleavage [meros (Gr.) = part]: In macrolecithal (much yolk) eggs, cleavage furrow cannot bisect the egg to produce two complete blastomeres. Usually, cleavage furrow is restricted within active cytoplasm of animal pole or periphery, and yolk packed vegetal pole remains almost undivided. Such type of cleavage is called meroblastic. In telolecithal eggs, cleavage is restricted within the disc-shaped area of animal pole containing active cytoplasm; the type is called **discoidal meroblastic cleavage** (Fig.3), and found in bony fishes, reptiles, birds and prototherian mammals. Again, in centrolecithal eggs, cleavage

occurs within the peripheral areal of active cytoplasm and called **superficial meroblastic cleavage** (Fig.3), found in insect.

7.5.2 Pattern of cleavage determined by orientation of blastomeres:

Amount of yolk is surely one factor to influence the pattern of cleavage. But there are inherited pattern of cell division resulting different types of cleavage. These cleavage patterns are superimposed upon holoblastic cleavages only and primarily controlled by position and orientation of mitotic spindle. Four such cleavage patterns are recognized:

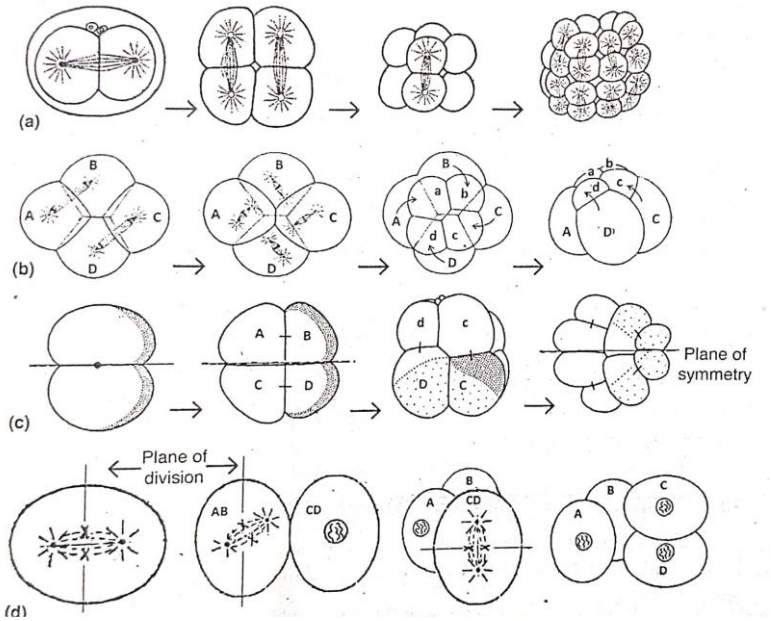

Figure 4: Four patterns of cleavage are recognized according to orientation of blastomeres - (a) Radial, (b) Spiral, (c) Bilateral and (d) Rotational.

a. **Radial cleavage:** When successive cleavage furrows are perpendicular to one another, equal sized blastomeres are arranged in radial symmetry around animal pole-vegetal pole axis. Radial cleavage (Fig.4) is found in echinoderms and *Amphioxus.*

b. **Spiral cleavage:** In annelids and mollusks, radial cleavage is exaggerated into a distinct pattern – the spiral cleavage (Fig.4), where one tier of blastomeres is rotated slightly in respect to the other adjacent ties. In this pattern, first two divisions are meridional, but the third is horizontal and occurs at an oblique angle. Such oblique pattern of cleavage is resulted from the oblique orientation of the mitotic spindles relative to animal vegetal axis. If the rotation of upper tier of blastomeres is clockwise, as viewed from animal pole, it is called **dextral spiral cleavage.** When the rotation is anticlockwise, it is called **sinistral spiral cleavage.**

c. **Bilateral cleavage:** Ascidian eggs, in addition to animal-vegetal polarity, have an anterior-posterior polarity due to asymmetric distribution of some cytoplasmic components. The first cleavage plane is meridional, passes through the animal-vegetal

axis. Resulted two equal blastomeres establish the left and right sides of the embryo. All further divisions on each side are mirror image of the other side. Throughout the cleavage of zygote, there is only one plane of symmetry, the median plane; hence the cleavage pattern is named as bilateral (Fig.4).

d. Rotational cleavage: In mammalian eggs, a characteristic pattern of cleavage is observed. The first cleavage is meridional and produces two equal blastomeres. In second cleavage, two blastomeres divide in different plane -one divides meridionally and other equatorially. Because of crosswise arrangement of cleavage and blastomeres, the pattern is called rotational (Fig.4).

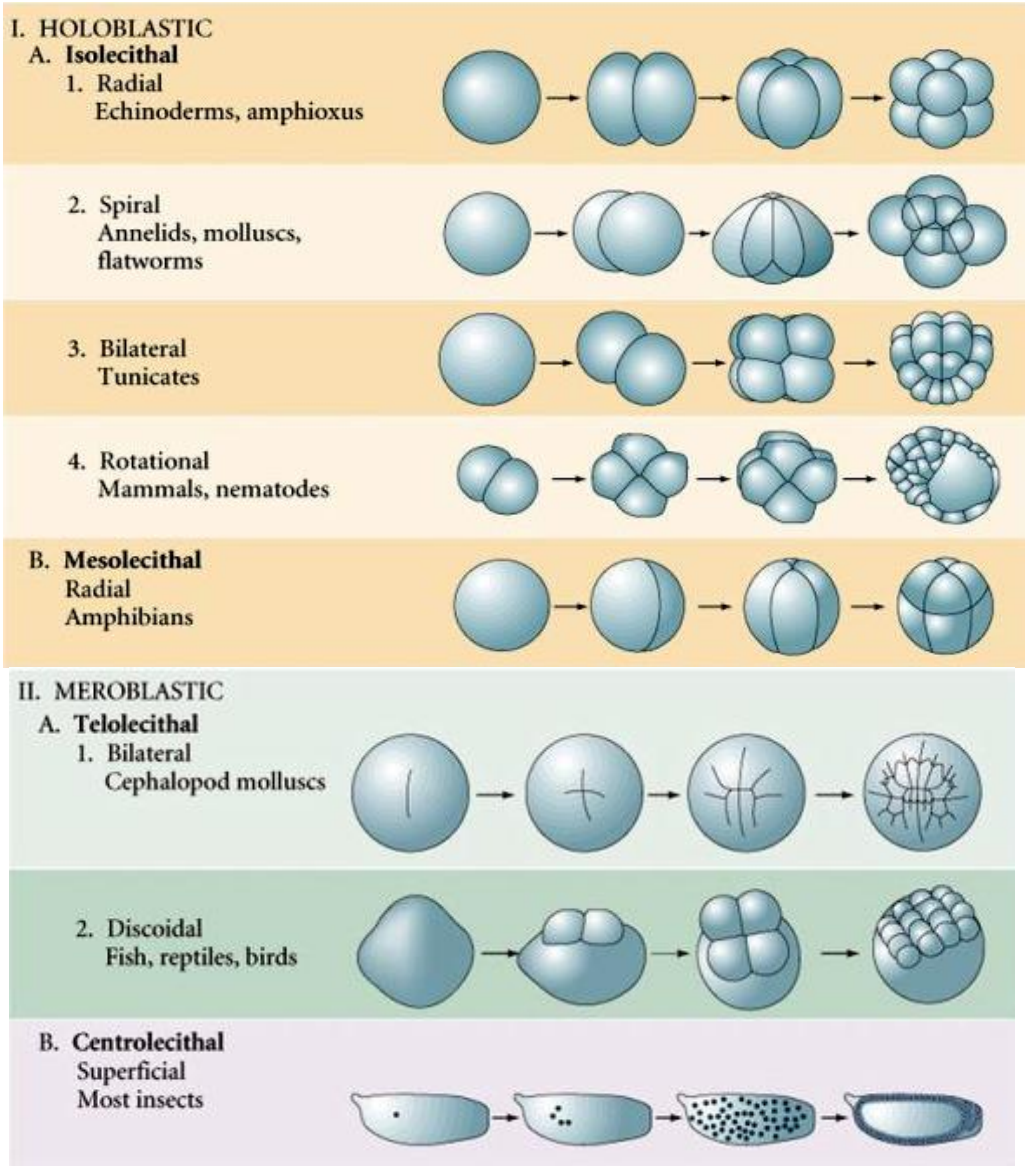

Figure 5: Holoblastic & Meroblastic

7.5.3 Pattern of cleavage determined by the Fate of Blastomeres:

I. **Determinate cleavage:** When blastomeres resulted from cleavage have precise position and rigid fate, the cleavage is called determinate. Blastomeres are assigned to unalterable fate and loss of any one results absence of that part of the embryo, which

derive from the said blastomere. Such as, when one blastomere is isolated from a two-celled stage, a half –larva is produced. Loss of one blastomere cannot be replaced by others and such pattern of developmental is called mosaic. Determinate cleavage is found in nematodes, annelids, mollusks and tunicates.

II. **Indeterminate cleavage:** When fate of blastomeres is less rigid and flexible in relation to position and destiny, it is called indeterminate cleavage. In such pattern, existing blastomeres can readjust and substitute the position and fate of a lost blastomere. So, loss of one or a few blastomeres has little effect on normal development of the embryo. Indeterminate cleavage is also called regulative development and observed in echinoderms and vertebrates.

7.6 GASTRULATION

Following the formation of blastula through cleavage process, an embryo enters into one of the most critical periods in its development-the **gastrulation** (Gr. Gaster= stomach). The process in which cells of embryo profoundly rearrange themselves through highly coordinated movement to achieve a multilayered body plan is gastrulation.

Blastula consists of many cells which are brought into their position during cleavage. During gastrulation, these cells are brought into new position with new neighbors and a multilayered embryo is formed. In triploblastic animals, gastrulation results in an embryonic stage called **gastrula** in which three germinal layers are established. These three germinal layers are outer ectoderm, inner endoderm and interstitial mesoderm. During cellular movement, cells forming endoderm and mesoderm are brought inside the embryo while ectodermal cells spread all over outside surface of the embryo.

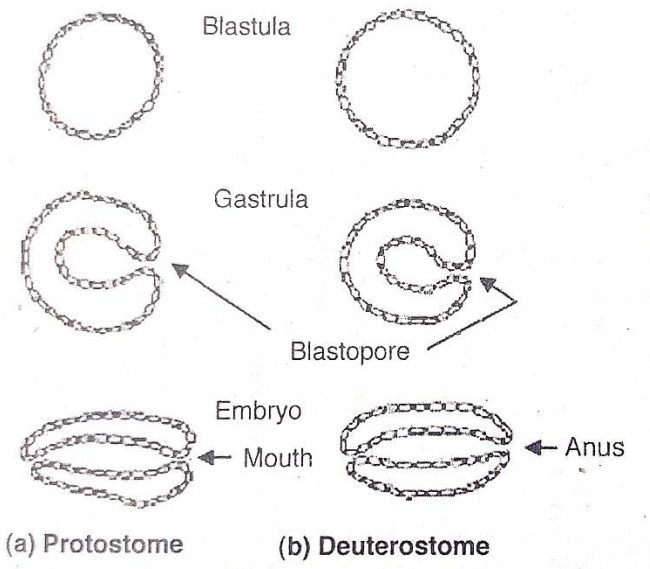

Figure 6: According to fate of blastopore, triploblastic metazoans are grouped into two -(a) protostomes, blastopore becomes mouth; (b) deuterostomes, blastopore becomes anus.

Embryos adopt two strategies to deal with gastrulation. The first mode is observed in embryo with little yolk *Amphioxus* or with moderate yolk like amphibians. In this mode, the gastrulation movements occur within a sphere. In *Amphioxus,* gastrulation begins with

inpocketing or invagination of blastoderm. As a result, a single layered hollow sphere transforms into a double –layered cup The new cavity within the cup is called archenteron. In frog gastrulation also begins with invagination and the new cavity thus formed is gastrocoel.

7.6.1 Unique features of Gastrulation:

- Gastrulation is characterized by profound but well-ordered rearrangements of cells. At the onset of gastrulation, cells acquire the capacity for undergoing directed movement. The event is called **morphogenesis** or **morphogenetic movement.**
- Control of development is entirely taken by embryonic genome form material genome. Actually, this shift or control starts during mid-blastula transition.
- Genetic reservoirs of cells are activated by more mechanisms that direct the synthesis of substantial amount of new RNA and proteins. Thus chemo-differentiation begins from gastrulation.
- Rate of cell division slows down dramatically and growth is very insignificant.

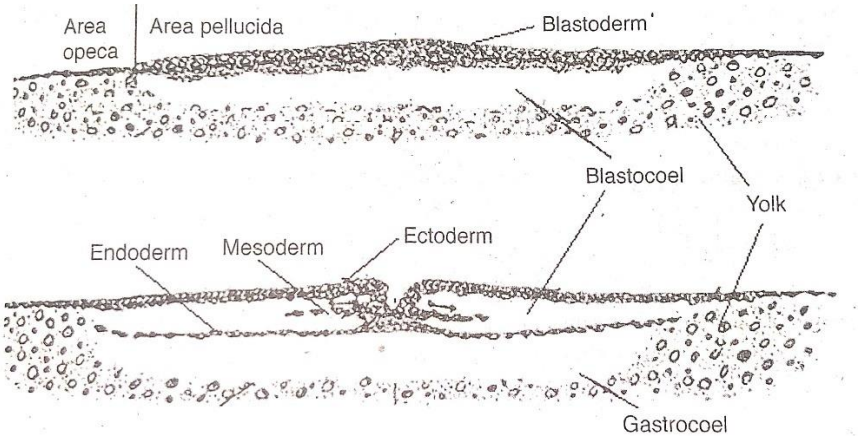

Figure 7: In birds and reptiles, large amount of yolk excludes invagination. Three germ layers are formed as two-dimensional sheet in animal pole.

7.6.2 Structure of Gastrula:

It is already stated that in the process of gastrulation, the spherical blastula transforms into a complex configuration of three germinal layers-the gastrula (Fig.8). The outer layer is ectoderm form which epidermis and nervous system of the organism develop. The inner layer endoderm mainly forms the primitive gut or archenteron. The interstitial layer is mesoderm, from which numerous structures develop. Ectoderm and endoderm are epithelia-closely packed sheet of cells resting on basement membrane. Mesoderm is sometimes epithelial but in other cases Mesenchymal, that is, loosely arranged cells with much extracellular materials.
A common feature of early gastrula of all organisms is a groove called blastopore, through which cells move inside. The fate of blastopore in adult is two, upon which triploblastic metazoans are also grouped into two. When blastopore becomes the mouth, the group is called **protostomes** (Fig.9). When blastopore becomes anus, the group is called **deuterostomes** (Fig.9) Deuterostomes mainly include echinoderms and chordates, rest of the triploblastic, groups are protostomes.

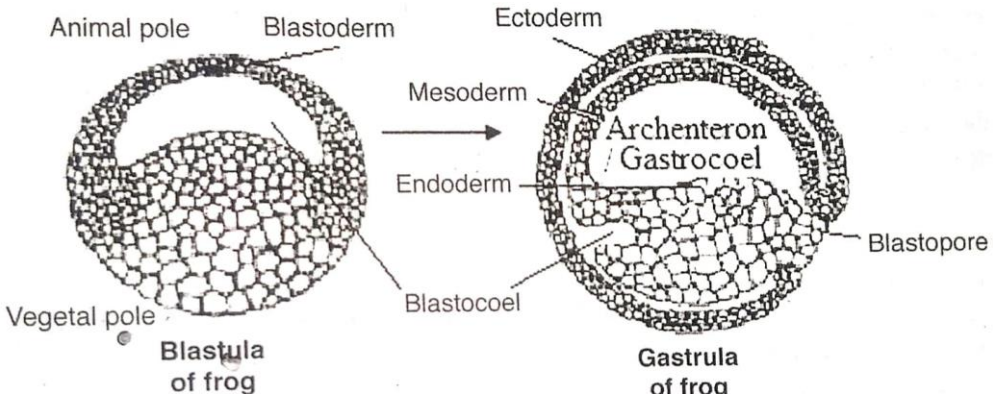

Figure 8: Gastrulation, in general, transforms blastula into gastrula with three germinal layers

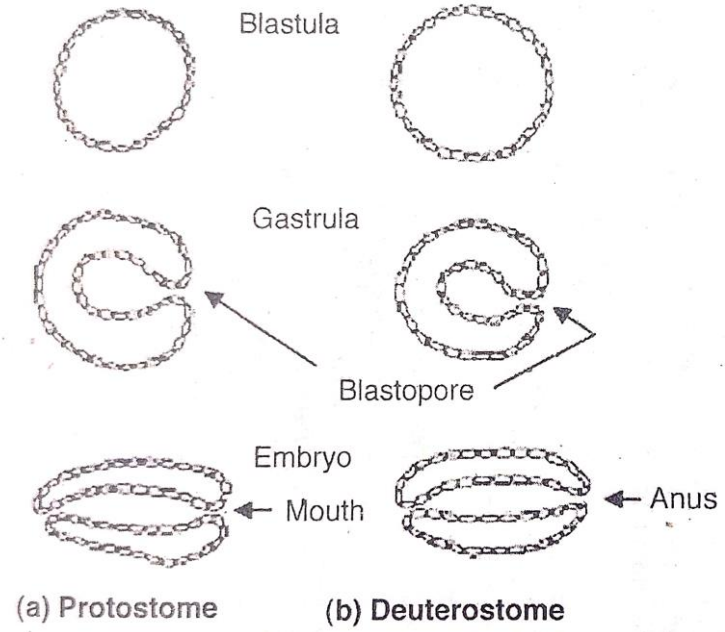

Figure 9: According to fate of blastopore, triploblastic metazoans are grouped into two -(a) protostomes, blastopore becomes mouth; (b) deuterostomes, blastopore becomes anus.

7.5.4 Morphogenic Movements during Gastrulation:

One of the primary features of gastrulation is profound and directed cell movement called morphogenetic movement. Because this chapter is related to the present context, some common types of movements are outlined in table1. In fact, different combinations of these movement types result in a wide variety of morphogenetic movements found in different animals.

The major morphogenetic movements during gastrulation result in the rearrangement of the blastomeres in blastula stage forming three germinallayer stage. But some movements continue into later stages of organogenesis. One of the great consequences of these movement is that the group of cells separated so far are brought closer. As a result, they undergo inductive interactions involved in the establishment of major organ systems in the organisms.

Invagination	Inpocketing of a sheet of cells	Formation of archenteron in *Amphioxus*
Involution	Inturning of cells over the internal surface of an outer layer	Mesoderm formation in Amphioxus
Delamination	Separation of a cell sheet from an original one	Hypoblast formation in birds
Convergent extension	Elongation of a cell sheet through convergence movement	Formation of primitive streak in chick
Epiboly	Spreading of cell sheet over a cell mass or yolk mass	Formation of epidermal ectoderm in amphibians
Amoeboid movement	Movement of single cell through own motility, usually by pseudopodia	Movement of neural crest cells
Intercalation	Wedging of individual cells between a layer of cells	Formation of archenteron on sea urchin
Ingression	Sinking of individual cells from a surface layer	Formation of mesenchyme in sea urchin

Table 1: Some common morphogenetic movements found during gastrulation

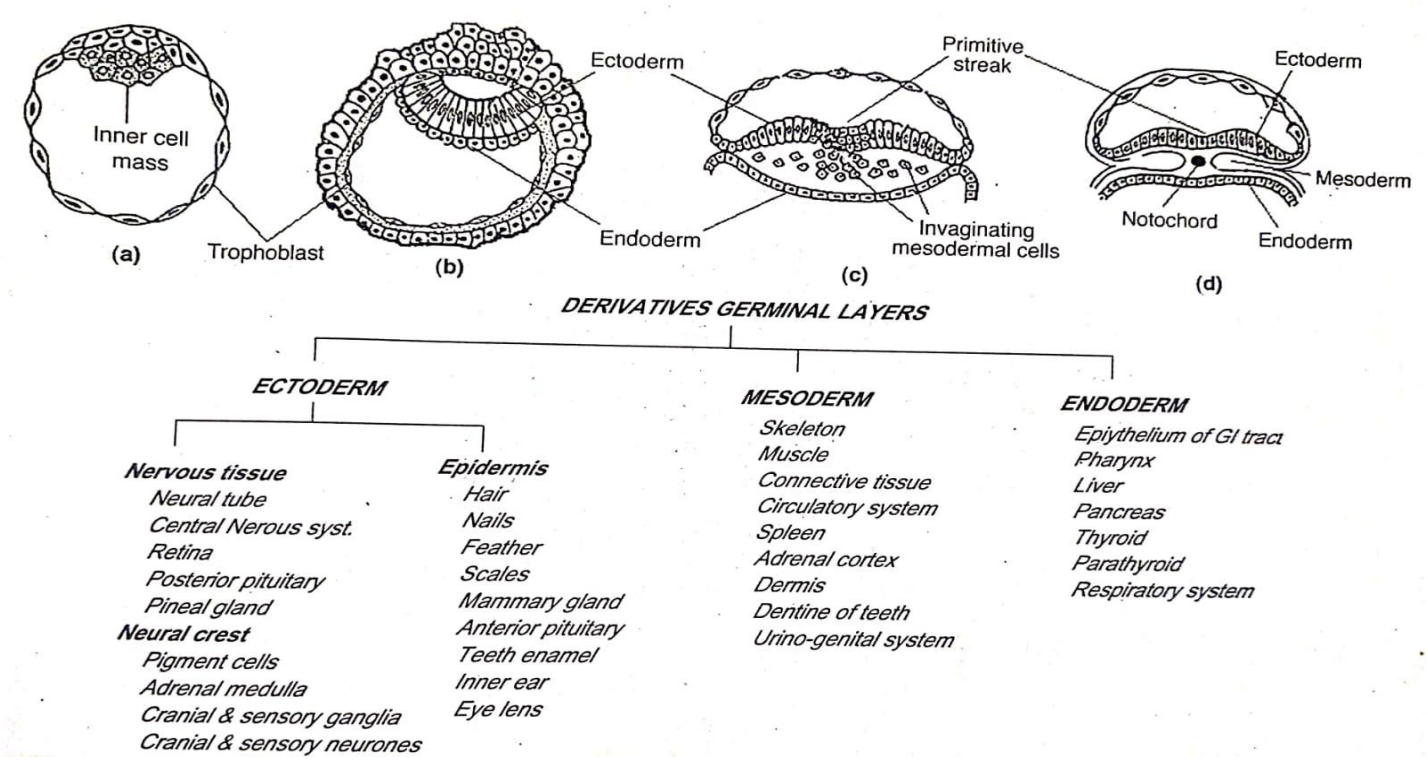

Figure 10: Gastrulation in man. (a) 5th day blastocyst after coming out of zona pellucida (b) 9th day embryo after implantation, establishment of three germ layers in 18th (c) and 17th (d) day embryo.

7.7 SUMMARY

Zygote formation marks the fusion of male and female gametes, resulting in the formation of a single-celled zygote, which serves as the foundation for embryonic development. Following this, cleavage begins, characterized by rapid mitotic divisions of the zygote into smaller cells called blastomeres. Cleavage can be classified as holoblastic (complete division) or meroblastic (incomplete division), influenced by the yolk content of the egg. The process is defined by properties such as reduced cell size and synchronized divisions in the early stages. Cleavage planes vary across species and may follow radial, spiral, bilateral, or rotational patterns, each contributing to the developmental symmetry and axis.

The orientation of blastomeres during cleavage plays a crucial role in determining the symmetry and developmental axis of the embryo. Additionally, the fate of blastomeres can be either determined, where the future developmental path of cells is fixed early, or indeterminate, where the fate remains flexible for a longer period. Gastrulation follows cleavage and is a pivotal phase in embryogenesis, during which the blastula reorganizes into a gastrula, establishing the three germ layers: ectoderm, mesoderm, and endoderm. This process is facilitated by morphogenetic movements such as invagination, epiboly, and convergent extension, which lay the groundwork for the formation of tissues and organs.

7.6 TECHINICAL TERMS

Cleavage, Blastomere, Holoblastic Cleavage, Meroblastic Cleavage, Radial Cleavage, Spiral Cleavage, Gastrulation, Germ Layers, Morphogenetic Movements.

7.9 SELF-ASSESSMENT QUESTIONS:

1. What is cleavage, and why is it important in embryonic development?
2. Explain the difference between holoblastic and meroblastic cleavage.
3. What are the key movements during gastrulation?
4. What is cleavage

(a) Patterns of cleavage determined by fate of blastomeres
(b) Gastrulation

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Prof. K. Sumanth Kumar

LESSON – 8
POTENCY, INDUCTION, COMPETENCE, DETERMINATION, IFFERENTIATION

AIMS AND OBJECTIVES

- Understand the concepts of potency, induction, competence, determination, and differentiation in developmental biology.
- Learn the different levels of potency and their role in cell specialization.
- Explore the mechanisms and importance of induction in tissue development.
- Analyze the role of competence in enabling cells to respond to developmental signals.
- Differentiate between determination and differentiation and their significance in cell fate.

STRUCTURE:

8.1 INTRODUCTION:

In developmental biology, a series of interconnected processes guide the transformation of a single fertilized egg into a complex multicellular organism. These processes include **potency**, **induction**, **competence**, **determination**, and **differentiation**, each playing a critical role in ensuring proper development and tissue specialization. Understanding these fundamental concepts provides insight into how cells acquire their specific roles and how complex structures form during embryogenesis.

Development is very dynamic process, in which cells touch and interact with each other in various ways, Sending and receiving a variety of chemical signals make an organism's body as an integrated unit rather than a mere collection of individual cells living independently. The ability of cells to interact with one another is a hallmark of multicellular organisms. Cell-cell interaction in embryological development allows an individual cell to determine its position within body, to achieve a distinct fate and to differentiate accordingly.

In the theory of regulative development, cells must interact with each other. But the central importance of cell-cell interactions in embryonic development was not really established until the discovery of the phenomenon of induction. Around 1900, Roux first made the distinction between tissues or structures that seems to develop strictly according to a fixed genetic program (determinate development) and others that are affected by adjacent tissues (regulative development). From this understanding, a new concept has developed in experimental embryology called **induction.** Importance of induction and other cell-cell interactions in the development was proved dramatically by Spemann and Mangoldin 1924 in their famous experiment with amphibian embryos. They showed that a partial second embryo could be induced by grafting a small region from dorsal lip of blastopore of a newt embryo to a new site of another embryo (Fig.1). Spemann and Mangold termed that small portion of dorsal lip of blastopore as organizer as it was appeared to be responsible for organizing a complete embryonic body.

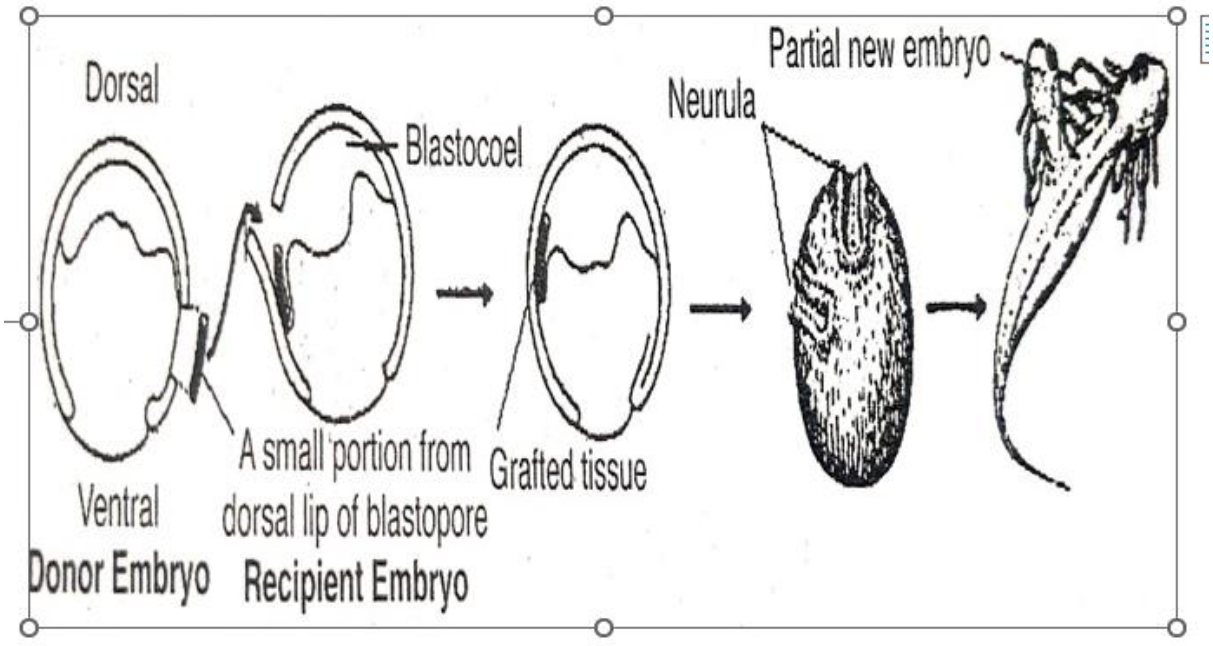

Figure 1: Grafting a small region from dorsal lip of blastopore of a newt embryo to a new site of another embryo can induce a partial new embryo.

8.2 POTENCY:

Cell potency is a cell's ability to differentiate into other cell types. The more cell types a cell can differentiate into, the greater its potency.

- The number of different cell types in the embryo increases as development proceeds.
- The potency of a cell is an intrinsic property and is greater than or equal to its fate. The fate of a cell depends on its potency + its environment (e.g. its contact with other cells in the embryo).
- Cell potency refers to the varying ability of stem cells to differentiate into specialized cell types. Cells with the greatest potency can generate more cells types than those with lower potency. Zygote considered a Totipotent cell.

- The zygote from that fusion of an egg cell and a sperm cell then begins cell divisions that are capable of forming the entire human body.

It is these cells that are totipotent, so called because their potential is 'total.' Types of Potency i)Totipotency ii) Pluripotency iii) Multipotency iv) Oligopotency v) Unipotentency

Totipotency ("ability for all [things]") is the ability of a single cell to divide and produce all of the differentiated cells in an organism. Spores and zygotes are examples of totipotent cells

Pluripotency, ("ability for many [things]") refers to a stem cell that has the potential to differentiate into any of the three germ layers: endoderm (interior stomach lining, gastrointestinal tract, the lungs), mesoderm (muscle, bone, blood, urogenital), or ectoderm (epidermal tissues and nervous system). However, cell pluripotency is a continuum, ranging from the completely pluripotent cell that can form every cell of the embryo proper,
This ability to become any type of cell in the body is called pluripotent. The difference between totipotent and pluripotent cells is only that totipotent cells can give rise to both the placenta and the embryo. As the embryo grows these pluripotent cells develop into specialized, multipotent stem cells.

Multipotency describes progenitor cells which have the gene activation potential to differentiate into discrete cell types. For example, a multipotent blood stem cell and this cell type can differentiate itself into several types of blood cell like lymphocytes, monocytes, neutrophils, etc.,

Oligopotency is the ability of progenitor cells to differentiate into a few cell types. It is a degree of potency. Examples of oligopotent stem cells are the lymphoid or myeloid stem cells.

Unipotentency cell is the concept that one stem cell has the capacity to differentiate into only one cell type. It is currently unclear if true unipotent stem cells exist.

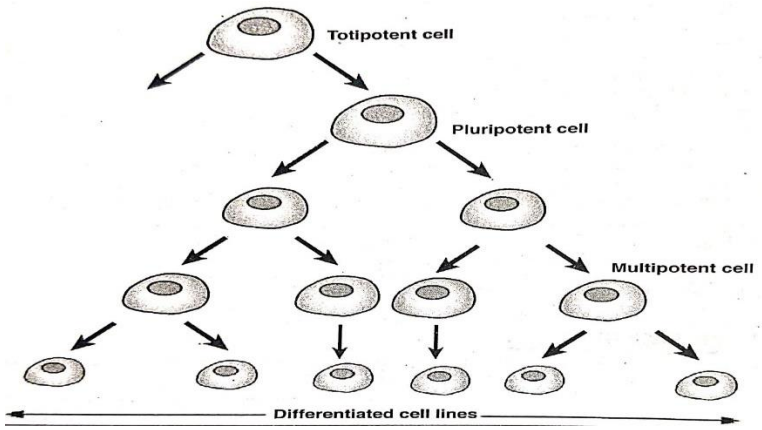

Figure 2: During development, potency of cells becomes progressively restricted and that leads to differentiation.

8.3 INDUCTION:

A simple definition of induction is “an interaction between cells that changes the fate of at least one partner”. Because cells interact in close range, induction is proximate interaction, and because it is a basic principle in embryonic development, it is also called embryonic induction. In inductive interactions, there are at least two components – the inducer and the

responder. **Inducer** produces signal(s) that changes the behavior of the other cell. **Responder** undergoes a change as a result of being induced. The ability of responder cell(s) to respond to a specific inductive signal is called **competence.**

Among several significances of induction in developmental process three are most apparent:

- Induction increases gradual complexity. As embryonic development proceeds, induction is the primary mechanism of generating arrays of different structures.
- Proximity of inducting and responding cells provides a guarantee that the structures developed from will be next to each other and in matching sizes. As we will discuss during development of eye, retina and lens are formed next to each other and in appropriate fitting size.
- Induction occurs at close range, so several inductive interactions can occur simultaneously. As for example, different parts of vertebrate brain induce key parts of nose, ear and eyes at the same time.
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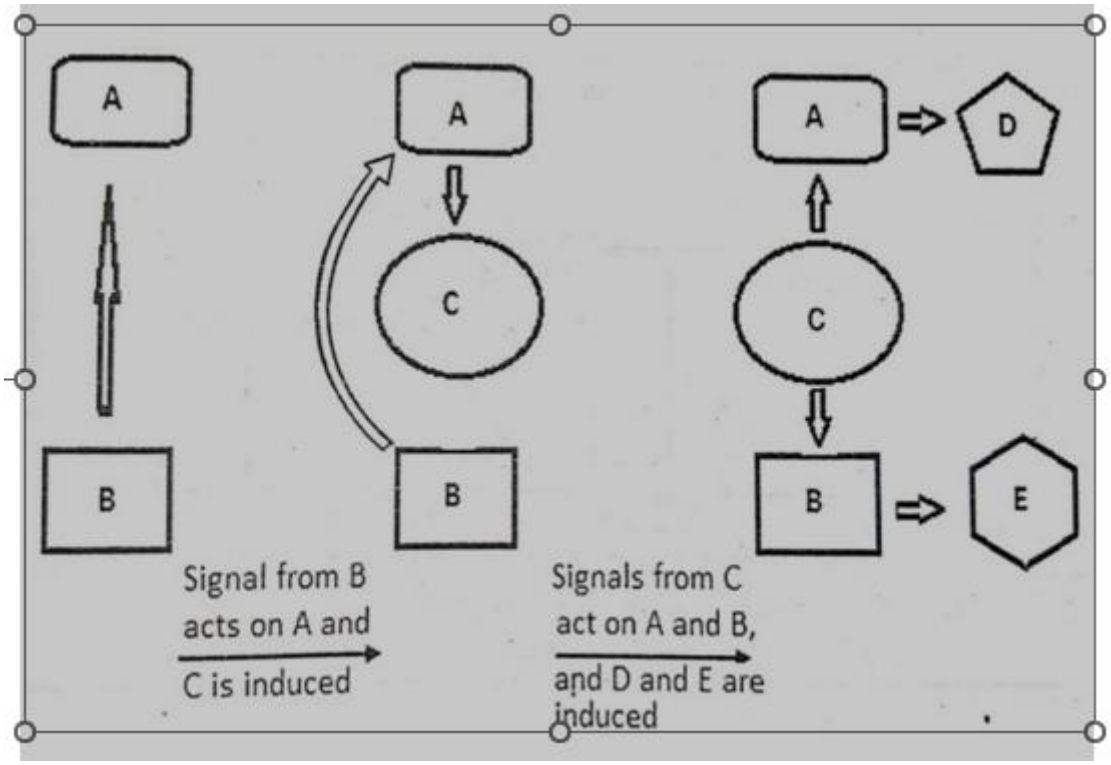

Figure 3: Sequential induction can generate many cell types from a few ones and thus complicated patterns are formed Induction, however, can be activating or suppressing for certain cell fate. Such as, formation of lens in vertebrate eye is promoted by inductive signals from pharyngeal endoderm, heart mesoderm and prospective retina. But lens formation is inhibited by signals form neural crest cells.

8.3.1 TYPES OF INDUCTIONS:

A) Sequential and Reciprocal Inductions

When two cell types are present, one of them can produce a factor that induces some of the neighboring cells to specialize in a third way. The third cell type, in turn, induces back to the other cell types nearby, generating to fourth and fifth cell type, and so on (Fig.3). This

strategy for generating a progressively more complicated pattern is called **sequential induction.** Body plan of a developing organism is formed chiefly through sequential induction. At first a rough miniature form is produced, which becomes elaborated with finer and finer details as development advances.

In the development of vertebrate eye, the lens induces the ectoderm above it to become cornea. After cornea forming ectoderm is induced by lens, the ectodermal cells become columner and secrete multiple layers of collagen. Mesenchymal cells from neural crest enter this area by using the collagen matrix and further differentiate cornea. Then thyroxine hormone dehydrates the tissues and makes it transparent. Thus, formation of cornea requires sequential inductive events.

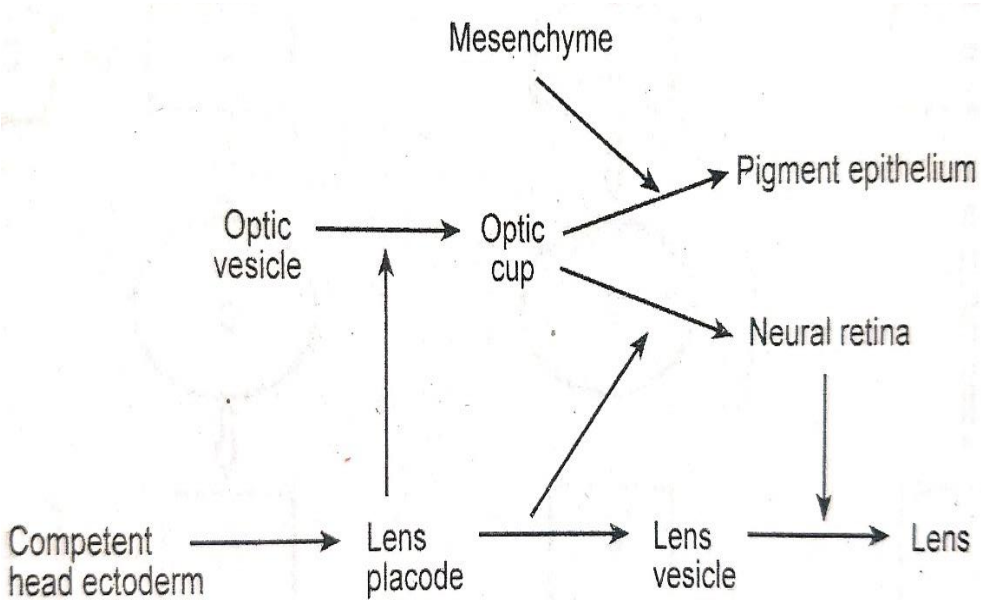

Figure 4: Formation of lens from epidermis and bilayered optic cup from optic vesicle is an ideal example of reciprocal induction.

Another strategy in inductive interaction is that the tissue being induced is inducing its inducer. This type of interaction is called **reciprocalinduction.** Once lens is formed during development of eye, it induces many other tissues. Among these tissues, one is optic vesicle itself, which induces the formation of lens. Under the induction of lens, optic vesicle becomes optic cup. The walls of optic cup are differentiated into two layers –inner neural retina and outer pigmented retina. (Fig.4)

B) Instructive and Permissive inductions:

When a signal from the inducer cell is necessary for the responder cell to differentiate in a particular pathway, it is called **instructive induction.** The responder cell is not capable to differentiate in that particular way in absence of the inducer cell. The general principles of instructive inductions are – In presence of tissue X, tissue B develops into a particular way.In absence of tissue X, tissue B does not develop into that particular way. In early gastrulation of X*enopus,* ectoderm develops into neural plate in presence of notochord, but fails to develop onto neural plate in absence of notochord – an example of instructive induction. When a responder cell is already committed to certain developmental fate but requires the inductive signal only to continue along that pathway, it is called **permissive induction.** In permissive induction, responding cell contains all potentialities to be expressed but needs a permission from the inducer cell, so that, it can express the specify traits. Many cells require the presence of **substrate adhesion molecule** (SAMs) like fibronectin for normal development. Fibronectin does not change the fate of the developing cell but only allow its expression. Apart from both instructive and permissive induction, there is a principle of default program. In absence of a particular inductive signal, responders do not die or make a chaos. In most cases, they have an alternate pathway of development, if they fail to receive the particular signal. As for example, in early *Xenopus* gastrula, neural plate forming ectoderm will develop into epidermis, if fails to get inductive signals from notochord.

8.3.2 Lateral Inhibition and Community Effect:

Two special types of inductions: are **lateral inhibition and community effect.** When particular developmental fate in one cell is inhibited by the signals from adjacent cells, it is called **lateral inhibition.** (This type of negative induction is also called suppressive induction). Initially, all cells are similar and have same potential to differentiate in the same way. In this stage, they all induce each other to repress differentiation. When individual cells start differentiation, their ability to repress neighboring cells increase but tendency to be repressed decrease. This state is theoretically possible by increased production of inhibitor signal and decreased production of receptor at the same time. Randomly, some cells produce more inhibitor signals, they themselves start to differentiate and suppress others. In this process of induction, a regularly spaced pattern is resulted in differentiating cells. The spaced pattern is dependent on strength and ranges of signals (Fig.5). In the neurogenesis of vertebrates, choices of neuronal progenitors are thought to be controlled by lateral inhibition.

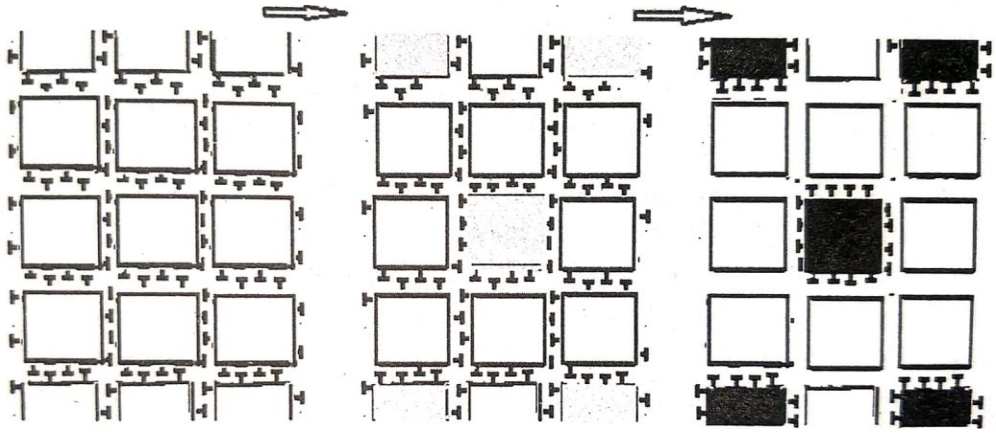

Figure 5: Spaced pattern is thought to be controlled by lateral inhibition and development on strength and ranges of signals

When change of cell fate requires the presence of a population of cells, it is called **community effect.** In this special type of induction, a cell secretes a signal that acts on its own receptor (called autocrine signal; see later in this chapter). Amount of signaling chemical produced by a signal cell is not enough for inductive effect. But a population of cells produce sufficient amount of signals that induce to change the fate of whole cell population. In *Xemopus,* isolated ectodermal cell form blastula form neuron but a portion of ectoderm tissue develops into epidermis in isolation. Cells of ectoderm in *Xemopus*blastula secrete a bone morphogenetic protein (BMP) that suppresses neuronal differentiation. But BMP produced by a single cell is not sufficient for this suppression.

8.4 COMPETNCE:

Competence is the ability of a cell or tissue to respond to specific inductive signals. Competence requires the presence of appropriate receptors and signalling pathways.

- **Features of Competence:**
- **Transient Nature:** Cells are competent only during a specific developmental window.
- **Molecular Basis:** Competence is conferred by the expression of specific receptors, transcription factors, and signaling molecules.

Example: During vertebrate eye development, ectoderm is competent to form a lens only when the optic vesicle signals it. Without these signals, lens formation will not occur.

8.5 DETERMINATION AND INTERMINATE GROWTH:

When the body and organs of an organism grow to a certain point and then their mass stays more or less constant, this type of growth is called determinate. Most mammals including humans follow this pattern of growth. They have a growth period, during which they grow up to the size that is characteristic of the species and sex, and then growth ceases.

In indeterminate pattern, growth occurs throughout the life of an organism, although rate of growth tends to slow down with age. Most other animals and plants undergo indeterminate growth. They have no definite growth period. This characteristic allows determining the age of a fish by examining the annual growth rings on its scale, or that of plant by examining annual rings in the trunk.

8.5.1Determination:

In early embryonic development, potency of a blastomere usually exceeds its fate. As development progresses, the range of its potency becomes more and more restricted, ultimately to its fate. The process by which potency is restricted to the fate of a cell is called **determination,** and the cell is called determined. As for example, if the fate of a cell is to become a neuron, and if after isolation and transplantation, it still forms neuron, then the cell is called determined for neuron development. Determination is the second step of commitment and usually irreversible. Properties of determination and as follows:

Character	Autonomous Specification	Conditional Specification	Syncytial specification
Mechanism	Specification by Differential acquisition of cytoplasmic determinants	Specification by Inductive interaction Between cells	Specification by interaction Between nuclei and cytoplasmic regions
Occurrence	After cellularization	After cellularization	Prior to cellularization
Fate of Blastomeres	Invariant, cells cannot change their fates	Variant, cells can change their fates	Variant cell fate is not rigid
Cell Migration	Occurs after Specification	Occurs before or During specification	Does not arise because There is no cellularization
Process of Development	Mosaic	Regulative	Regulative
Examples	Most invertebrates	All vertebrates and Some invertebrates	Insects

Table 1: Characters of three types of specifications

1. Determination is a stepwise process in which a cell loss its potency, that is commitment to develop in one pathway by excluding others. Determination is the loss of competence to follow alternative pathways of development. The process definitely needs element of instruction. For example, presumptive neural plate region in amphibian blastula and early gastrula develops into epidermis in isolation. Therefore, becoming epidermis is the fate of these cells is absence of further instruction. But to become neural tissue, those cells require additional instructions during gastrulation.

2. Embryonic cells are destined to their fates by several mechanisms. In majority animals, there are some components unevenly distributed in egg cytoplasm and are called **localized cytoplasmic determinants.** These components are asymmetrically divided into blastomeres during cleavage; as a result, blastomeres become biased to certain lineages. Subsequent steps of cell determinations may occur through signal among equivalent cell or between non-equivalent cells. When equivalent cells exchange signals, all of them may attain some determined state or they may compete to attain a preferred state of determination. Interaction between non-equivalent cells creates new types of cells in their interacting zone. This phenomenon is called embryonic induction, observed during morphogenetic movement in gastrulation and organogenesis when cells meet new neighbors and exchange inductive signals.

3. Cell determination can be viewed as embryonic pattern formation. Differentiated cells are organized in spatial pattern in organs, systems and in organism. This organized body patterns are progressively established from earliest stage of development and go on before differentiation. Such intial pattern of most embryos is animal and vegetal poles in blastula. Later inductive interactions produce germinal layers and organ rudiments. The final body pattern is resulted from a hierarchy of determined events in which each step is forwarded on the basis of previous step.

4. When a determined cell divides during normal development, its daughter cells are similarly determined. For example, division of a cell determined to form heart cell the mechanism is least understood. A series of commitment events occur during vertebrate development. A scheme showing major steps is presented in (Fig.6).

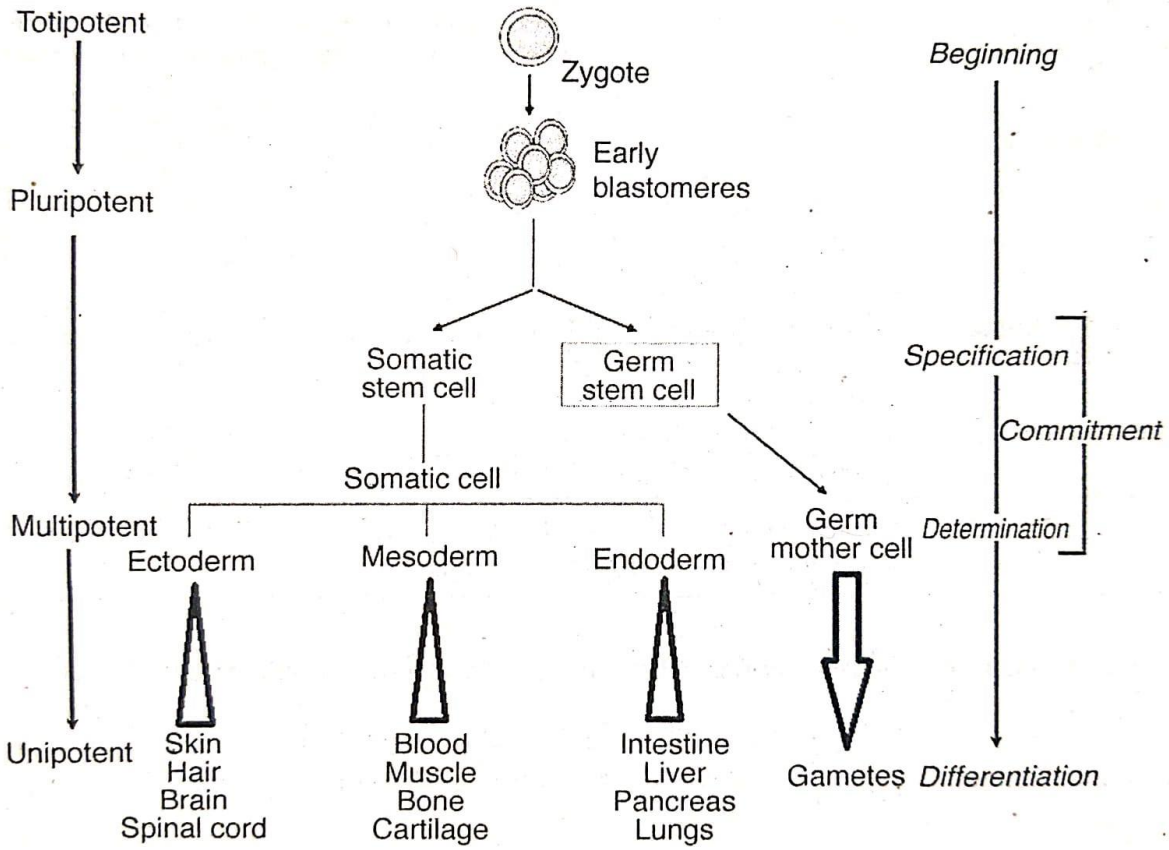

Figure 6:A simple scheme of series of commitments of the vertebrate embryonic cells to become differentiated cell types in adult body. As cells undergo hierarchy of determinative steps, they progressively loss potency (from top to bottom). At late embryo stage, final developmental decisions make the cell differentiated. The boundary between specification and. differentiation depends upon the embryonic cell types.

8.6 DIFFERENTIATION:

Figure 7: Laurent Chabry (1855-1894).a French Medical student, first demonstrated that each cell of tunicate embryo appeared to develop autonomously. This is first record of autonomous specification, hence cell commitment and differentiation, (b): John Tyler Bonner (b. 1920-) identified in 1952 that differentiation is one constructive process of development that induces cellular movement.

8.6.1 Differentiation and class o differentiation:

An organism may be composed of trillions of cells, but number of cell types is relatively very few. Cell type means a mature cell with a definite morphology and a specialized function, such as, a neuron or a muscle cell. All mature cells are formed from some common and unspecialized cells through differentiation.

A simple definition of differentiation is becoming of a cell different from others, Differentiation is a process through which cells become structurally and functionally specialized to give rise distinct cell type. A mature cell after attaining structural and functional specialization is called differentiated. In fact, differentiation of a cell depends upon the protein it synthesizes. Since all cells in an organism's body are genetically identical, then differentiation involves differential gene expression.

Sometime differentiation is classified into several types, such as morphological, functional, behavioral, chemical etc. A differentiated cell performs special functions, which are distinct from the basic functions to all cells (kitchen functions). Such as, a nerve cell conducts nervous impulses, a muscle cell contracts, a hepatocyte secretes bile etc. A cell performing a special function may be called **functionally** or **behaviorally differentiated.** The ability to perform special functions is often evident by visible structure of the cells, such as processes in the nerve cells, myofibrils in the muscle's cells. These cells can be designated as **morphologically differentiated** (Fig.8). Morphological specializations are not always apparent, but secretary vesicles in secretary cells, matrix in bones are also morphological expression of their functional differentiation.

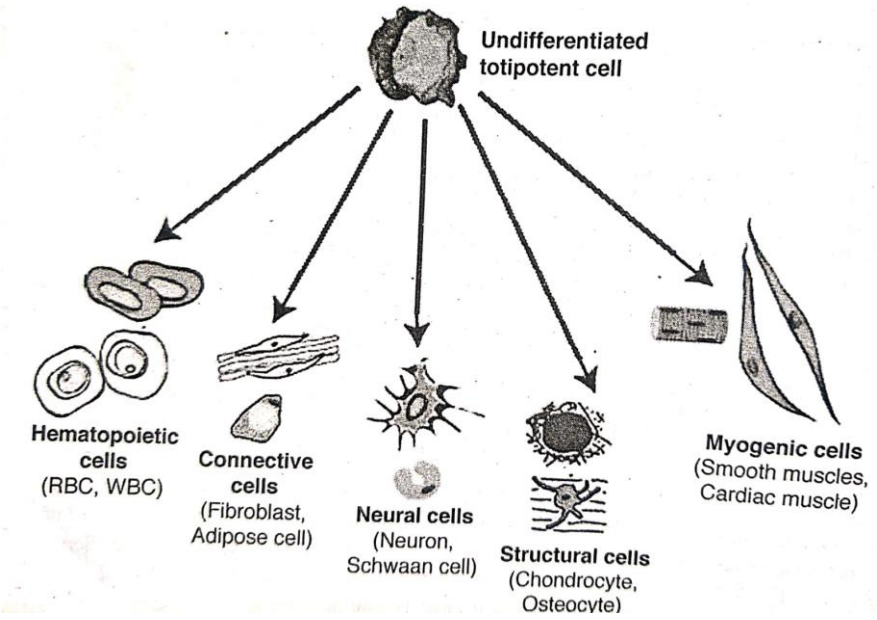

Figure 8: Cells are usually differentiated functionally and/or morphologically.

Whether a cell is differentiated functionally, it must be based on its chemical change or **chemical differentiation.** Both function and morphology of a cell is based on its chemical constitution. Such as, production of myofibrils requires synthesis of actin and myosin; production of bile requires enzymes. Many special chemicals are important in the life of a cell; they may be proteins or non-proteins. The difference between protein and non-protein is that, the latter can be synthesized in the cells with the help of enzymes, which are again proteins. So, proteins- enzymatic or non-enzymatic, produced by a cell determines its special function and morphology that is differentiation. For this reason, differentiation if often viewed as production of unique protein patterns. And it is evident that chemical differentiation is the basic of functional and morphological differentiation.

But proteins are not made by proteins; rather they are synthesized in the cells according to specific codes in the DNA of chromosomes or simply genes. Therefore, all credits of cell differentiation, whether functional, morphological or even chemical, ultimately goes to the genes. We shall discuss genetic control of cell differentiation in latter part of this chapter.

8.6.2 Phases of differentiation:

A cell may give rise to many other cell types or may become a single type or may die. Whatever may be the fate of a cell, there are two extremes in the life of a cell; in one extreme the cell may be structurally and biochemically identical to other cells destined for other fate. In another extreme, the cell is fated to become a specialized cell, such as neuron and the fate is almost assured. The process by which a cell reaches from the first extreme to the second one is differentiation.

Differentiation is lengthy process and a cell makes developmental decisions before it shows any visible changes. These developmental decisions involve changes in biochemistry and function of cells and the process is called **commitment.** Following each decision in developmental hierarchy, cell fate becomes restricted and cells are said to be committed to a certain specialized fate. Process of commitment can be divided into two steps-specification and determination. Specification – **the first step, is a labile phase** and the cell is directed (or specified) to follow a certain developmental pathway. A specified cell is capable to differentiate autonomously when placed in a neutral environment. But a specified cell may change its developmental fate when placed in a different environment, that is, undergoes re-specification. This means that commitment at this step is reversible.

The second step of commitment is determination and irreversible. A determined cell can follow its differentiation autonomously even it is placed in a different environment. A simple scheme of commitment is shown in (Fig.9).

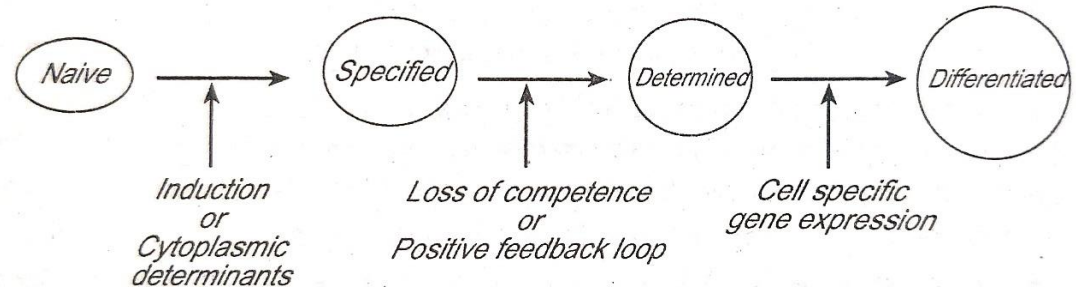

Figure 9: simplified scheme of cell commitment

Specification:

There are three modes of specifications:

1. **Autonomous Specification:** When a particular cell is isolated from an early embryo and it will produce the same cells which it should produce as a part of theembryo, it is called **autonomous specification.** Again, the embryo from which the particular cell is taken will lack those cells, which are to be produced by the missing cell. Autonomous specification results determinative development, where cells cannot change fate if the blastomere is lost. The pattern of development due to autonomous specification is also referred to as mosaic development, because the embryo seems to be made up of a mosaic of independent self-differentiating parts. Such type of specification is found in many invertebrates, particularly in annelids, mollusks and tunicates.

2. Certain proteins and mRNAs called morphogenetic determinants are placed in different regions of egg cytoplasm of these embryos. When the egg or early blastomeres divide, those determinants are distributed asymmetrically to different daughter cells. Specification of cell types is due to these morphogenetic determinants. Experimental studies have revealed that when a blastomere is removed from 8-celled tunicate embryo, the embryo lacks those structures to be produced by that particular blastomere. Moreover, the isolated blastomere produces those structures which are missing in the embryo. Cytoplasmic segregation of the morphogenetic determinants responsible for this pattern is confirmed biochemically (Whittaker, 1983).

3. **Conditional Specification:** This mode of specification involves interaction with other cells. Originally, each cell has much greater potency than its fate, so that it can produce many different cell types. Cell fate is restricted by its interaction with other neighboring cells. Because cell fate depends upon the conditions in which it develops, this type of specification is called conditional. If a particular cell is removed from an embryo, it cannot fulfill its normal fate, because it lacks the necessary interaction. Furthermore, remaining cells in the embryo can compensate for the missing cell. This filling in of missing parts is called **regulation** and the pattern of embryogenesis due to conditional specification is referred to as **regulative development.** Conditional specification is characteristics of all vertebrates and a few invertebrates like sea urchin.

4. An embryo is referred to as harmonious equipotential system (Driesch, 1892) because each cell in it gives up most of its potential in order to produce a part of the single complete organism. Theoretically, each cell could have become a complete animal but did not. Then instead of becoming autonomous entities, cells cooperate with each other. Recent experiments reveal that gradual restriction of fate of neighboring cell is due to negative induction events. When skeletal mesodermal cells are removed from early gastrula of sea urchin, an equal number of gut precursor cell form skeletal mesoderm. Therefore, skeletal mesodermal cells have restrictive effect on gut precursors and prevent them to form skeletal mesoderm. In fact, presence of neighbor cells, even of same kind, restricts the potencies of both partners. A part from influence of neighboring cell, cell fates may be specified by morphogens.

5. **Syncytial Specification:** A third type specialized strategy for specification employed by insects is syncytial specification. In this type of specification, interactions occur between different parts of cells, not between cells. The zygote nucleus divides.

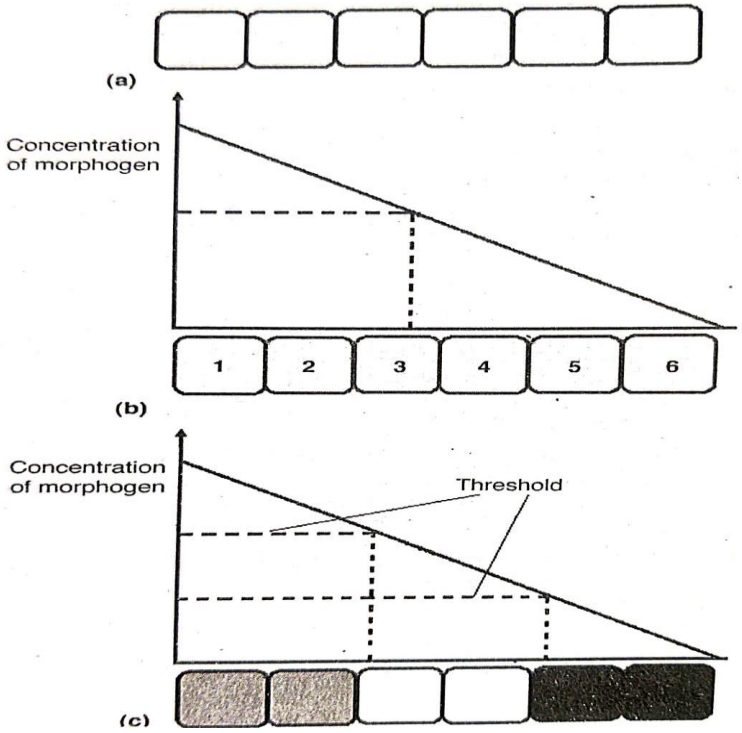

Figure 10:A schematic view of positional information sensed by cells by a gradient of diffusible morphogens several times producing thousands of nuclei, but no cytokinesis occurs. Hence a syncytial condition observed in the egg cell. Because egg cytoplasm is not uniform, interactions between different diffusible regulators in cytoplasm and individual nuclei determine the cell fates prior to cellularization.

Syncytial specification is evident in the development of *Drosophila.* Fertilized egg contains two diffusible proteins – Bicoid and Nanos. Concentration of Bicoid protein is highest in anterior portion of egg and gradually declines in the posterior portion. Reversely, concentration of Nanos protein is highest in the posterior portion of the egg and gradually declines at the anterior. Thus long axis of *Drosophila* shows two opposing gradients – Bicoid from anterior to posterior and Nanos from posterior to anterior. The result is that each region of egg is unique by different ratio of these two proteins. When egg nuclei divide, daughter nuclei occupy different regions of egg cytoplasm. These nuclei receive positional information by the combined ratio of these two proteins along the anterior-posterior axis and specify accordingly. Nuclei exposed to high Bicoid but low Nanos concentrations have their genes activated to produce the head region of the fly. Similarly, nuclei exposed to moderate concentration of both Bicoid and Nanos proteins are specified to become thorax. Again, nuclei-exposed to low Bicoid and high Nanos concentration are instructed to become abdominal structures.

8.6.3 Gene control in differentiation:

There is no doubt that Multicellular organisms consist of different cell types, which differ in both morphology and function. The differences are so extreme, such as between a neuron and

a red bold cell, that it is difficult to believe that they have same genome. Again, cell differentiation is usually irreversible.

There are three hypotheses (Fig.11) regarding control of differentiation. The first hypothesis proposed by Weismann and Roux was that cells become different due to differentialloss of genetic materials. Each cell type inherits that fraction of genome that is necessary for its own fate. The second hypothesis was selective gene amplification, which forwarded that cells replicate those specific segments of genome that are used most. The third hypothesis is based on principle of differential gene expression. Differential gene expression means different types of cells express different set of genes of their genetic materials. Now it is well established that cell differentiation is a change in gene expression, not any change in cell's genome.

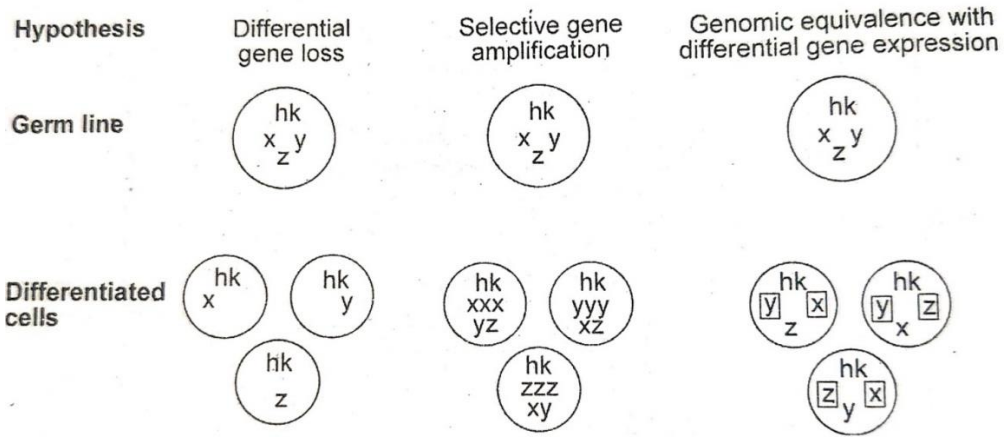

Figure 11: Three hypotheses about cell differentiation. hk = house-keeping genes; x, y, z = tissue specific genes; box indicates inactive genes A cell types (except gametes) in a multicellular organism contain the same DNA, no matter how they differ in look or function. This concept is called **genomic equivalence.** Cells preserve their genome during differentiation is evident from a classic experiment. When the nucleus of a skin cell from an adult frog is injected into an enucleated frog egg, it gives rise to a normal tadpole (Fig. 12). A tadpole contains full range of differentiated cells, so the donor skin cell nucleusdoes not loss

any part of its DNA sequences during differentiating into skin cell. Similarly, a single cell form carrot when kept in culture,

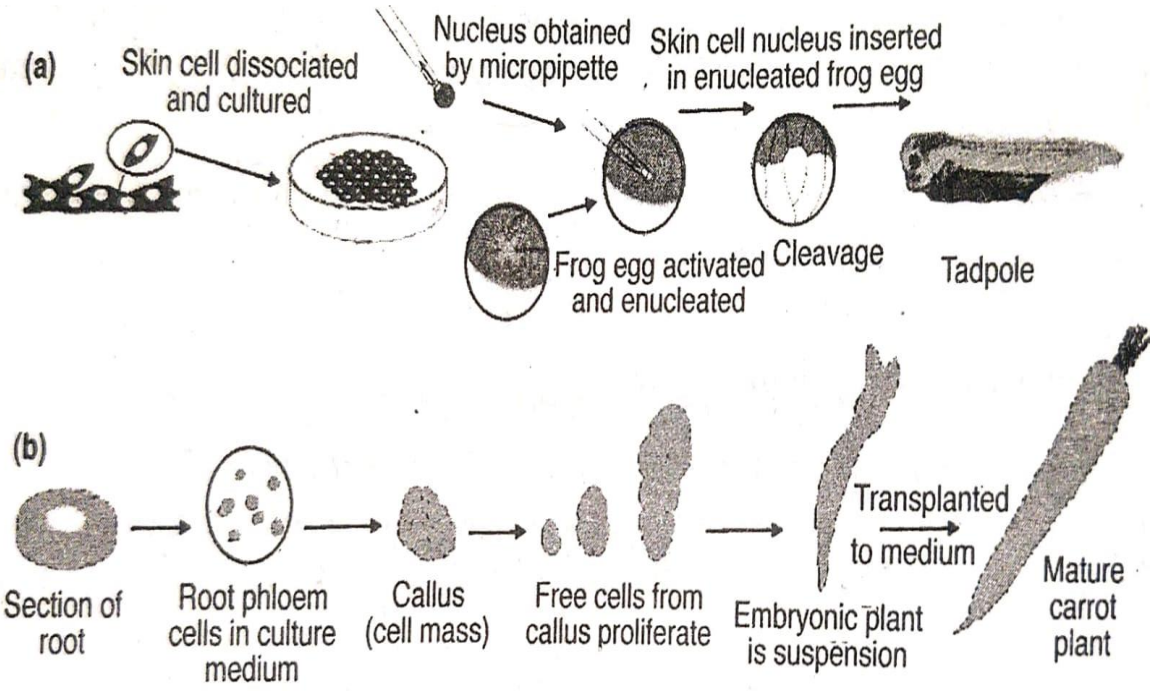

Figure 12:A differentiated cell contains the entire genome necessary for development of a complete organism. (a) Nucleus from the skin cell of an adult frog can gives rise to normal tadpole; (b) A single cell from carrot can produces a new carrot plant in culture. can gives rise to an entire carrot plant (Fig.12). Comparison of genomes of different types of cells has been done by modern recombinant DNA technology. The general rule is that, there may be differential gene expression but no change in the DNA sequences of the genes. [One exception to this general rule is observed in development of immune system of mammals, where DNA rearrangement takes place in genome.

8.7 SUMMARY:

Potency, induction, competence, determination, and differentiation are fundamental concepts in developmental biology that govern the transformation of a single fertilized egg into a multicellular organism. Potency describes a cell's potential to differentiate into various types, ranging from totipotent to unipotent. Induction involves signaling molecules from one group of cells influencing the developmental pathways of neighboring cells, which is crucial for tissue and organ formation. Competence is the ability of cells to respond to these inductive

signals, which depends on the presence of specific receptors and signaling pathways. Determination marks the irreversible commitment of a cell to a specific fate, even before any observable changes occur. Differentiation follows determination and involves the acquisition of specialized structures and functions, resulting in the formation of distinct cell types such as neurons, muscle cells, or blood cells. Together, these processes ensure proper development and tissue specialization in multicellular organisms.

8.8 TECHINICAL TERMS:

Competence, receptors, signaling pathways, determination, irreversible commitment, differentiation, specialized structures.

8.9 SELF-ASSESSMENT:

1. Define potency and describe its different levels.
2. What is induction, and why is it important in development?
3. Explain the concept of competence with examples.
4. Differentiate between determination and differentiation

8.10 SUGGESTED READINGS:

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Prof. K. Sumanth Kumar

LESSON - 9

CELL AGGREGATION AND DIFERENTIATION

AIMS AND ONJECTIVES

- To understand the molecular basis of axis and pattern formation in *Drosophila*, amphibians, and chick embryos.
- To study the genetic and biochemical pathways involved in dorsal-ventral patterning in *Drosophila* oocyte and embryo.
- To compare and contrast axis formation mechanisms in invertebrate and vertebrate models.
- To explore the role of signaling molecules like BMPs, Wnt, and Hedgehog in axis specification.
- To appreciate the evolutionary conservation and divergence of patterning mechanisms.

STRUCTURE:

9.1 INTRODUCTION

Axis and pattern formation are fundamental processes in developmental biology that establish the body plan of an organism. These processes ensure that the various regions of an embryo develop into specific tissues and organs in an organized manner. In model organisms like *Drosophila* (fruit fly), amphibians, and chick embryos, axis formation and dorsal-ventral (DV) patterning have been studied extensively. These studies have provided valuable insights into the conserved molecular mechanisms and signaling pathways driving embryonic development.

Drosophila melanogaster serves as a classical model for understanding genetic control over axis formation, while amphibians and chicks offer insights into vertebrate embryonic development. This document delves into axis and pattern formation in these models, highlighting the regulatory molecules and signaling mechanisms involved.

9.2 AXIS FORMATION

In the foundation of body plan of most animals, the embryo must develop three axes- the anterior-posterior axis, the dorsal-ventral axis and the left-right axis. The line extending from head to tail (or mouth to anus) is anterior-posterior axis. The line extending from dorsum (back) to ventral (belly) is the dorsal-ventral axis. The line between the two lateral sides of the body is left-right axis. It seems that the embryo knows which organs are to be developed on either side and which organs belong to one our other side. A typical axis in an organism has various body parts arranged from pole to pole in a specific order. Such as anterior – posterior axis of a mammal includes head, neck, thorax, belly and so on. Then how the axes are specified during development is an important question. A most common way for determination of one pole of an axis is by cytoplasmic determinants and their presence following a concentration gradient. Among other ways of specification of axis include point of sperm entry and series of interactions during subsequent development. In this context, we shall discuss axis formation only with the example of frog *Xenopus.*

9.2.2 Determination of Anterior-Posterior Axis:

The anterior-posterior axis in frog develops during gastrulation from previous animal-vegetal axis. The animal-vegetal axis in amphibians establishes during oogenesis by the presence of germinal vesicle on animal pole and mitochondrial cloud in between germinal vesicle and vegetal pole. Accumulation of yolk protein in the form of yolk platelets also contribute to the establishment of animal-vegetal axis. About 70% of total yolk protein is accumulated in vegetal pole and there is a gradient of yolk platelets. Largest yolk platelets are accumulated in vegetal pole, smallest in animal pole and intermediate sizes in the intermediate region.Spatial order of the germ layer rudiments at blastula stage contribute to the establishment of anterior-posterior axis. Most blastomeres of animal half form endoderm. Blastomeres of the intermediate zone develop into mesoderm. During gastrulation, endoderm and mesoderm move inward and ectoderm expand to cover the whole embryo. The area of ectoderm remains close to animal pole will form the anterior structures of embryo-like brain, head epidermis etc. Opposite area of ectoderm will form the posterior structures like spinal cord, trunk and tail epidermis. Then animal –vegetal axis of blastula of frog is translated into anterior-posterior axis of embryo, at least of ectoderm. For mesoderm and endoderm, this transformation is complicated by morphogenetic movements during gastrulation.

9.2.3 Determination of Dorsal-Ventral Axis:

Dorsal- ventral axis arises from cytoplasmic arrangements occur during the first cell cycle after fertilization –between egg activation and first cleavage. Proper establishment of dorsal – ventral axis in amphibians has a regular relation to the point of sperm entry. A gray crescent forms near the egg equator and usually opposite to the point of sperm entry. The gray crescent marks the future dorsal side of the embryo where the blastopore will form and the neural plate will develop later.

Following sperm entry, a rotation of egg cortex relative to endoderm occurs. During cortical rotation, cytoplasmic components originally positioned near the vegetal pole are transported to the prospective dorsal side of the embryo. In this movement, microtubules play crucial roles. Upon reaching the prospective dorsal side, these cytoplasmic components interact with mesoderm inducing factors to form dorsal mesodermal structures, particularly notochord.

Prospective notochord, also known as Spemann's organizer, induces overlying ectoderm to form neural plate. The same organizer also induces neighboring mesodermal cells to make lateral structures, like somite's, nephrotomies, and lateral plates. Thus, full set of mesodermal organs along the dorsal -ventral axis is formed.Interests are shown to know the molecular mechanisms of dorsal-ventral axis formation. The most likely molecule that establishes the dorsal pole of the embryo is β -catenin. During cortical rotation, β-catenin is found in association with microtubules that move cytoplasmic components from the vegetal region to the dorsal side of the embryo. Removal of β-catenin stops formation of dorsal-ventral polarity and a radially centralized embryo is formed. It is also observed that some growth factors like fibroblast growth factor (FGF) and transforming growth factor- β (TGF- β) plays as diffusible signal in the induction of mesoderm and origin of Spemann's organizer.

9.2.3 Determination of Left-Right Axis:

Most animals have a median plane defined by anterior-posterior and dorsal –ventral axes and they are called bilaterally symmetrical relative to that median plane. The lateral halves of a bilaterally symmetrical animal are termed as left and right sides, which differ in their handedness because their medio-lateral axes point towards opposite direction. The median plane of the future embryo is placed over the widest part of gray crescent that is centered over the meridian of greatest cortical displacement toward animal pole after sperm entry. The first cleavage furrow often bisects the gray crescent resulting first two blastomeres, each of which will become a lateral half of the embryo. In many animals, some inner organs are not positioned symmetrically though they are bilaterally symmetrical externally. This condition is called.

This asymmetry is not random, rather consistent, such as in normal human stomach is positioned to left and most of liver to the right of the abdominal cavity. Thus left-right asymmetry is a very regular one.

The origin of left-right asymmetry is thought to be dependent on some molecules having handedness. When such handed molecules are oriented with regard to anterior-posterior and dorsal-ventral axes, they determine the left-right polarity. These molecules cause oriented transport of signals, which in turn, may direct appropriate gene expression for left-right asymmetry and morphological development occurs accordingly.

9.3 AXIS AND PATTERN FORMATION IN DROSIPHILA:

The general body plan of Drosophila is same in the embryo, the larva, and the adult possessing a distinct head end, repeating segmental units and a distinct tail.
In the repeating segmental units three segments form thorax, while another eight segments form the abdomen. The first thoracic segment has legs; the second thoracic segment has legs and wings and the third thoracic segment has legs and halters (balancing organs).

9.3.1 Anterior-Posterior Axis Formation:

The follicular epithelium surrounding the developing oocyte is broken by two signals which involve the same gene, *gurken*organized by the oocyte nucleus.
In the oocyte nucleus, *gurken*gene is localized between the nucleus and the cell membrane and is translated into *gurken* protein.

The time at which oocyte nucleus is very close to the posterior tip, *gurken* signal which results in the "poster ionization" of the follicle cells is received by these follicle cells through a receptor protein encoded by the torpedo gene (Figure:1)Specification of anterior-posterior axis during oogenesis

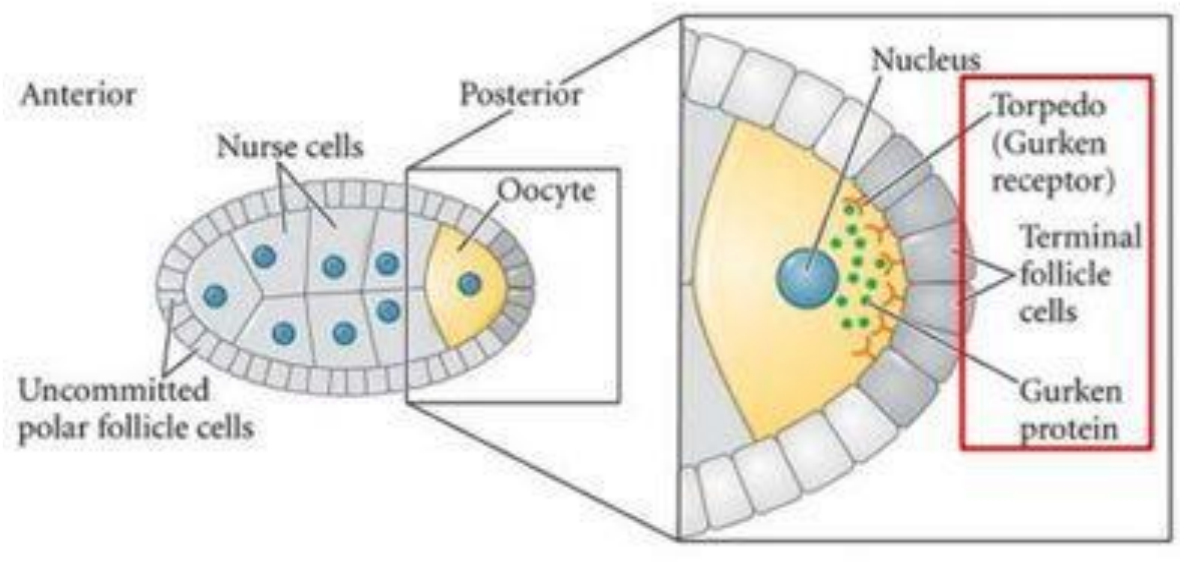

Figure :1 Specification of anterior-posterior axis during oogenesis

1.3.2 Anterior-Posterior Body Pain:

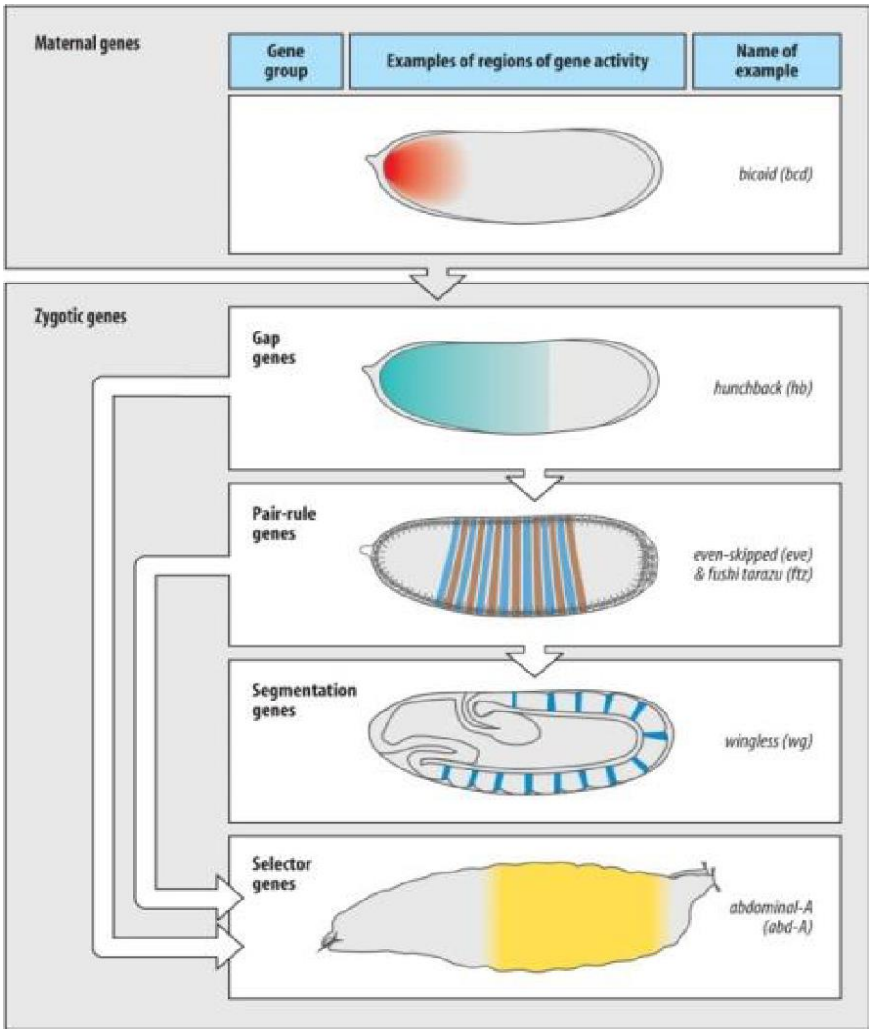

Figure 2:maternal genes and zygotic genes

1. Gap genes

Gap genes are the first such zygotic genes to be expressed and encode transcriptional factors. These are called so because mutations in gap genes cause gaps in the segmentation pattern.Gap genes define regional differences and are activated or repressed by the maternal effect genes. These genes are expressed in the anterior-posterior domain. Examples of gap genes are hunchback, kruppel, and knirps, giant, tailless etc.

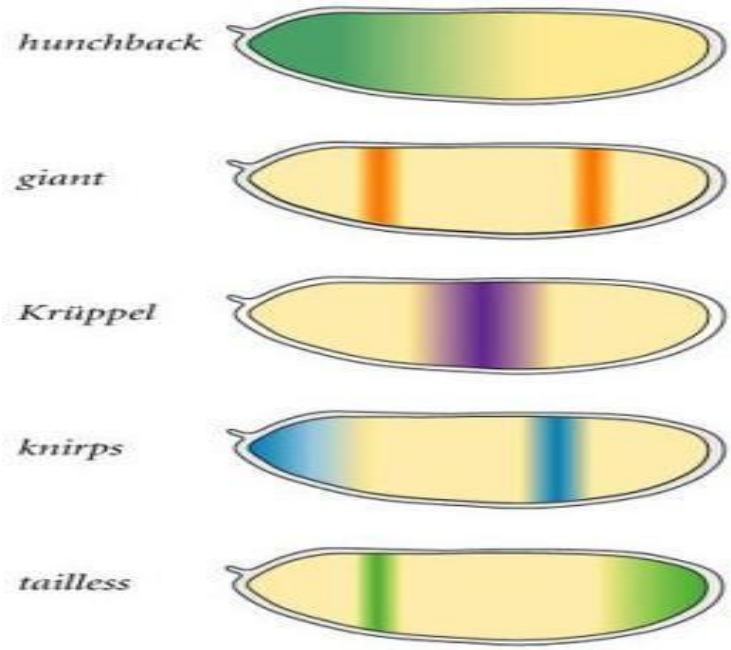

Figure 3: Gap genes

2. Pair-Rule Genes:

Pair-rule genes divide the embryo into periodic units and their transcription is regulated by differing combinations and concentrations of the gap gene proteins. Their transcription results in a striped pattern of seven transverse bands perpendicular to the anterior-posterior axis.
The primary pair-rule genes, hairy, even-skipped, and runt are expressed in seven stripes where each stripe corresponds to every second para segment. There are pair-rule genes which define odd numbered para segments (e.g., even-skipped), whereas others define even-numbered para segments (e.g., fushitarazu).

Bicoid and hunchback proteins activates the even-skipped gene, though the boundaries of the stripe are defined by kruppel and giant proteins by repressing even-skipped at posterior and anterior edge of the stripe respectively. In contrast, fushitarazu are not regulated by the gap genes,
but they may depend on the prior expression of primary pair-rule genes such as even-skipped and hairy.

3. Segment polarity genes:

Segment polarity genes are activated by the pair-rule proteins. The mRNA and protein products of the segment polarity genes divide the embryo into 14-segment-wide units and establish the periodicity of the embryo.
segment polarity genes are activated in response to pair-rule gene expression and have two important functions to perform:

- They reinforce the parasegmental periodicity established by the earlier transcription factors.
- Establishing cell-to-cell signalling and cell fates within each Para segment.

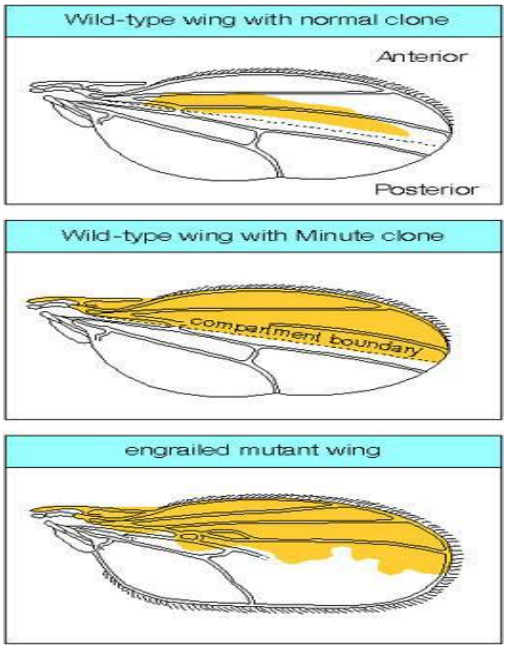

Figure 4: anterior and posterior compartments in the wing
Demonstration of the boundary betweenanterior and posterior compartments in the wing by marked cell clones.

4. Homeotic Selector Genes:

Homoerotic selector genes which determine the developmental fate of each segment are regulated by the interaction of the protein products of the gap, pair-rule, and segment polarity genes at the same time.

Specification of each segment is defined by homeotic selector genes. In Drosophila two homeotic gene clusters are present named as bithorax complex and Antennapedia complex and the chromosome region containing these complexes are referred to as the homeotic complex (Hom-C).

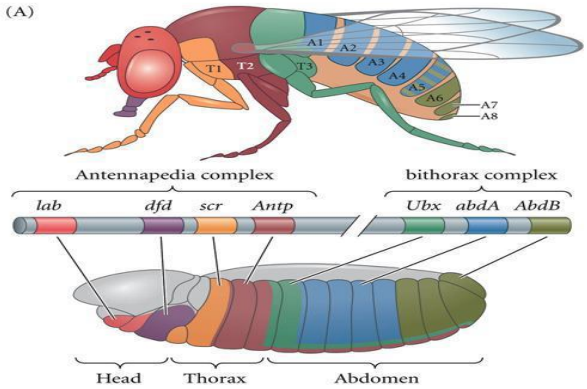

Figure 5: Expression of homoerotic selector gene in *Drosophila*

Bithorax complex: these are responsible for diversification of posterior segments. It contains three genes,
Ultrabithorax: mutation in this gene causes transformation of halteres into wings, i.e. fly results in four wings (Figure 6).

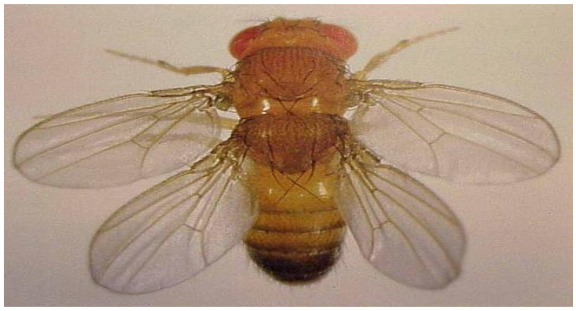

Figure 6: fly with four wings

Abdominal A (abd A) and Abdominal B (abd B):segmental identities of abdominal segment is regulated by these two genes.

Antennapedia complex: It is a complex of five genes,

1. labial (*lab*)
2. deformed (*dfd*)
3. Antennapedia (*Antp*)
4. sex combs reduced (*scr*)
5. proboscipedia (*pb*)

labial and *deformed* genes are involved head segment specification, whereas *Antp*and *scr*are responsible for thoracic segment specification. Gene *pb* is active only in the adult flies, due to its mutation transformation of labial palp of mouth into legs occurs.

9.4 DORSAL-VENTRAL PATTERNING IN OOCYTE:

The movement of oocyte nucleus occurs towards anterior dorsal position with increase in volume of oocyte. Message of *gurken*gene is localized in crescent between the oocyte nucleus and the oocyte cell membrane. The product of *gurken*gene is *gurken* protein which forms an anterior-posterior gradient along the dorsal surface of oocyte. *gurken*gene is present only in oocyte, whereas *torpedo* is active only in the somatic follicule cells. Follicle cells contain Torpedo receptor protein which receives *gurken* signals. The Torpedo signal inhibits the expression of *pipe* gene, because of which Pipe protein is produced only in the ventral follicle cells.

9.5 DORSAL-VENTRAL PATTERNING IN THE DROSOPHILA:

Dorsal and Ventral Pattern in the Embryo Product of the gene dorsalis involved in the Dorsal-ventral patterning of the embryo. Mother gene *dorsal* produces Dorsal protein which is placed in the oocyte by nurse cells. Dorsal protein is also known as ventral morphogen. In the syncytial blastoderm of early Drosophila embryo Dorsal protein is present everywhere, but in late embryo Dorsal protein is translocated only in the ventral part. During ventral specification Dorsal enters the nucleus and represses the genes.

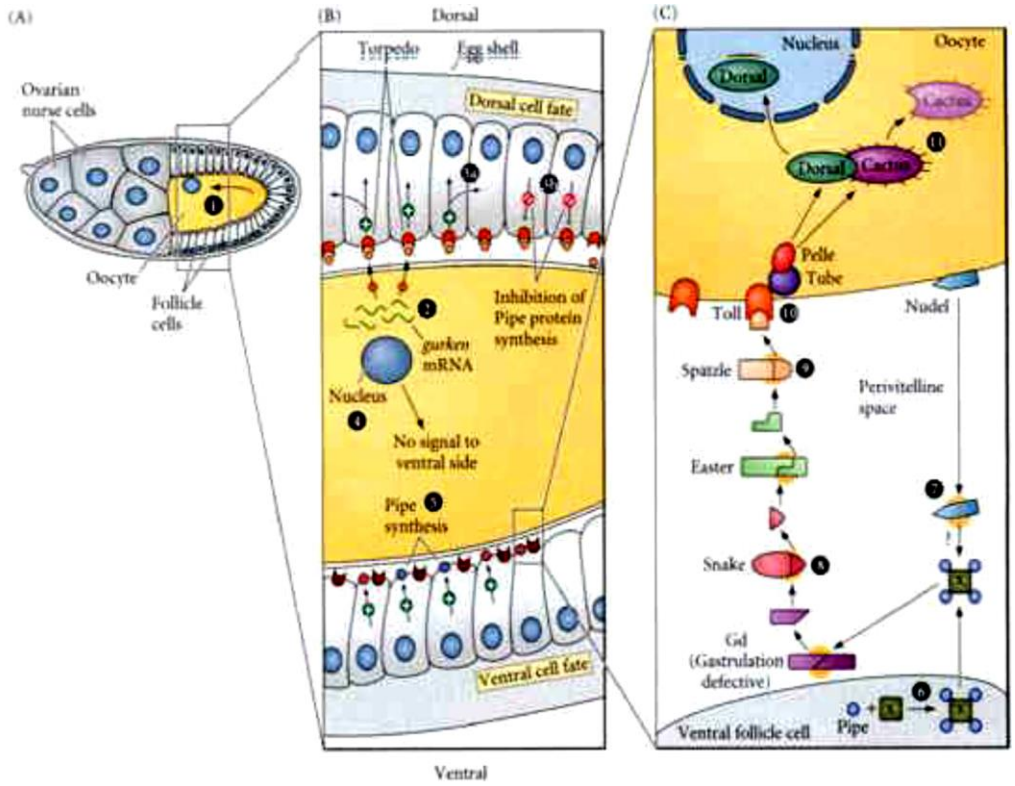

Figure 7: Dorsal and Ventral Pattern in the Embryo

- Twistand snailare activated where the inter nuclear concentration of Dorsal is highest. These genes are responsible for development of cells as mesoderm and for gastrulation.
- Rhomboidgene is activated where low level of Dorsal protein is present, and it acts as future neuroectoderm. In more ventral region they are repressed by Snail protein.
- Decapentaplegic, Tolloid and Zerknulltare repressed by Dorsal proteins (which is present mainly in the ventral region), so are expressed in the dorsal region of the embryo (where there is no Dorsal protein in the nuclei).
- Zerknulltgene is expressed in the dorsal most region of the embryo and forms amino serosa.
- Decapentapelagicgene is involved in the specification of dorsal part of embryo where no Dorsal protein is present and also specifies dorsal ectoderm. *dpp*is a member of TGF-β family of cytokines. Decapentapelagic is homolog of bone morphogenetic protein-4 (BMP-4) present in vertebrates.

9.6 AXIS FORMATION IN AMPHIBIANS:

9.6.1 Determination of Dorsal-Ventral Axis:

Dorsal- ventral axis arises from cytoplasmic arrangements occur during the first cell cycle after fertilization –between egg activation and first cleavage. Proper establishment of dorsal – ventral axis in amphibians has a regular relation to the point of sperm entry. A gray crescent forms near the egg equator and usually opposite to the point of sperm entry. The gray crescent marks the future dorsal side of the embryo where the blastopore will form and the neural plate will develop later.

Following sperm entry, a rotation of egg cortex relative to endoderm occurs. During cortical rotation, cytoplasmic components originally positioned near the vegetal pole are transported to the prospective dorsal side of the embryo. In this movement, microtubules play crucial roles. Upon reaching the prospective dorsal side, these cytoplasmic components interact with mesoderm inducing factors to form dorsal mesodermal structures, particularly notochord. Prospective notochord, also known as Spemann's organizer, induces overlying ectoderm to form neural plate. The same organizer also induces neighboring mesodermal cells to make lateral structures, like somite's, nephrotomies, and lateral plates. Thus, full set of mesodermal organs along the dorsal-ventral axis are formed.Interests are shown to know the molecular mechanisms of dorsal-ventral axis formation. The most likely molecule that establishes the dorsal pole of the embryo is β-catenin. During cortical rotation, β-catenin is found in association with microtubules that move cytoplasmic components from the vegetal region to the dorsal side of the embryo. Removal of β-catenin stops formation of dorsal-ventral polarity and a radially centralized embryo is formed. It is also observed that some growth factors like fibroblast growth factor (FGF) and transforming growth factor- β (TGF- β) plays as diffusible signal in the induction of mesoderm and origin of Spemann's organizer.

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9.6.2 Determination of Anterior-Posterior Axis in Amphibians:

Spatial order of the germ layer rudiments at blastula stage contribute to the establishment of anterior-posterior axis. Most blastomeres of animal half form endoderm. Blastomeres of the intermediate zone develop into mesoderm. During gastrulation, endoderm and mesoderm move inward and ectoderm expand to cover the whole embryo. The area of ectoderm remains close to animal pole will form the anterior structures of embryo-like brain, head epidermis etc. Opposite area of ectoderm will form the posterior structures like spinal cord, trunk and tail epidermis. Then animal-vegetal axis of blastula of frog is translated into anterior-posterior axis of embryo, at least of ectoderm. For mesoderm and endoderm, this transformation is complicated by morphogenetic movements during gastrulation.

9.7 AXIS FORMATION IN CHICK EMBRYO:

Body axes in chick are formed during gastrulation but axis specification begins during early cleavage.

9.7.1Formation of anterior-posterior axis:

An initial radial symmetrical blastoderm of chick becomes a bilaterally symmetrical due to gravity. Lighter components of yolk of chick ovum come to lie below one side of blastoderm (probably due to spinning of ovum in hen's reproductive tract). As a result, one end of blastodermbecomes elevated from which primitive streak formation starts. This end initiates the posterior of the embryo.

Box 1: The chick organizer

The specific portion of blastoderm from where the gastrulation is initiated is the **Posterior Marginal Zone** (PMZ) Apparently, this PMZ contains cells, those act as equivalent of the amphibian Nieuw Koop center. Just anterior to this PMZ, the organizer of the chick embryo is formed. The epiblast and cells in the anterior portion of Koller's sickle becomes Hensen's node of chick embryo is known as equivalent of amphibian dorsal lip of blastopore. The reason is that gastrulation begins in this site and the cells of this site become chorda-mesoderm. When this region is transplanted into another location of gastrula, it can organize a second embryonic axis.

Anterior identity of chick embryo is established with the help of hypoblast and presumptive endoderm (the lower layer). This lower layer secretes Cerberus and other head inducer proteins and induces the anterior end.

9.7.2 Formation of dorsal –ventral axis:

The dorsal-ventral axis in chick embryo is defined by the orientation of blastomeres with respect to yolk. While animal pole is up, ventral is down and towards the yolk mass. This axis is created by a difference of pH between inside and outside of the blastoderm; when sub germinal space is inside, the albumin is the outside. The sub germinal space has pH 6.5, while that of albumin is 9.5.

9.7.3 Formation of left-right axis:

The distinction between left and right side of chick embryo is controlled by two proteins-Nodal, a paracrine factor and Pitx2, a transcription factor.

When the primitive streak reaches its maximum length, on the left side of embryo, Sonic hedgehog protein activates Cerberus. Cerberus prevents BMP from repressing Nodal, which activates Pitx2-crucial for directing the left-specific features.On the right side of the embryo, transcription of Sonic hedgehoggene is inhibited by the expression of activin. Absence of Sonic hedgehog protein ultimately blocks the expression of Pitx2-the left specific factor. However, limited expression of activin protein to the right side only is a mystery.

The anterior-posterior axis in frog develops during gastrulation from previous animal-vegetal axis. The animal-vegetal axis in amphibians establishes during oogenesis by the presence of germinal vesicle on animal pole and mitochondrial cloud in between germinal vesicle and vegetal pole. Accumulation of yolk protein in the form of yolk platelets also contribute to the establishment of animal-vegetal axis. About 70% of total yolk protein is accumulated in vegetal pole and there is a gradient of yolk platelets. Largest yolk platelets are accumulated in vegetal pole, smallest in animal pole and intermediate sizes in the intermediate region.Spatial order of the germ layer rudiments at blastula stage contribute to the establishment of anterior-posterior axis. Most blastomeres of animal half form endoderm. Blastomeres of the intermediate zone develop into mesoderm. During gastrulation, endoderm and mesoderm move inward and ectoderm expand to cover the whole embryo. The area of ectoderm remains close to animal pole will form the anterior structures of embryo-like brain, head epidermis etc. Opposite area of ectoderm will form the posterior structures like spinal cord, trunk and tail epidermis. Then animal-vegetal axis of blastula of frog is translated into anterior-

posterior axis of embryo, at least of ectoderm. For mesoderm and endoderm, this transformation is complicated by morphogenetic movements during gastrulation.

9.8 SUMMARY:

Axis and pattern formation are central to embryonic development, establishing the body plan in organisms. In *Drosophila*, anterior-posterior and dorsal-ventral axes are defined by maternal effect genes, morphogen gradients, and zygotic gene expression. The dorsal-ventral patterning of the oocyte is governed by signaling pathways like Toll and Dpp (Decapentaplegic). During early embryogenesis, gradients of morphogens such as Dorsal protein play pivotal roles.

In amphibians, the Spemann organizer directs dorsal-ventral and anterior-posterior axis formation through gradients of Wnt, BMP inhibitors, and Nodal-related signals. Similarly, in chick embryos, Henson's Node functions as an organizer, with signaling pathways like FGF, Wnt, and Sonic Hedgehog contributing to axis specification.

Together, studies in these organisms reveal both the diversity and conservation of mechanisms underlying embryonic patterning. The findings provide critical insights into developmental biology and evolutionary processes.

9.9 TECHINICAL TERMS:

Axis formation, pattern formation, dorsal-ventral axis, anterior-posterior axis, maternal effect genes, zygotic genes, morphogen gradients.

9.10 SELF-ASSESSMENT:

1. Axis & Pattern formation in Drosophila
2. What role do maternal effect genes play in axis formation in *Drosophila*?
3. How does the Spemann organizer contribute to dorsal-ventral patterning in amphibians?

9.11 SUGGESTED READINGS:

1. Longo FJ. 1987. Fertilization. Chapman & Hall, London.
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Prof. G. Simhachalam

LESSON-10

POST EMBRYONIC DEVELOPMENT

AIMS AND OBJECTIVES

- Understand the concept of metamorphosis and its significance in the animal kingdom.
- Compare the different types of metamorphosis in insects, amphibians, and other organisms.
- Explore the physiological, ecological, and evolutionary implications of metamorphic changes.
- Define and explain the process of retrogressive metamorphosis and its unique characteristics.

STRUCTURE

10.1 INTRODUCTION:

METAMORPHOSIS:

In many animals, embryonic development ends with the formation of a stage that does not resemble a young adult-called larva. Larva is a free-living and normally sexually immature stage in life cycle. Most often larval stage has separate life style and separate name than the adult stage, and specific functions like growth and dispersal. The transition from the larval to adult stage entails an amazing sequence of events known as metamorphosis: we generally use to say that larva undergoes metamorphosis to become adult.Existence of larval forms is so widespread in animal world that it is expected to have some biological pay off. Adult sea urchin leads a sedentary life; the pluteus larva of sea urchin can travel on ocean currents, hence help in dispersal. Adults of many species of moths live for a brief period, they do not eat, even have no mouth parts; their post-embryonic larval stage or caterpillar feeds for several months and only growth period of the life cycle. In many land-dwelling amphibians, larval forms are transition from the embryo's strictly aquatic existence to adult's terrestrial existence.

If the living conditions experienced by larva are more hospitable than those experienced by adult, the time of life cycle spend as larva and as adult may vary adaptively. As for example,

larval forms of some species of lamprey exist for several years. When larvae metamorphose into adults, they exist for a few weeks of breeding and die. Similarly, silk moths have a long period of larval existence in comparison to adult life.Metamorphosis is reactivation of developmental process, during which the entire organism is remodeled. The changes not only occur at morphological level, but also at physiological and behavioral level. In fact, metamorphosis prepares an organism for its new mode of life style during adult stage. Even though metamorphosis is widespread, we shall restrict our discussion in two groups of animals, one invertebrate the insects and one vertebrate - the amphibians.

10.2 METAMORPHOSIS IN INSECTS:

Post-embryonic stages of insects (and other crustaceans) are encased within rigid exoskeleton, called cuticle. Cuticle is a non-cellular layer containing protein, chitin, wax and calcium carbonate, and secreted by underlying epithelial layer. Because the cuticle has limited capacity for expansion, growth of body requires periodical shedding of it with the shedding of old cuticle, a new one is formed by underlying epithelial layer. The new cuticle is larger and softer, thus allow growth. The process of replacement of older cuticle by the new one is called molting or ecdysis, and the stage between successive molts is called an instar. Development of insects and other arthropods is punctuated by molting, number of which varies with species**.**

10.2.1 Life Cycle in Insects

Insects have three major strategies of development. Springtails (order Collembola) and silver fish (order Thysanuran) have no larval stage, so they undergo direct development, and are called **ametabolous** (Fig.1). After hatching, these insects have a transitory stage called **pronymph.** After pronymph stage, they appear as a miniature adult (preadult) and grow larger through successive molts until reaching adult stage.

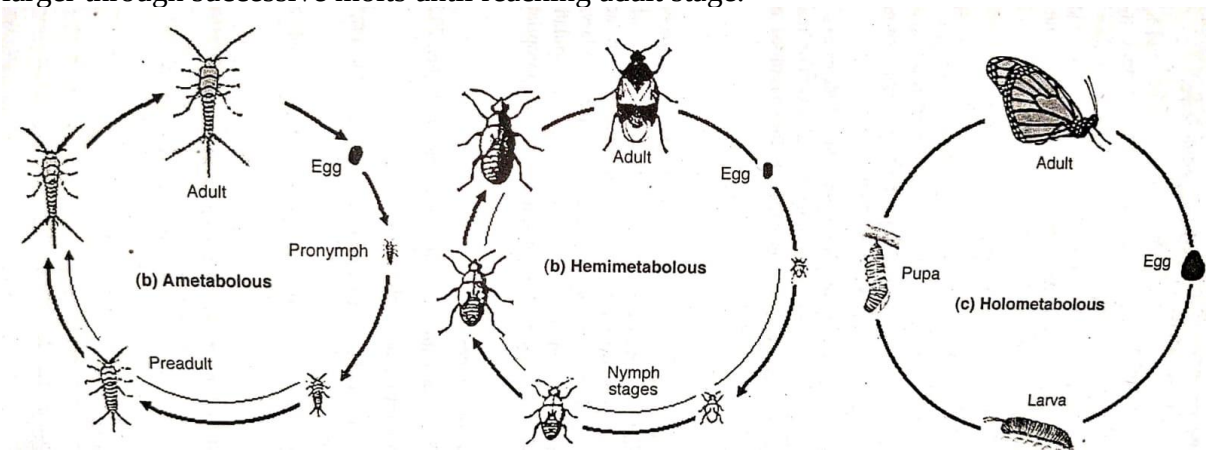

Figure 1: Types of life cycle in insects. (a) Ametabolous, (b) Hemimetabolous and (c) Holometabolous**.**

Grasshoppers (order Orthoptera), dragon files (order Odonata) and bugs (order Hemiptera) undergo indirect development through **hemimetabolous** (incomplete) metamorphosis (Fig.1). They hatch into a paranymph stage, which then molts into a **nymph –** a stage alike immature adult. Through successive molting and nymphal instars, these insects grow in size; develop wing, genital organs and other adult structures. The sexually mature adult with wings is called **imago,** which emerges after final molt. In many other insects like files (order Diptera), moths and butterflies (order Lepidoptera), beetle (order Coleoptera), post-embryonic stages

hatch from egg is called larva (a caterpillar or grub or maggot). Larva grows larger, though successive instars and undergoes series of molts. After final larval instar, the insect undergoes an encasement, the process is called **pupation** and the encased stage is called **pupa.** The molt of final larval instar to become pupa is called metamorphic molt.

During pupation, adult structures develop to replace larval structures and after definite time period (influenced by external environment), adult insect or imago emerges from pupal case. This type of development is called holometabolousor complete metamorphosis (Fig.1).

10.3 METAMORPHOSIS IN AMPHIBIANS:

The class of vertebrates designated as amphibians is due their dual life (amphi=double; bios = life). That means; to complete their life cycle, they undergo partly aquatic-partly-terrestrial existence. The morphological changes occurred during leaving aquatic existence and entering terrestrial existence is generally metamorphosis in amphibians.In anurans (frogs and toads), metamorphic changes are more dramatic; include almost all organs and regarded as complete metamorphosis. In urodeles (salamanders and newts), changes are less drastic than anurans and in some partial metamorphosis occurs. Metamorphic changes in amphibians, mainly in anurans are summarized in table 1.

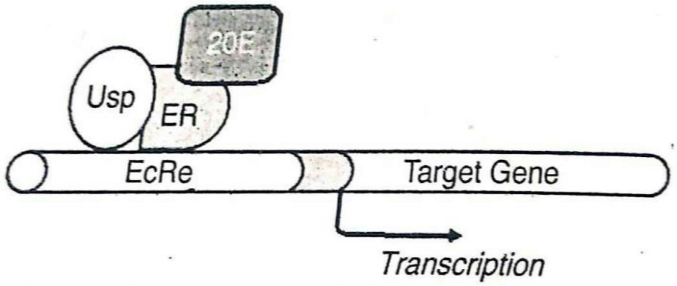

Figure 2: Usp and 20E receptor protein (ER) heterodimer with 20E regulates gene transcription.

Table 1: Important metamorphic changes in amphibians

Organ System	Larval	Adult
Locomotory	Tail with tail fin	Legs. Tail absent in anurans but retained in urodels. In some fins are also retained.
Respiratory	Gills, skin, lungs	Lungs and skin. In some urodels, external gills are retained.
Nutritional	Herbivore, long spiral gut, small mouth.	Carnivore, short gut, wide mouth with long tongue.
Excretory	Mostly ammoniotelic	Mostly ureotelic
Nervous and senses	Lateral line sense organ, no nictitating membrane and eyelids	Nictitating membrane and eyelids present, no lateral line sense organ.
Retinal pigment	Prophyropsin	Rhodopsin
Integument	Thin epidermis and dermis; no mucous or granular gland	Stratified epidermis with keratin; well developed dermis with mucous and granular glands.

10.3.1 Morphological and Biochemical changes during Amphibian metamorphosis:

Amphibian metamorphosis is initiated by hormones, mainly Thyroxine (T_4) and Triiodothyronine (T_3). These hormones are transported through blood circulation to the larval organs, which respond either of four ways.

- Development of new structures
- Cell death
- Remodeling of existing structures , and
- Biochemical specification

1. Development of New Structures:

- Limbs of the adult amphibians emerge on metamorphosing tadpole. Paired hind limbs appear first, followed by paired forelimbs (Fig. 3). As they grow out from body axis, new neurons proliferate and differentiate in spinal cord, which send axons to the newly formed limbs.
- Both nictitating membrane and eyelids emerge in the eye. At the same time, position of eye becomes frontal from their original lateral position in larva (Fig.3). With this movement of eye position, adult frogs acquire a binocular field of vision.Paired lungs develop and circulatory system also changes with development of carotid and systemic arch.

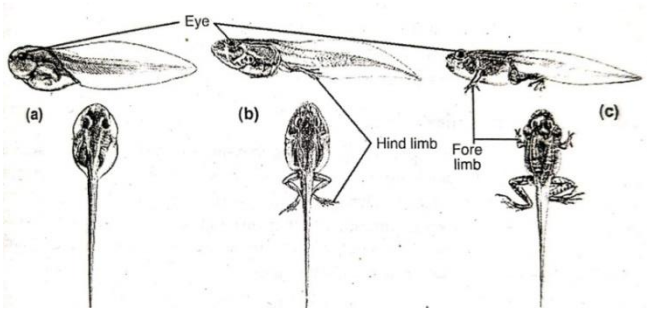

Figure 3: In Metamorphosis of amphibians, new structures develop in tadpole (a). Paired hind limbs appear first, followed by forelimbs (b). Position of eye becomes frontal from lateral in tadpoles developing a binocular vision in adult (c). Upper row - lateral view; lower row - dorsal view.

2. Cell Death:

- Degeneration of tail occurs. It is evident that first part of tail regresses by means of programmed cell death or apoptosis. In later metamorphosis, remnants of larval tail are destroyed by phagocytosis.

- Gills those are important for larval respiration degenerates.
- When adult red blood cells become functional, larval red blood cells are digested by macrophages in liver and spleen.
- Lateral line sense organ that helps in hearing and swimming also degenerates.

3. Remodeling of Structures:

- Larval intestine is long and coiled, designed for digesting plant diets. Larval intestine is converted into shorter intestine of adult frog for carnivorous diet. The cells of adult intestine are derived from functional cells of larval intestine.
- Adult nervous system is remodeled as neurons grow and innervate new organs. However, during restructuring, some neurons die, some are born and some change their specificity.
- Almost all structural component of head in adult is remodeled along with the change in the shape of skull and lower jaw. Some skeletal elements die, some proliferate and some are remodeled.

4. Biochemical Respecification

In association with morphological changes, certain new proteins are induced in existing adult cells. Tadpoles are ammonotellic while adult frogs are mostly ureotelic an adaptation to terrestrial life. During metamorphosis, liver cells start to synthesize enzymes which are necessary to produce urea from ammonia and certain carbon-di-oxide. It is known that T3 induces a transcription factor that activates expression of urea-cycle genes and suppresses genes for ammonia synthesis.

10.4 RETROGRESSIVE METAMORPHOSIS:

In majority cases of metamorphosis, we witness that adult individuals develop more advanced features than their larval forms. It is not that larvae are less adapted, but adult structures are better than their larval counterparts. Naturally we denote this type of metamorphosis as progressive. But there are examples in animal kingdom, where key features of the group are present in larvae disappear in adults after metamorphosis. Such type of metamorphosis is called retrogressive.
Retrogressive metamorphosis is excellently evident in urochordates, particularly in ascidians. Ascidians or sea squirts are solitary or colonial marine chordates, adult are sessile, but larvae are free-swimming.

1. Larva:

Larvae are also called ascidian tadpoles, usually do not feed, swim actively for a few days and select a suitable substratum to settle permanently as adult. The free-swimming larva exhibit chordate characters: -

a. Small pharynx bearing slits,
b. Tubular nerve cord extending into tail, and
c. A flexible notochord.

At the beginning of pharynx, there is a distinct endostyle. Pharyngeal slits open into bilateral atrium that opens to exterior through atrial pore. In solitary species of ascidians, gut is not well-developed in non-feeding larva and anus is absent. In majority colonial species gut is fully differentiated with mouth and anus. Their feeding begins shortly after settlement. The heart and pericardium arise from pharynx and lie behind endostyle.

In most solitary and colonial species, notochord is tubular. Walls of notochord are made up of single layer of epithelial cells covered by a sheath of collagen fibers. The epithelial layer encloses a lumen that is fluid-filled. As a result, the notochord is tubular, closed at both ends and is turgid.Brain composed of a rudimentary cerebral ganglion and a large visceral ganglion that sends nerve to various parts of body. A hypophysis is present beneath cerebral ganglion. Dorsal nerve cord arise from visceral ganglion has a neurocoel surrounded by ependymal cells and nerve tracts. Dorsal nerve cord with notochord and tail muscles form axial complex. A sensory vesicle near the atrial pore includes a light-sensitive ocellus and a gravity-sensitive otolith. These sensitive structures help the larva as navigational equipments, during swimming and searching a place for attachment.The active body of larva is covered by an acellular tunic secreted by underlying epidermis. The tunic is covered by an outer and inner cuticular layer. From outer cuticle tail fin of larva arise. At the anterior part of larva, adhesive papillae are formed from epidermis, which help the larva to attach to a suitable substratum.

2. Metamorphosis

After a short free-swimming life, larva attaches with a suitable substrate in a shaded place. The attachment is made by the adhesive papillae and the larva immediately begins to metamorphose. Most of the chordate features in larva, namely notochord, nerve cord and tail disappear.

The epithelial cells in the notochord contract and separate from each other. The fluid from the lumen leaks and the notochord becomes limp. The axial complex is actively absorbed into body. Within body, the axial complex is broken down by phagocytosis and its components are used to rebuild the young adult. Outer tunic layer, sensory vesicles and visceral ganglion are lost. The pharynx persists and enlarges, slits in pharyngeal wall increase in number and each slit subdivides repeatedly to form small openings called stigmata. The pharynx becomes barrel-shaped and called branchial basket. After attachment, the metamorphosing tadpole starts feeding.

3. Adult

In sedentary life, adult ascidians need only feeding system, circulatory system, nervous system and reproductive system. Whole animal is enclosed in a tunic composedof unique protein tunicin and a polysaccharide resembling cellulose. Tunic is almost transparent, below which a single-layer epidermis is present. Within tunic, branchial basket, atrial cavity and visceral system are enclosed, and the basal part of tunic helps the individual to attach with substratum.

Water circulates through the body of adult ascidians. Water enters into branchial basket through incurrent siphon. Small finger like sensory tentacles encircling incurrent siphon testthe quality of entering water and prevent entrance of large particles. Water passes from branchial basket to atrium through numerous stigmata. From atrium, water exits through excurrent siphon.

Within branchial basket, endostyle produces mucous, which bind the food particles. Row of cilia, lining the internal chamber of branchial basket collect the sheet of mucous containing food and convey it to the gut.

Tubular heart contracts to push blood out to organs and tunic. After several minutes, blood flows in reverse direction in same vessel to return the blood to heart. Blood contain ameobocytes, which are phagocytes.

Nervous system includes a cerebral ganglion, located between two siphons. From this ganglion, nerves arise to supply siphons, gills and other visceral organs. A sub neural gland below this ganglion persists from larval hypophysis, function of which in adult is not known. Solitary ascidians reproduce sexually, but colonial ascidians reproduce both by sexually and asexually. All ascidians are hermaphrodites but self-fertilization, usually does not occur. Asexual reproduction in colonial ascidians involves budding.

10.5 SUMMARY:

Metamorphosis is a transformative biological process in which an organism undergoes significant physical and physiological changes during its life cycle. In insects, it occurs as either complete metamorphosis, with distinct stages (egg, larva, pupa, adult), or incomplete metamorphosis, which lacks a pupal stage. Amphibians exhibit metamorphosis through dramatic changes, such as the transition from aquatic larvae (e.g., tadpoles with gills) to terrestrial adults (e.g., frogs with lungs). Retrogressive metamorphosis, seen in organisms like tunicates, involves a simpler adult form compared to the larval stage, adapted for specific ecological niches. These processes are vital for survival, resource allocation, and evolutionary adaptation.

10.6 TECHINICAL TERMS:

larva, Pupa, Nymph, Tadpole, Gills to Lungs Transition, Retrogressive Metamorphosis.

10.7 SELF-ASSESSMENT QUESTIONS:

1. What are the key differences between complete metamorphosis, incomplete metamorphosis, and retrogressive metamorphosis?
2. How does metamorphosis in amphibians help them adapt to both aquatic and terrestrial environments?
3. Discuss the ecological and evolutionary significance of retrogressive metamorphosis in certain organisms.

10.8 SUGGESTED READINGS:

1. Longo FJ. 1987. Fertilization. Chapman & Hall, London.
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LESSON - 11

REGENERATION

AIMS AND OBJECTIVES

- to understand regeneration in hydra, planaria, amphibians, crabs
- to study about compensatory regeneration
- types of regeneration

STRUCTURE:

11.1 INTRODUCTION

Regeneration is the biological process by which organisms restore or replace lost or damaged tissues, organs, or entire body parts. This ability varies greatly among different species, ranging from simple cellular repair to complex regrowth of limbs or body structures. It is a fascinating phenomenon that showcases the diverse strategies organisms use to survive injuries and maintain functionality.

11.2 TYPES OF REGENERATION

Throughout the animal kingdom, stem cells are involved to regrowth tissues or organs that have been lost. We can designate such regrowth or repair as normal and routine regeneration. There are numerous examples of stem-cell mediated regeneration, such as, continuous production and replacement of blood cells form hematopoietic stem cells in bone marrow; regrowth of hair shafts from stem cells in hair follicles etc. but such routine regeneration is not the topic of discussion in this chapter. The regeneration mechanisms, which are not routine and peculiar in specific animals, are three types:

- When regeneration occurs mainly by repatterning of existing tissues and re-establishment of boundaries, it is called morphallaxis**.** In this process, there are little growth and seen in *Hydra.*
- The regeneration process that involves dedifferentiation of adult tissues to form undifferentiated mass of cells, which are again respecified into lost structures, is called epimorphosis**.** There is new growth of correctly patterned structures. Epimorphosis is seen in planarian flatworms and in limb regeneration of urodeles amphibians.
- A third type of regeneration is found in mammalian liver, called **compensatory** regeneration. In this process, each differentiated cell type divides and produces cells like itself and they maintain their differentiated functions. There is no dedifferentiation of adult cells; hence no mass of undifferentiated tissues is formed.

11.3 REGENERATION IN HYDRA:

A. Morphallaxis regeneration in hydra

Hydra is freshwater coelenterate (phylum Cnidaria). It has a hollow tubular body, about 0.5 cm long, with head at one end foot at another. Head or distal end consists of a conical **hypostome** with mouth at its tip and a set of tentacles surrounding the mouth. The tentacles are used to catch small prey animals upon which *Hydra* feed. The foot or proximal end is the **basal disc,** which enables *Hydra* to stick to an underwater substratum (Fig.1).

Hydrais diploblastic animal that is having only two germ layers (Fig.1). Body wall is composed of two cell layers –outer epithelium or ectoderm and inner epithelium or endoderm. Two layers are separated by a basement membrane and there is no true mesoderm layer. There are about 20 different cell types in the body of *Hydra,* which include nematocytes (stinging cells), secretory cells, nerve cells, interstitial cells etc. Interstitial cells act as stem cells, which can give rise to other cells. But interestingly, interstitial cells have little role in regeneration of this genus.

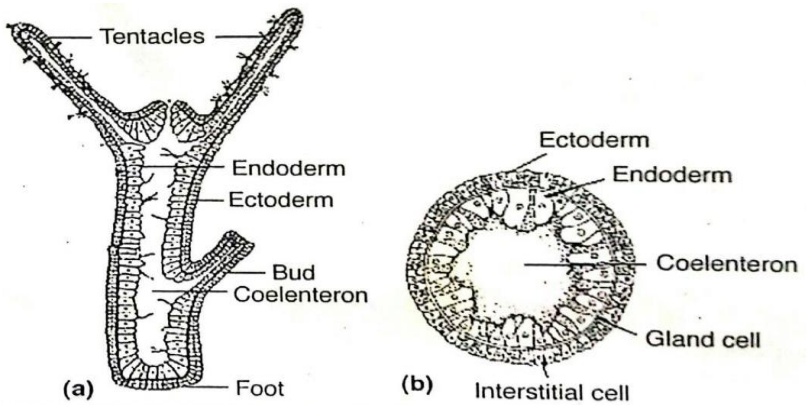

Figure 1: Structure of an adult Hydra (a) and a transverse section showing two germ layers (b)

B. Body of *Hydra* is under dynamic growth

Like most animals, body of *Hydra* is not stable. A well- nourished *Hydra* is always in a dynamic state of growth. Cells of both epithelial layers constantly undergo mitosis, and as the tissues grow, cells are displaced along the body column towards head or foot. In order to maintain size, an adult *Hydra* needs to shed the excess cells continuously. Cells are shed through two extremities-at the tips of tentacles and at the basal disc. In this process of

continuous growth, cells are continually changing their relative positions along the body column. Thus, each cell plays several roles, which depends upon the age of the cell.

Most excess cells are used when new Hydrais produced from the body column by asexual budding. (Hydracan reproduce sexually too, but only in unfavorable conditions like low temperature, crowding etc.). Budding generally occurs at body column at about two-third of the way down from head. During budding, an evagination occurs in the body wall that forms a new column. Ultimately, a head develops in the distal end of the column and it detaches from the parent body as a small new Hydra. During continuous growth, changing position and role, and during asexual budding, there must be some dynamic mechanisms for repatterning of cells. These mechanisms enable Hydrato have a remarkable capacity of regeneration.

When the body column of a *Hydra* is cut transversely into two halves, the lower portion regenerates a head and upper portion a foot (Fig.1). If the body column is cut transversely in several small pieces, each piece regenerates to become small Hydra(Fig.1). The structure cells will regenerate at cut surface depends on their relative position, indicating a well – defined polarity**.** The polarity is even maintained in small pieces, the distal end regenerates a head and proximal end become basal disc.When a small piece of body column regenerates, there is no initial increase in size. Thus, regenerated Hydrabecomes a small one, which after feeding attains a normal size. Even, a piece of body column lacking interstitial cells (stem cells) regenerate normally. Since each cell retains its plasticity, the regeneration of Hydrais called morphallaxis**.**HeadofHydra acts both as organizer and inhibitorEach part of body column of Hydraalong apical –basal axis is potentially able to form both head and a foot. But there is series of morphogenetic gradients that allow the head to form at one part and basal disc at another.

Early grafting experiments showed that a small portion of hypostome region of *Hydra* into middle (gastric) region of another Hydrainduced a new body axis with complete head with tentacles (Fig.2). Similarly, grafting of a portion of basal region in the same region of another Hydrainduced a new body column with basal disc at its tip (Fig.2). Thus, it appears that Hydrahas two organizing regions, one at each end-the hypostome and basal disc, which give Hydraits overall polarity.

Grafting experiments also indicate that the hypostome produces an inhibitor of head formation, effectiveness of which becomes lesser with distance from the head. (This inhibitory property normally prevents inappropriate head formation in intact animals). When a body fragment of just below the head (subhypostomal region)

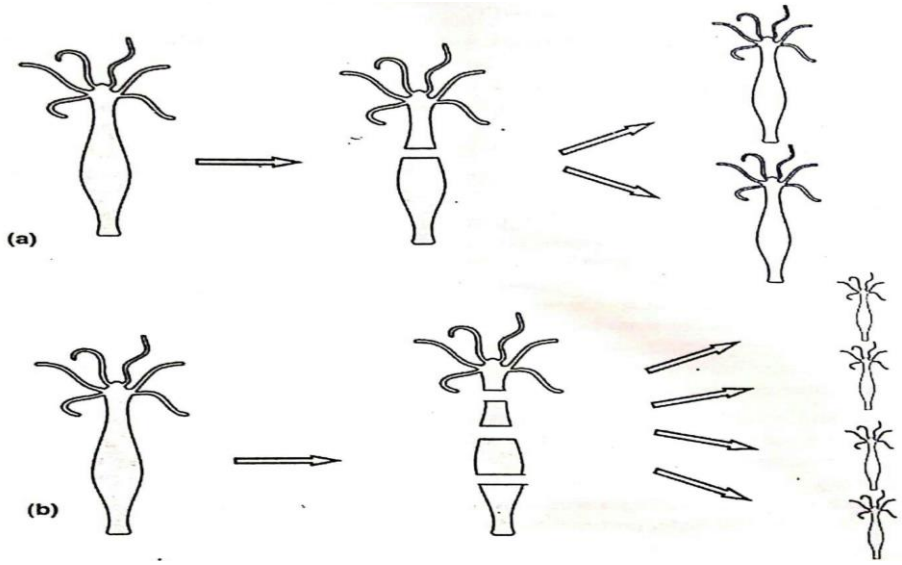

Figure 2: Regeneration in Hydra (a) when cut transversely into two halves and (b) when cut transversely into several pieces.

(i) Grafted into gastric region, no new head is induced and the grafted tissue simply absorbed into body (Fig. 3.14).
(ii) Grafted into same region and the head of host Hydrais removed, the graft tissue induce a new axis with head (Fig. 3.14).
(iii) Grafted near the foot, the graft tissue induces a new body axis with head, while the original head of the host is in position (Fig. 3.14).

Results from these experiments suggest that:
(a) Formation of additional head is normally prevented by an inhibition mechanism.
(b) The inhibitory mechanism act through a gradient with its highest effectiveness at the head.
(c) When head is removed [experiment (ii)], effectiveness of head inhibitory Mechanism is lost.

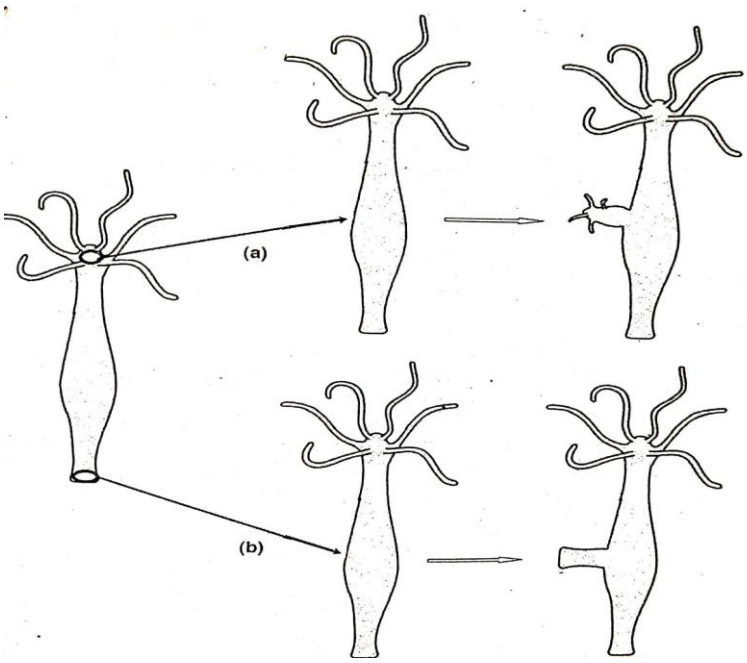

Figure 3: Grafting experiments in Hydra. (a) Hypostome region into middle position of another.

Hydra, (b) basal region at same position of another Hydra.

C. Basal disc activation and inhibition gradient

Similar like head activation and inhibition gradients, there is a source of both foot activation and inhibition at the basal disc. The gradient of head inhibition is highest at head region, lower down the body column and lowest at basal disc. Similarly, gradient of foot inhibition is highest at basal disc, lower upward the body column and lowest at head. The inhibition gradients for the head and the foot are crucial in determining where a bud can form.

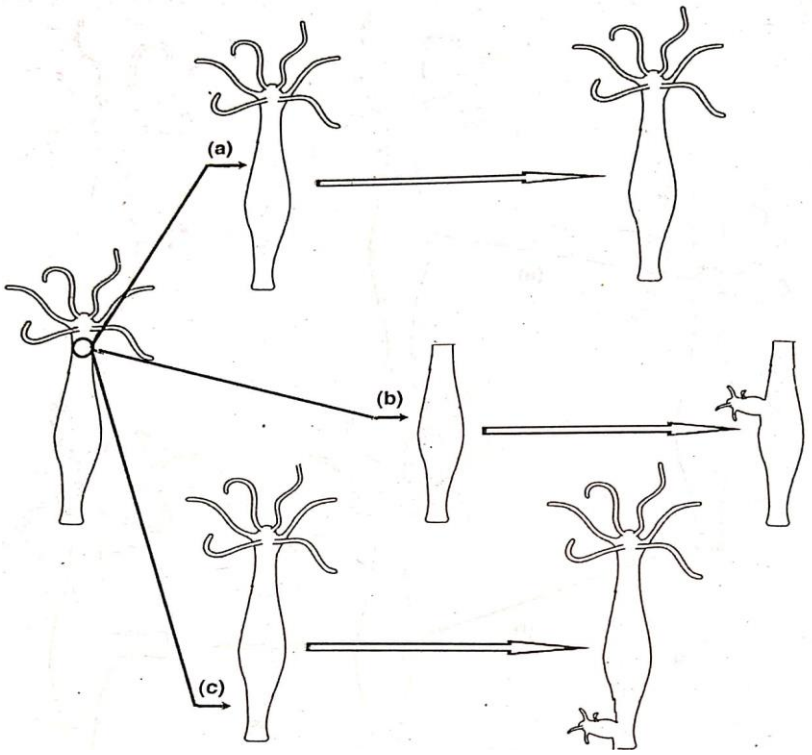

Figure 4: Results of grafting of subhypostomal region into (a) gastric region.

(b) gastric region but head is removed and (c) foot region of another hydra
The region of body column of a growth *Hydra* that is about two –third down the trunk has the both inhibitor at minimal. This region is known as bud formation region.

D. Two gradients caused by head in *Hydra*

Experiments with regeneration of *Hydra* indicate that two gradients are set up along the body column by the head, one is a gradient in positional value and other is a gradient of head inhibitor (Fig 4). The gradient in positional value seems to control two incidences –resistance to head inhibition and head –inducing ability.

The gradient in resistance to head inhibition can be determined by the ability of different regions of the body to suppress head formation. This gradient in resistance decreases with distance from the head. Thus, when region 1 (resistance to head inhibitor is high) is transplanted near foot (insufficient head inhibitor), a new head is regenerated. But when region 5 (resistance to head inhibitor is low) is transplanted near foot, inhibition is sufficient to prevent head regeneration (Fig.5).

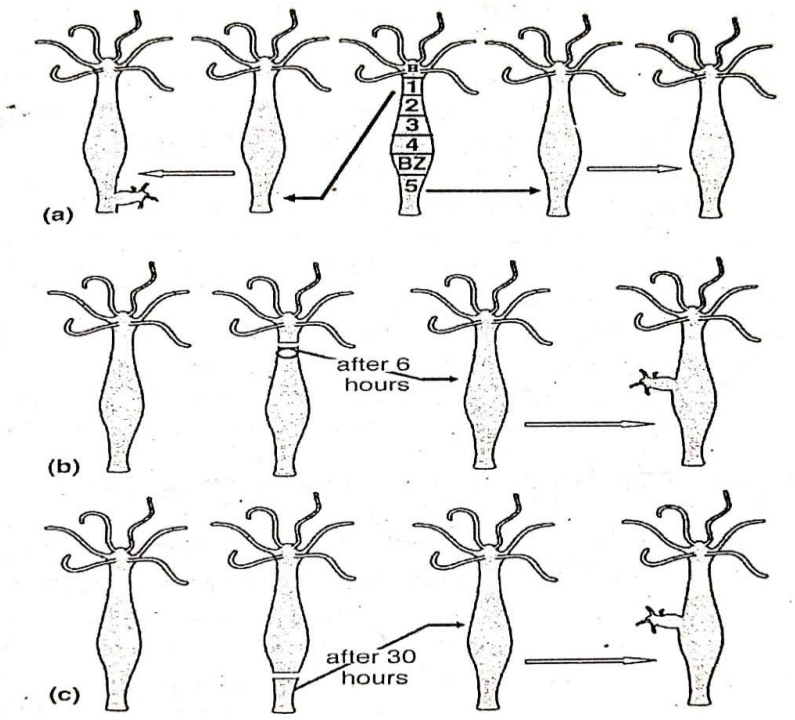

Figure5: Head of Hydra set up a gradient in positional value along body column, which control resistance to head inhibition (a), and head indueing ability (b) & (c).BZ Budding zone; H = Hypostome.

The gradient in head –inducing ability also decreases with distance from head i, e, high at head end low at foot end. This gradient in head –inducing ability is determined by a gradient in positional value, which is evident from the differences in time required for different regions of body column to acquire head –inducing property. It is already mentioned that region 1 from an intact *Hydra*, when transplanted to gastric region of another individual, no axis is induced. When the head of donor Hydraus cut, region 1can induce a new axis when transplanted 6 hours after head amputation (Fig.5). The region 5 forms a new axis when transplanted 30 hours after amputation (Fig.5). Thus, more down the axis of amputation, longer the remaining cells take to acquire head –inducing property.

A simple model assumes that head inhibitor is a diffusible factor that is secreted by head. The factor diffuses down the body column and is degraded at foot end. The gradient in positional value is supposed to be an intrinsic property of cell. Both gradients are linear, decreases constantly with distance form head.

According to this model

- **i** In an intact *Hydra,* level of inhibitor is greater than the threshold set by the positional value, hence head regeneration is inhibited.
- **ii** After head removal, concentration of inhibitor started falling, lowest at the cut end. As the inhibitor concentration falls below threshold set by local positional value, the positional value increases at that enc. So, when the head region is removed, the first necessary step in regeneration of *Hydra* is to specify a new head region at the cut end.
- **iii** Once positional value increased to normal alike head region, the cells start to secrete inhibitor, which prevent another head formation elsewhere. After new head specification, inhibitory gradient is re-established but that takes time. In the meantime, the regenerated small *Hydra* grows to adult size.

E. Chemicals and genes affecting regeneration of *Hydra*:

Chemicals of activators and inhibitors are found to be peptide in nature. Three peptides associated with head activation are Heady, Head Activator and Hym-301. First two are key molecules for head formation and inhibition of bud; the third peptide has a role in regulating the number of tentacles. Several other small peptides are known to affect foot formation, but details of their functions are yet to be sort out.

Several genes are found active in head (hypostome) organizer area, which suggests that a set of signals is conserved over millions of years of evolution and acts as organizer throughout animal kingdom. The Hox *genes,* which control body pattern in many animals, are involved in regional patterning of Hydra. Some *Hox* genes express in regional pattern and seem to be involved to specify head –foot positional values.

A Hydraversion of goosecoid *gene,* an organizer specific gene in vertebrates, is expressed in the hypostome region, just above where tentacles will appear. When hypostome is brought in contact with trunk of an adult *Hydra,* it induces the expression of *brachyury* gene. The usual tissue in which *Brachyury* expresses in mesoderm that *Hydra* lacks; it suggests that the head of *Hydra* correspond to blastopore of other animals. Another organizer-specific gene is *Hywnt,* the *Hydra wnt*homolog, has restricted expression to the apical tip of body axis, which is the *Hydra* head organizer. During head regeneration both *wnt*and *Hydra β-catenin* genes are expressed at the tip within one hour after head amputation. A significant upregulation of *Hydra β-catenin* expression is observed in the prospective budding zone, just before evagination begins.

Understanding regeneration in *Hydra* allows insight into organizer and developmental gradients, which evolved early in animal kingdom. So, it is logical that complex body patterns of higher animals evolved from simple body plan of animals like *Hydra.*

11.4 REGENERATION IN PLANARIA:

Planarians are free-living flatworms (phylum Platyhelminthes), primitive but organized. They are triploblastic, bilaterally symmetrical, acoelomate and unsegmented. Except digestive and simple nervous system, they lack circulatory, respiratory and skeletal structures. They reproduce through two methods – asexually by fission and sexually through producing sperms and eggs by males and females respectively.

Planarians have long been known for their high regenerative capacity. If a Planaria is cut into two halves, the anterior half will regenerate a new tail from the cut surface and the posterior half will regenerate a head from its cut surface. Even a small fragment cut from its body can give rise to an intact animal. Apparently, regenerative power of planaria seems like of *Hydra,* but there are basic differences in the mechanisms of regeneration.The process of regeneration in planarians involves two main events – blastema formation and pattern formation.

A. Blastema formation:

When a planaria is cut, there is a muscular contraction limiting the area of the cut. The epithelium around the wound rapidly closes up and the cut surface is covered by a thin film of epidermal cells from the stretched old epidermis. Below the wound epithelium, mass of undifferentiated cell accumulates in a few layers to give rise to an outgrowth, called

regeneration blastema.Blastema is generally used to designate regeneration bud containing undifferentiated proliferating cells. But in planaria, there is no cell division in regeneration blastema. The cells in blastema of planarians come dividing neoblast cells in the proximal region. The blastema grows by continuously incoming cells, which then differentiate over a few days to form the missing body part.

Planarians can grow or shrink on a daily basis through continuous cell turnover, and it depends on food supply. Neoblast cells with large nuclei consist about 20% of total cells in the body of planarians. Neoblasts are the only dividing cell population in planarians and perform as stem cells. When one neoblast cell divides, one daughter cell produces a differentiated cell in the body and other daughter cell remains as stem cell. Neoblasts have developmental totipotency and can produce 12-15 histologically distinguished and differentiated cell types found in the body of planarians. The presence of neoblast cells in body is evitable for regeneration in planaria, because irradiation of these cells results in failure of regeneration.

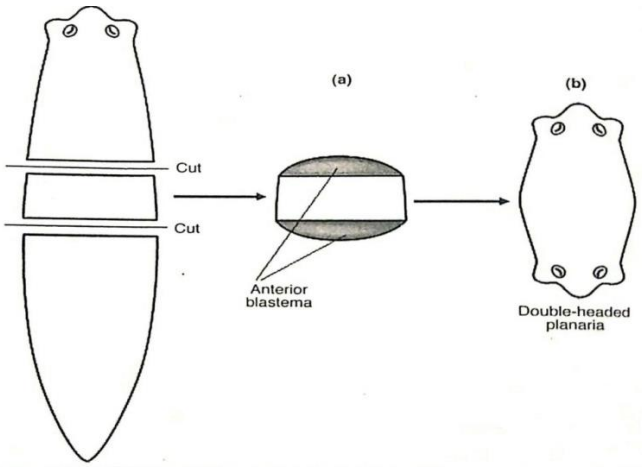

Figure6:A short segment from middle part of body of Planaria forms anterior blastema at both ends (a) resulting a double-headed worm (b).

11.5 REGENERATION IN AMPHIBIANS:

A. Pattern formation:

The cells in the blastema have to decide first about that of polarity, whether to be a head dud or tail bud. Little is known about determination of polarity, but it is suggested that origin of would epithelium is important. When the wound epithelium originates from dorsal epidermis, it becomes anterior blastema producing head. Similarly, if the wound epithelium originates from ventral epidermis, it becomes posterior blastema producing tail.

Involvement of some mechanism to control polarity is suggested by errors in regeneration. In some species of planaria, short segment from middle part of body form anterior blastema at both ends resulting a double headed worm (Fig.6). In this case, blastema in posterior end of the segment lacks some signal that normally causes it to become tail blastema. Existence of some signaling process associated with planarian regeneration is also evident. When a second head is grafted near the original one and the original one is amputated, its regeneration is prevented by the grafted one (Fig.7).

The undifferentiated cells originating after division of neoblasts accumulate within blastema and then differentiate into new structures following a distal-proximal sequence. Ultimately,

lost structural pattern is restored in proportion of normal body size. The epithelium enclosing blastema cells induce expression of several homebox genes. These genes, such as *otx, pax6,* probably maintain the cell proliferation below the wound surface and induce some early pattern formation.

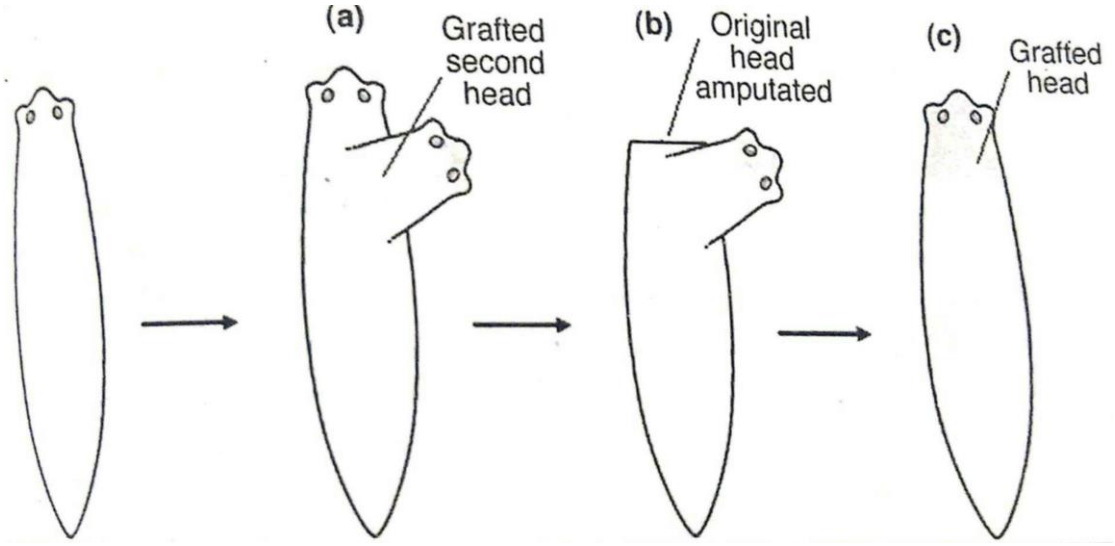

Figure 7: When a second head is grafted near original head (a) and the original one is amputated (b), its regeneration is prevented (c).

There are two theories existing on the origin of blastema cells. First is neoblast theory**,** which is current view and put forward that neoblast cells give rise to the cell population involved in regeneration. Recent electron microscopic studies put forward a dedifferentiation theory**.** According to this theory, differentiated cells below blastema undergo dedifferentiation during regeneration. Gland cells lose secretary properties, muscle cells lose their fiber system and they transform into neoblasts. Thus, planarian regeneration includes both normally existing neoblasts and transformed neoblasts from other cell types. The regeneration of planaria should have a special class between morphallaxis and epimorphosis.

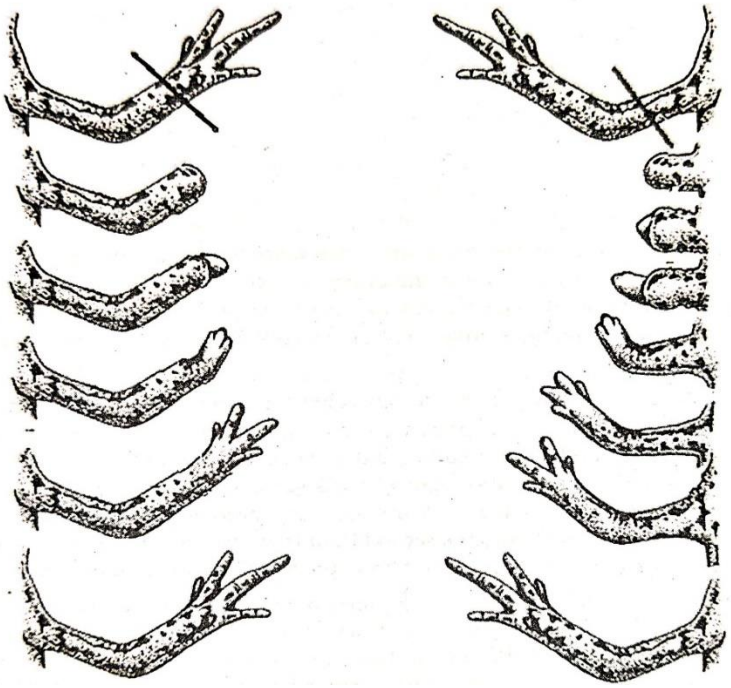

Figure 8: Urodel amphibians have remarkable capacity of regeneration of only the missing part. When a wrist is amputated, only the wrist is regenerated (left figures). When arm is amputated in middle region (right figures), entire portion is regenerated.

B. Regeneration in urodele amphibians:

A remarkable capacity of regenerating body structures like limb, tail, jaws etc. is found in urodele amphibians. An adult limb of urodele consists of many differentiated cell types which are organized and arranged to form the structure. So, the central question of regeneration in urodeles is the origin of cells, which give rise to regenerated structures. Are there any reserve cells or the existing differentiated cells change their character and dedifferentiate to give rise to all other cell types? Another point is that only the missing part is reconstructed; for example, when a wrist is amputated (Fig.8), only the wrist is regenerated. So, there is a positional knowledge about where the limb is severed on regeneration involves new growth, it is epimorphic in nature.

C. Formation of Regeneration Blastema

Following amputation of a salamander limb, there is a rapid formation of plasma clot. Within 8-12 hours, epidermal cells from remaining structure migrate over the wound surface to form a woundepidermis(Fig.9). Initially single –layered, the wound epidermis proliferates and forms an apicalectodermalcap(Fig.9).

Within 3-4 days of amputation, the cells below ectodermal cap undergoes dedifferentiation. Fibroblasts, nerve cells, muscle cells, bone cells all lose their differentiated properties and detach from each other. Genes in these differentiated tissues are down-regulated. Thus, at the cut edge, mass of a dedifferentiated, indistinguishable proliferating cells aggregated below the ectodermal cap. The cell mass is called regenerationblastema(Fig.9) and continue proliferation. In the next weeks, the limb regenerates and these cells differentiate into muscle, cartilage, connective tissue etc. (Fig.9). Such process of conversion of one tissue into another is called transdifferentiation or metaplasia**.**

Cells of blastema originate locally from the mesenchyme tissue of the stump, near the site of amputation. Majority of them come from dermis but also from cartilage and muscle. Multinucleated muscle cells, which have stopped mitosis, can be changed to uninucleate cells in culture medium, in presence of thrombin. Thrombin is familiar as one key protein in blood clotting, but also involved in dedifferentiation in some cases. Msx1 is a transcription factor that is known to prevent myogenic differentiation in mammals. Its characteristic expression is found in undifferentiated mesenchymal cells and dedifferentiated muscle cells, which undergo regeneration.

Whether cells differentiated into muscle or cartilage in the regenerating limb remain true to its type or whether dedifferentiated cells can differentiate into different cell types, is a question. In urodele amphibians, the second statement is correct. Cultured limb muscle myotubes, which are multinucleated and left cell cycle, are labeled and introduced into regenerating limbs. Labeled unnucleated cells are found in blastema after a week. These unnucleated cells, derived from myotubes, proliferate and give rise to new muscle cells as well cartilages. Thus, blastema creates an environment that induces cells to dedifferentiate. These cells then act as progenitor cells for regenerating limb. Thus, blastema provides necessary environment containing cues for both dedifferentiation and redifferentiation into other cell types.

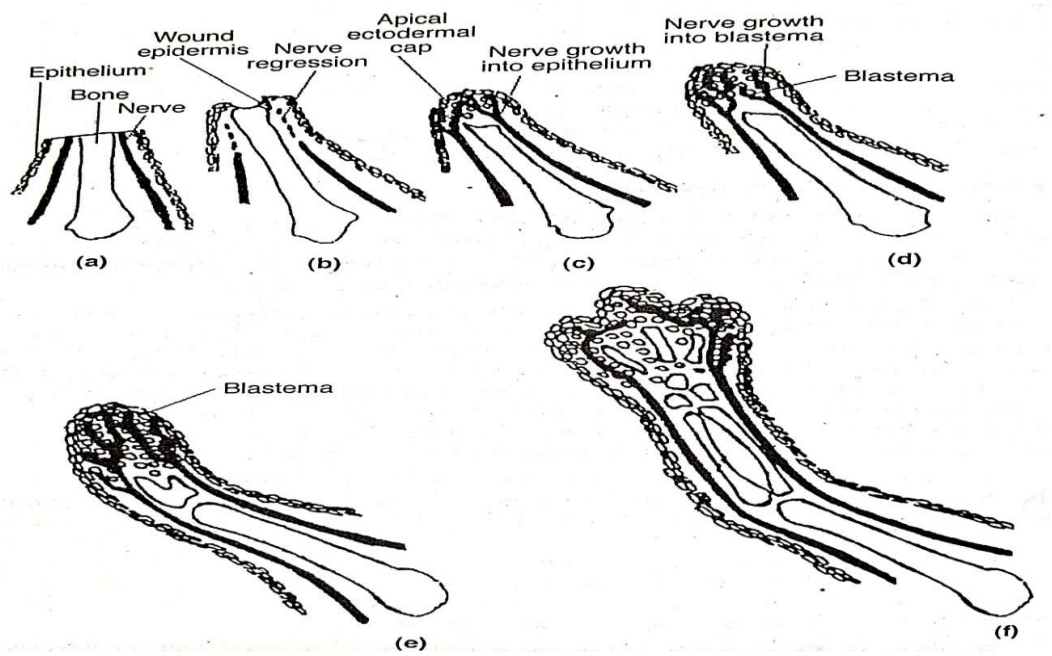

Figure9: Urodel amphibians have remarkable capacity of regeneration of only the missing part. When a wrist is amputated, only the wrist is regenerated (left figures). When arm is amputated in middle region (right figures), entire portion is regenerated.

Normally mature skeletal muscle cells never divide, so –entering cell cycle by the muscle cells is a key feature of salamander limb regeneration. During differentiation of vertebrate muscle, precursor cells withdraw from cell cycle after myoblasts fuse into myotubes. The regenerating cells of salamander contain Rb protein (a product of *Rb* gene) that is involved in cell cycle regulation, but it is inactivated by phosphorylation, so the cell re-enter cell cycle and divide. Local activation of thrombin is also associated with the ability of muscle cells to re-enter cell cycle.

D. Requirements for proliferation of Blastema Cells

Proliferation of blastema cells is dependent on several factors:

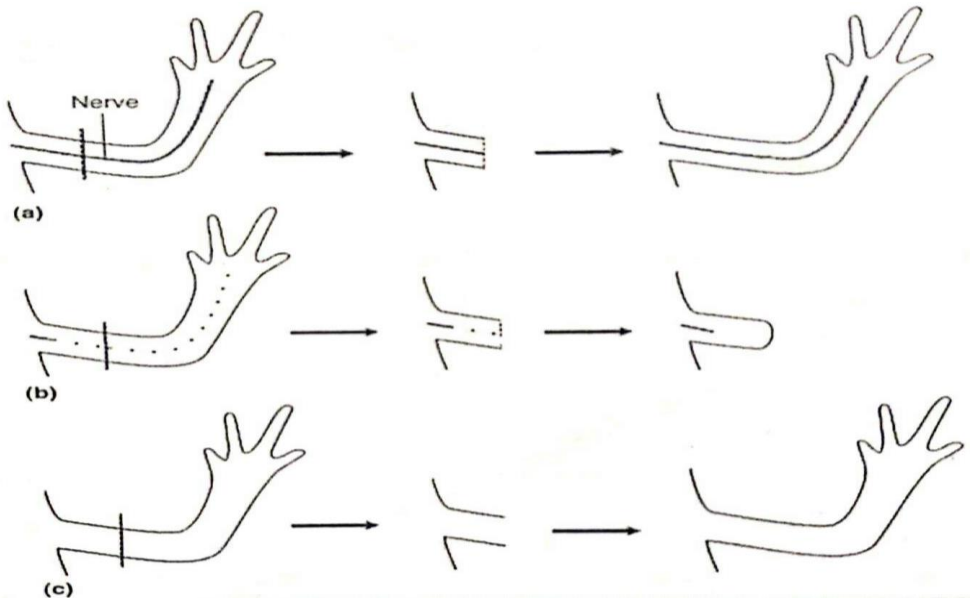

Figure 10: Proliferation of blastema cells in urodels is dependent on nerve supply except the limbs which are without nerve at early development. Regeneration of (a) Normal limb, (b) Denervated and (c) Aneurogenic limb.

1. Normal limb generation requires presence of minimum nerve supply (Fig.10). In limbs denervated before amputation, a blastema is formed but failed to grow. Nerve cells, therefore, seems to release some growth factor, that is essential for proliferation of blastema cells. Glial growth factor, a member of neuregulin family, is one likely candidate for maintaining high rate of cell division.

In an interesting experiment, it is found that when nerve is removed from a limb in early development, it can regenerate complete limb (Fig.10) in absence of any nerve supply. It suggests that dependence of proliferation of blastema on nerve is imposed on the limb after the ingrowth of nerves.

2. The wound epidermis provides the signals to the underlying cells to dedifferentiate forming blastema and also its proliferation. The signals include Fibroblast growth factors (Fgf1 and Fgf2 is also important in regeneration by restoring the expression of a developmental gene *Distal –less* (Dx1) in the epidermis. Local application of Fgf2 can result in regeneration of denervated limb.

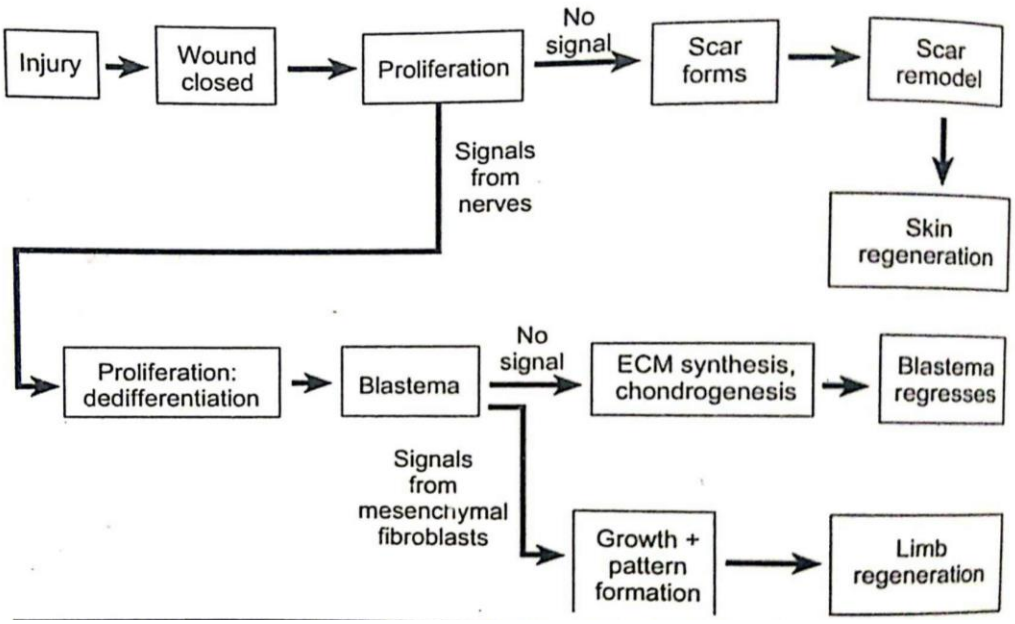

Figure 11: A model is forwarded that includes both nerve-dependent and mesenchyme-dependent steps of regeneration of urodel limb

Fgf2 servers as an angiogenesis factor because regenerating limb requires normal blood supply after amputation. Fgf2 also promotes mitosis and patterning of limb generation. Apical mesenchyme of blastema release some other Fgf2 (4, 8, 19), which are necessary as positional signals for successful regeneration.
A model of limb regeneration (Fig.11) is forwarded that include both nerve-dependent and mesenchyme-dependent steps:

- After amputation, dermal cells proliferate and migrate over wound.
- Without signals from neurons, these cells form a scar and allow only healing (skin regeneration).
- In presence of neural signals these cells proliferate and differentiate to form blastema.
- Without signals from mesenchyme, the blastema regresses.
- In presence of mesenchymal signals, the blastema is patterned to regrow the limb.

E. Positional value and Pattern Formation in Regeneration of Urodele Limb:

Whether limb regeneration and embryonic limb development involve same mechanisms is not known but some relationship must exist between the two. Regeneration always follows the direction distal to the amputated surface and allows replacement of lost parts. If the hand is cut at wrist, only carpals and digits are regenerated; if cut at the middle of humerus, regeneration occurs distal to that point. Thus, positional value along the axis is of great importance. The blastema appears to have morphogenetic autonomy; when grafted to a neutral location permitting growth, it regenerates structures appropriate to the position from which it was taken.

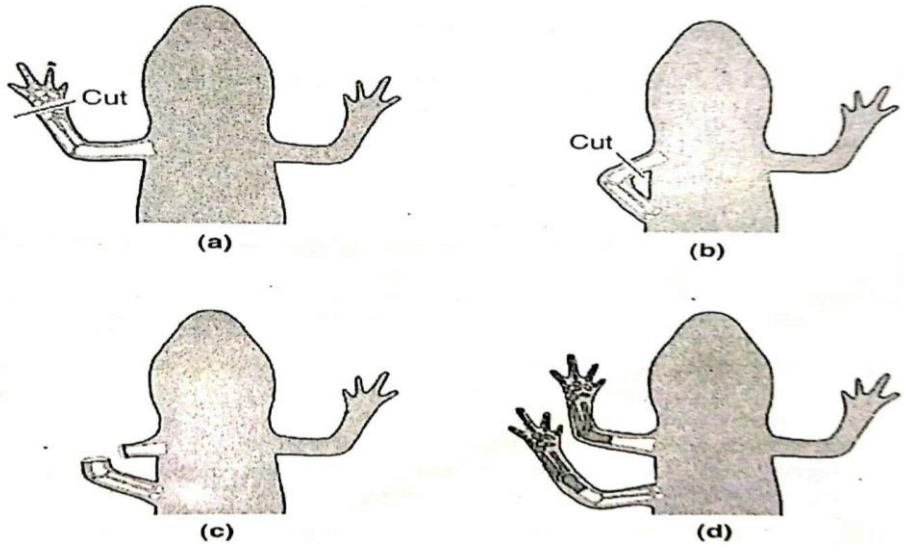

Figure 12: Distal end of limb of an urodel is amputated at wrist (a) and inserted into belly of same animal (b). When this limb is cut at mid-humerus level (c), radius and ulna are formed at proximal level (d).

A classic experiment showed that blastema is not simply replacing missing parts. Distal end of a limb is amputated at wrist and inserted into belly of same animal to establish a blood supply to that part. This limb was then amputated at mid-humerus level. Both cut surfaces regenerate distally, the attached part to the belly had a radius and ulna at proximal level (Fig.12).

Blastema is larger in size than embryonic limb bud. But it has a set of positional value along its proximal –distal axis, alike that set up during embryonic development. The retention of embryonic process, like the ability to specify new positional values is one of the main features of epimorphic regeneration in urodel amphibians.

Cells in amputated stumps can recognize the discontinuity in positional values. When a distal blastema is grafted to a proximal stump, the stump and blastema have different positional values. A normal limb is regenerated from the proximal stump by intercalary growth and cells from wrist blastema give rise to entire limb (Fig.13).

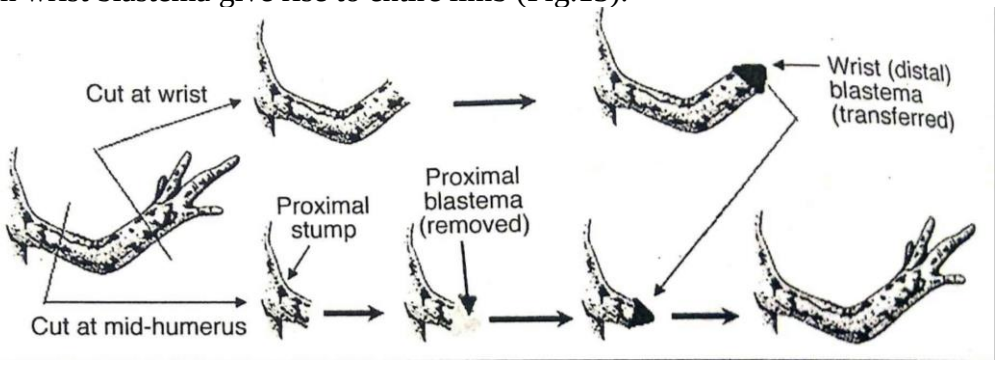

Figure 13: A wrist (distal) blastema can form entire limb from proximal stump.

Knowledge of pattern formation comes from the molecular basis of positional information. Recently, a cell –surface protein is identified that is expressed in a graded manner along the proximal –distal axis of salamander blastema. A cell –surface protein, known as Prod1, is equivalent of mammalian cell –surface protein CD59. Concentration of Prod1 has about twofold difference between proximal and distal blastema. Probably this protein guides the cells to determine positional identity.

Gradient in Prod1 evidently shows that cell-surface proteins are involved in regeneration. Transplantation experiments suggest that proximal –distal positional values in urodel limb regeneration are encoded as a graded property at the cell surface. Cell behavior relevant to axial specification is a function of the expression of this property. At present, it is not clear to what extent blastema cells inherit positional value and to what extent they are subject to signals inducing expression of positional values.

Relationship between *Hox* gene expression and positional value is becoming understood for both embryonic limb development and limb regeneration. Though initially *Hox* gene expression in regenerating limb is not same as embryonic limb development, but later becoming same as limb regenerate. During embryonic limb development, *Hoxa* genes are expressed with temporal and spatial co-linearity along proximal –distal axis. During regeneration of salamander limb, two *Hox* genes –*Hoxa* and *Hoxd*are expressed together in the cells of stump within 48 hours of amputation. Experiments show that most distal region of blastema is divided into distinct zones within 4 days, which will become the proximal –distal regions of the limb.

F. Role of Retinoic Acid in Limb Regeneration:

Retinoic acid (RA) has crucial effect on regeneration of amphibian limb. RA is synthesized in wound epidermis of regenerating limb and form a gradient in the blastema along proximal-distal axis. This gradient probably informs the cells about their position in limb axis. RA can differentially activate the *Hoxa* gene across blastema and specify pattern in the regenerating limb. During normal regeneration, probably RA is secreted by wound epidermis and /or apical ectodermal cap. This RA induces gene activation for cell proliferation, down –regulates specific genes for differentiated cells and activates *Hox* genes, which inform the cells about their position in the limb and how much they have to grow.

When regenerating limb is exposed to RA, the blastema becomes proximalized, that means the regenerating limb has been cut at a more proximal place. When a limb is cut at middle of radius and ulna, and treated with RA, the regeneration occurs not only distal to the cut but also production of a new limb. Thus, RA can change the proximal –distal positional value of blastema towards more proximal. RA probably affects proximalization by increasing the concentration of Prod1. RA change positional values in blastema by activating *meis*homebox gene, which are involved in specifying proximal identity.

11.6 REGENERATION IN CRABS:

Crabs have the incredible ability to regenerate lost limbs, such as claws or legs, which is vital for their survival. This process is intricately linked to their molting cycle, allowing the growth of new limbs under the protection of a newly formed exoskeleton. Regeneration helps crabs maintain their functionality and adapt to environmental challenges.

Process of Regeneration in Crabs

1. **Autotomy:** Crabs can voluntarily shed damaged or injured limbs at specific breakpoints to prevent further harm or escape predators.
2. **Limb Bud Formation:** Following autotomy, a limb bud forms at the site of the lost appendage. This bud is made up of undifferentiated cells that proliferate and differentiate to develop a new limb.

3. **Molting Cycle:** The regeneration process is closely tied to molting. Since the exoskeleton limits growth, the new limb develops underneath it. When the crab molts, the old exoskeleton is shed, revealing the partially regenerated limb.
4. **Growth and Maturation:** The newly regenerated limb is initially smaller and less functional. Over successive molting cycles, the limb grows and matures until it matches the original in size and capability.

Importance of Limb Regeneration

- **Survival:** Allows crabs to escape predators even after losing limbs.
- **Functionality:** Restores critical functions like locomotion and feeding.
- **Adaptation:** Demonstrates their resilience and ability to recover from injuries, maintaining ecological balance.

11.7 COMPENSATORY REGENERATION:

Compensatory regeneration is observed in higher organisms, where damaged tissues or organs recover their functional capacity without restoring the original structure. Unlike complete regeneration, which restores both form and function, compensatory regeneration emphasizes functional recovery.

Examples of Compensatory Regeneration

1. **Liver Regeneration in Mammals:**

- The liver can regenerate its functional capacity after partial removal or damage.
- Remaining liver tissue undergoes cellular proliferation to compensate for the loss.
- The regenerated tissue does not replicate the exact structure of the original but restores vital functions like metabolism and detoxification.

2. **Kidney Regeneration:**

- In certain species, kidneys exhibit limited compensatory regeneration.
- Surviving nephrons increase their functionality to compensate for damaged ones.

3. **Heart Regeneration in Zebrafish:**

- Zebrafish can regenerate damaged cardiac tissue, restoring heart function through the replacement of lost cells.

Mechanisms of Compensatory Regeneration

1. **Cellular Proliferation:** Surviving cells divide and expand to replace lost or damaged tissues.
2. **Tissue Remodeling:** Existing tissues reorganize to optimize functionality.
3. **Gene Activation:** Specific genes are activated to support repair and functional recovery.

Importance of Compensatory Regeneration

- **Survival in Higher Organisms:** Enables recovery from injuries while maintaining essential bodily functions.
- **Functional Recovery:** Prioritizes restoring critical processes over structural replication.
- **Evolutionary Adaptation:** Demonstrates the ability of organisms to thrive despite injury or tissue loss.

11.8 SUMMARY

Regeneration occurs in various forms across the animal kingdom, with distinct mechanisms tailored to the organism's complexity. Hydra regenerates through morphallaxis, reorganizing existing tissues, while Planaria employs epimorphosis, forming a blastema for new growth. Amphibians demonstrate limb and tail regeneration, a remarkable ability to replace lost structures. Crabs regenerate claws as a survival strategy, linked to molting cycles. Compensatory regeneration, seen in higher organisms, focuses on restoring function rather than structure, as observed in liver regeneration. These processes illustrate the adaptability and resilience of life.

11.9 TECHINICAL TERMS:

Crabs, LimbRegeneration, Molting, Autotomy, Exoskeleton, Compensatory Regeneration, Functional Restoration, Tissue Repair, Cellular Proliferation.

11.10 SELF-ASSESSMENT QUESTIONS:

1. How is limb regeneration in crabs related to their molting cycle?
2. What is compensatory regeneration, and how does it differ from complete structural regeneration?
3. Why is compensatory regeneration significant for higher organisms?
4. Types of regeneration
5. Explain regeneration in amphibian
6. Explain regeneration in insects

11.11 SUGGESTED READINGS

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Prof. V. Venkata Ratnamma

LESSON - 12

HARMONAL REGULATION

AIMS AND OBJECTIVES

- To understand the role of hormones in regulating metamorphic changes in insects and amphibians.
- To analyze the specific hormonal pathways involved, such as ecdysteroids and juvenile hormones in insects and thyroid hormones in amphibians.
- To examine the interplay between hormonal signals and environmental factors during metamorphosis.
- To highlight the comparative aspects of hormonal influence across different species of insects and amphibians.

STRUCTURE

12.1: INTRODUCTION:

Metamorphosis, a profound biological transformation, is a hallmark of development in many insects and amphibians. It involves dramatic changes in morphology, physiology, and behaviour, enabling these organisms to transition between distinct life stages, such as larva to adult in insects or tadpole to frog in amphibians. Central to this process is the precise regulation of hormones, which act as chemical messengers to coordinate growth, tissue remodelling, and functional adaptation. In insects, hormones like ecdysteroids and juvenile hormones govern the timing and nature of metamorphic events. Similarly, in amphibians, thyroid hormones play a pivotal role in initiating and controlling metamorphic progression. The interplay of these hormones ensures that metamorphosis aligns with environmental cues and developmental requirements, making it a fascinating area of study in developmental biology and endocrinology.

12.2 HARMONAL REGULATION IN INSECTS:

1. Hormonal Influence on Insect metamorphosis:

The general pattern of hormonal regulation on metamorphosis is very similar among insect species. Three hormones are recognized to play crucial role in molting and metamorphosis – prothoracicotropic hormone, ecdysone and juvenile hormone.

Prothoracicotropic hormone (PTTH) is, in fact, a neurohormone secreted by the neurosecretory cells so –called brain (Fig.1). PTTH is a peptide hormone with 109 amino acids and molecular weight about 40,000; its release is triggered by neuronal, hormonal or environmental signals. PTTH stimulates the production and release of ecdysone form the prothoracic gland situated near the brain.

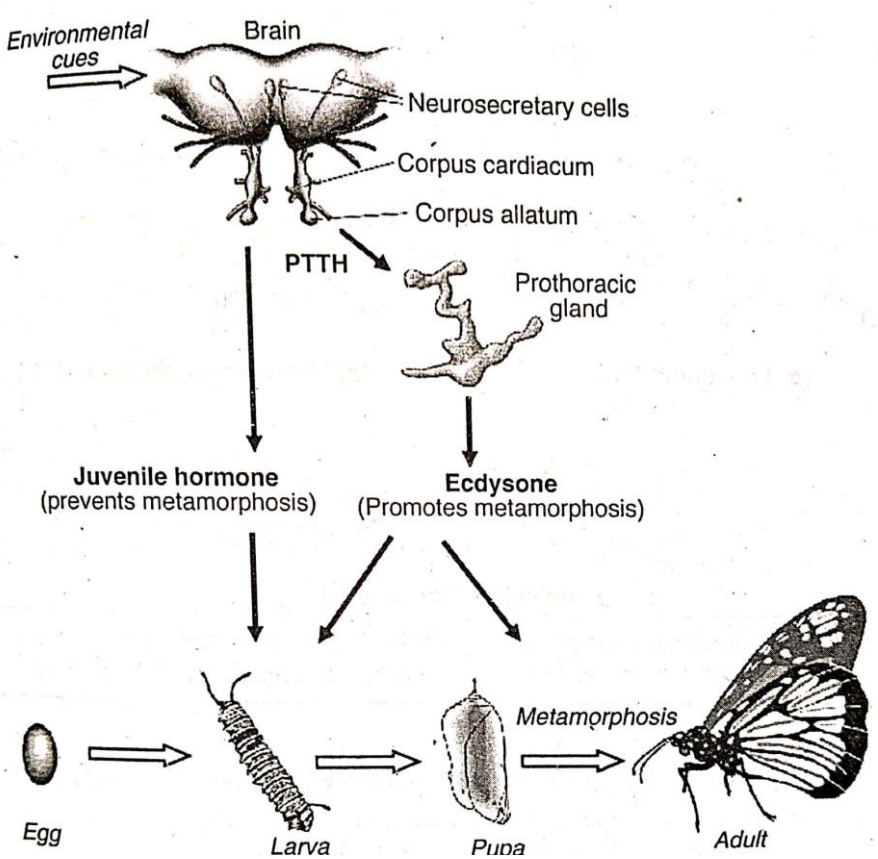

Figure 1: Neurosecretory cells of so-called brain of a holometabolous insect, which secretes a neurohormone PTTH to trigger metamorphosis.

Ecdysone (Ec) is a steroid ($C_{27}H_{44}O_6$) molecule (Fig.2) and is a prohormone that is modified in peripheral tissues and fat bodies in 20-hydroxyecdysone (20E) [Fig.2]. 20E ($C_{27}H_{44}O_7$) is the active form that initiates and coordinates each molt and regulates changes in gene expression occurring during metamorphosis.

Juvenile hormone (JH) is secreted by a pair of endocrine glands, the corpora allata, situated and attached to the posterior of the brain. JH is a hydrophobic molecule of acyclic sesquiterpenoid ($C_{16}H_{26}O_3$) class (Fig.2). JH does not cause molt, but play a vital role in its regulation. When JH level is relatively low, 20E stimulates pupal molt. In some insects,

corpora allata and prothoracic glands are fused into one called ring gland, as observed in *Drosophila.*

Hormonal interactions during molt and metamorphosis:

PTTH secretion produces waves of ecdysone production, hence initiating pulses of 20E. During each larval molt, concentration of 20E in hemolymph rises to trigger a change in commitment of epidermal cells. A second pulse of 20E initiates differentiation processes during molting, stimulated by 20E, epidermal cells of body surface withdraw from cuticle and produce a molting fluid containing a proenzyme. This proenzyme, upon activation, digests the old cuticle. The epidermis then generates a new cuticle that is initially distensible and expands as the larva (now in next instar) grows in size. Then the new cuticle hardens and becomes nonelastic; the cycle is repeated.

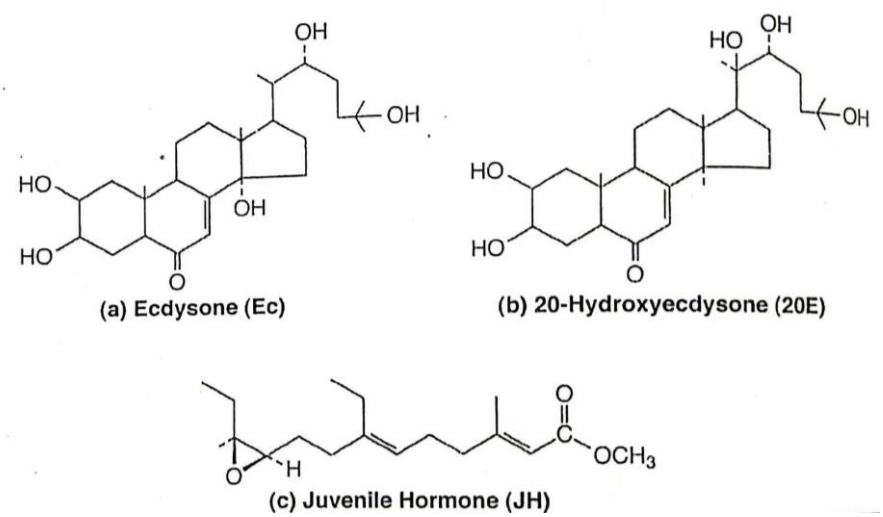

Figure 2: Hormones playing crucial role in molting and metamorphosis in insects.
(a) Ecdysone (Ec) (b) 20-Hydroxyecdysone (20-E) and (c) Juvenile Hormone (JH).

It is JH that determine whether the result of a molt will be an increase in larval size or pupation or metamorphosis. As long as JH is present in relatively high concentration, molts result in a new larval instar. In last larval instar, neuronal signal from brain to corpora allate inhibits JH production. A drop in JH level causes PTTH secretion from brain, which in turn, stimulates secretion of ecdysone from prothoracic gland. This pulse of pulse of 20E in presence of low level of JH causes pupal development. In the final step, 20E acts in absence of JH, the imaginal disc differentiates and the molt results in adult.

During metamorphosis of *Drosophila,* two major pulses of 20E occur. At the end of third instar, first pulse of 20E initiates morphogenesis of leg and wing imaginal discs. At this time larval hind gut degenerates and larva stops eating. The second pulse of 20E occurs several hours after the first one; it activates pupa-specific characters and later molt in absence of JH. The result is adult fly. Beside PTTH, 20E and JH, several other factors are involved in insect metamorphosis. A cascade of hormones controls the events of eclosing-muscular movements and rupture of the puparium that allow the emergence of adult fly. There are receptor molecules in the target tissues, which are3 synthesized at particular time to make the tissue responsive.

Like other steroid hormones, 20E diffuses across cell membranes and interacts with cytoplasmic receptor proteins. In order to the active, 20E –receptor complex requires (ER) another protein produced by a gene known as *ultraspiracle (usp)* in *Drosophila.*Usp protein dimerizes with 20E receptor protein (ER); the heterodimer with 20E becomes an active complex that regulates gene transcription.

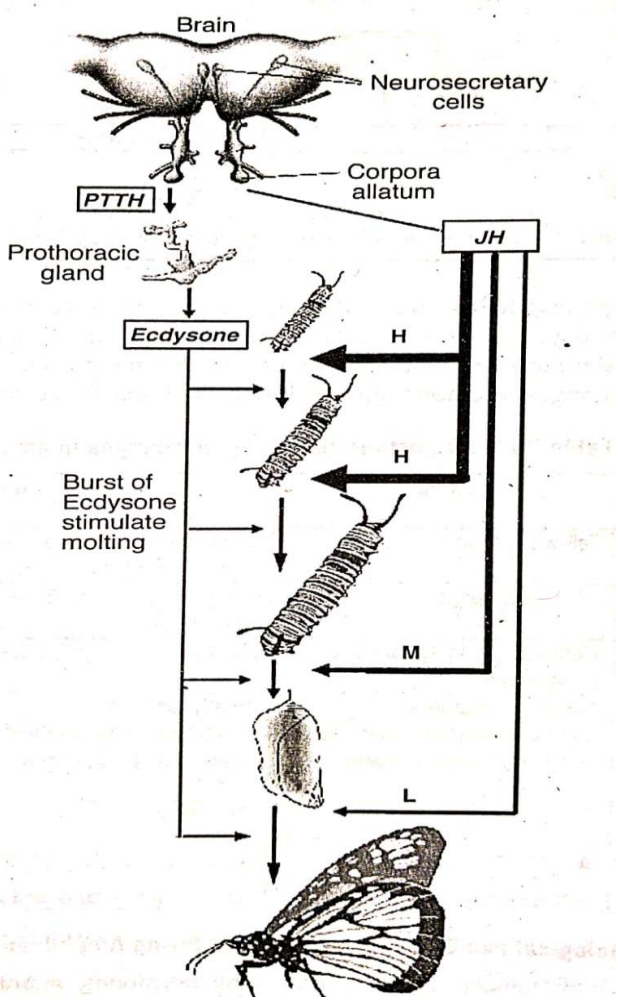

Figure 3: It is JH that determine whether molt will be an increase in larval size or pupation or metamorphosis. Burst of JH secretion - H = high, M = Moderate and L = low.

12.3 HARMONE REGULATION IN AMPHIBIAN METAMORPHOSIS:

Role of thyroid hormones in metamorphosis of amphibians was demonstrated in 1912. When tadpoles were fed extract of horse thyroid gland, they metamorphosed prematurely. Complementarily, surgical removal of developing thyroid gland of tadpoles was done in 1916. Such thyrodectomized tadpoles failed to metamorphose and grew into giant tadpoles. Later, it was found that hypophysectomy produced similar effect. Subsequent studies evidently showed that amphibian metamorphosis is regulated by thyroid hormones - tetraiodothyronine/thyroxine (T4) and triiodothyronine (T3) [Fig. 20. 10]. In absence of T4 and/or T3 metamorphosis will not occur, the tadpoles simply grow into large aquatic creatures.

It is now known that some environmental factors, especially temperature, and an endogenous developmental program in brain trigger the secretion of a hypothalamic hormone - the Thyrotropin-Releasing-Hormone (TRH). TRH stimulates

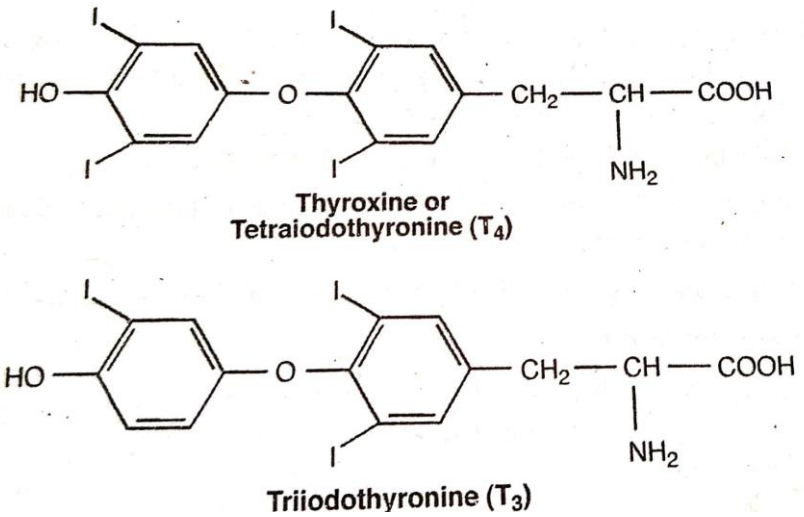

Figure 4: Thyroid hormones play a key role in amphibian metamorphosis. (a) Thyroxine or Tetraiodothyronine (T3), (b) Triiodothyronine (T4).

Anterior pituitary to synthesize and release Thyroid-Stimulating-Hormone (TSH) or thyrotropin. TSH controls the function of thyroid gland to synthesize and release T4 and T3 (Fig. 20.11). Interestingly, the effective hormone in tadpoles to drive TSH release pituitary is Corticosterone-Releasing-Hormone (CRH) that also triggers the release of Adrenocorticotropic Hormone (ACTH). ACTH stimulates adrenal cortex to secrete corticosteroids, which partly regulate the production of enzyme deiodinase (type II) to convert T4 to T3 in target tissues. Corticosteroids also modify the action of thyroid hormones in some target tissues. Recently known that thyroid hormones stimulate pituitary to secrete another hormone - Prolactin, which also play some role in regulating metamorphosis in amphibians.

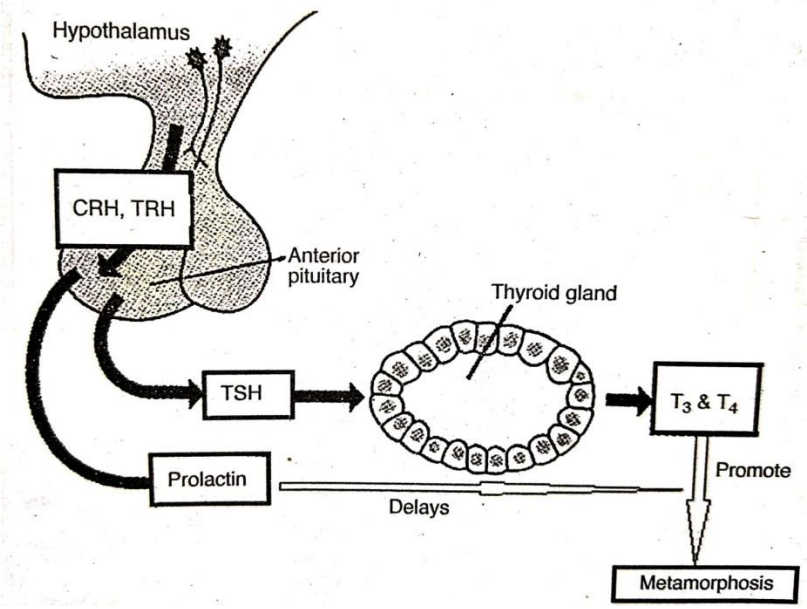

Figure 5: Hormonal interaction in regulating thyroid function during metamorphosis in frog

12.3.1 Role of Prolactin in amphibian metamorphosis:

It is now known that thyroid hormones stimulate pituitary gland to secrete prolactin, which has some regulatory role in metamorphosis. When mammalian prolactin is injected into tadpoles, they show slower metamorphic changes. It is suggested that prolactin interferes

with the T3 and T4 receptors. Recent findings reveal that prolactin level increases as metamorphic climax approaches (Fig.4). Experiments with transgenic tadpoles expressing high level of prolactin show that prolactin prevent tail resorption. Such tadpoles, however, metamorphose, but juvenile frogs appear with persistent tail. There are many questions about interaction between T3 and prolactin; there is no doubt that prolactin counteracts some of many effects of T3.

12.3.2 Metamorphic events are regulated by levels of thyroid hormones:

Sequence of metamorphic changes is controlled by concentration of thyroid hormones. In fact, thyroid hormone concentration increases during the progressive changes of metamorphosis. Some changes occur early, when thyroid hormone concentration is low; some changes occur later after the thyroid hormone concentration is higher. These kinds of observations support that each of the different local responses to T3/T4 has a threshold concentration. This threshold model is useful in understanding amphibian metamorphosis, but it cannot throw any light on the mechanism involved. Again, molecular studies reveal that timing of events in amphibian metamorphosis is even complex than just hormone concentration.

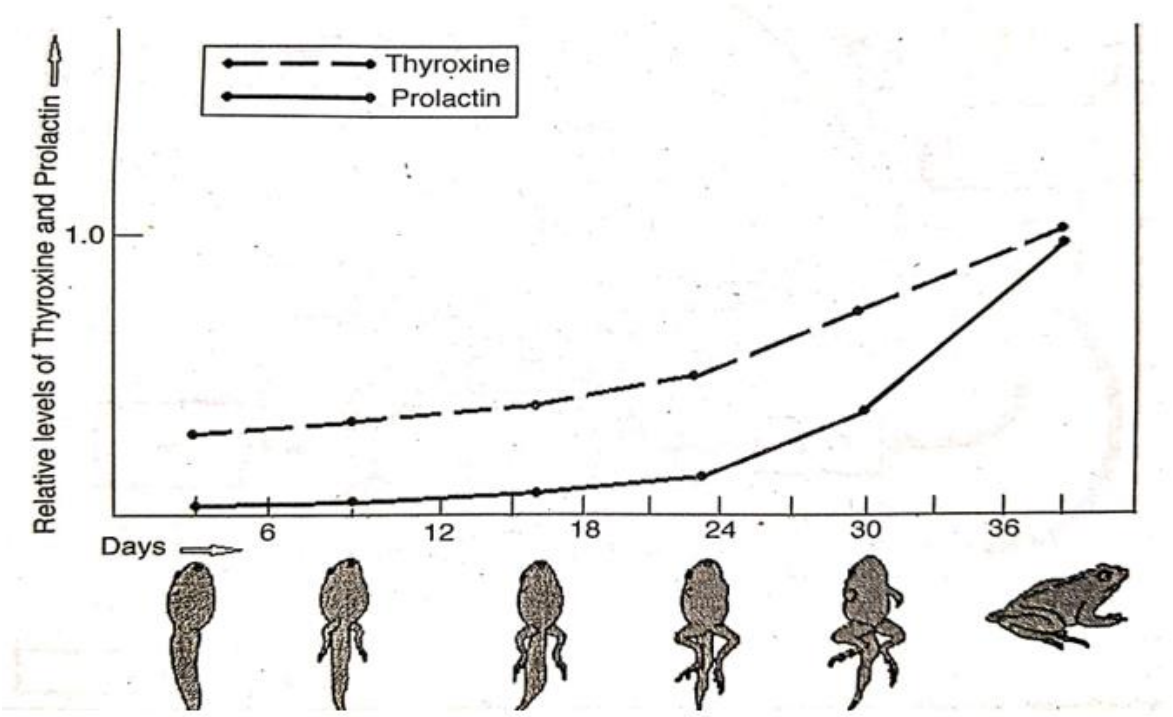

Figure 6: Relative levels of Thyroxine and Prolactin during metamorphosis in amphibians

Depending on thyroid hormone concentration, metamorphosis in amphibians can be divided into stages. In the initial stage premetamorphosis, thyroid glands of tadpoles start secreting low amount of T4 and very low amount of T3. This initial T4 secretion is brought about by CRH. In this stage, limb growth begins and continues. In prometamorphosis stage, when mature thyroid glands produce more hormones, lungs begin to develop and changes in head region begin. In metamorphic climax stage, concentration of T3/T4 rises dramatically. In this stage, major changes like resorption of gill, remodelling of gut, tail resorption etc. occur. The high level of T3/T4 produce a negative feedback loop (Fig.7) to lower TSH and probably

CRH productions, so that thyroid hormone secretion is appropriate for juvenile frog/toad. In fact, metamorphic events are down-regulated once metamorphic climax is reached.

12.3.3 Response of thyroid hormones is tissue/organ specific:

T3 alone causes many changes in metamorphosis of amphibians. The same hormone causes some tissues to develop and differentiate while causing other tissues to degenerate. The type of response (differentiation or degeneration) is determined by other factors present in different tissues.Degeneration of tadpole's tail occurs relatively rapidly, because bony structures do not extend to tail. Tail regression takes place by apoptosis followed by enzymatic digestion by the macrophages. Tail epidermis lacks epithelial stem cells and fails to generate new skin like head or trunk epidermis.

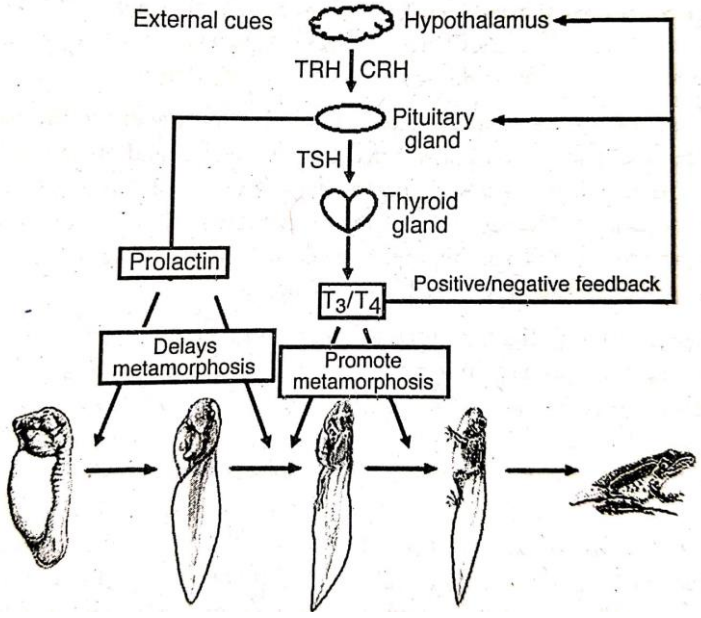

Figure 2: Level of T3/T4 produces a positive/negative feedback loop to higher/lower secretion of TSH/CRH, which in turn results thyroid hormone secretions appropriate to juvenile frog/toad.

Metamorphosis not only involves dramatic morphological changes, some fundamental metabolic pathways also altered. Rod segments of tadpole's retina contain porphyropsin, which is vitamin-A_2 based visual pigment. Vertebrates inhabiting freshwater have vit-A2 based visual pigment in their eye. During metamorphosis, the metabolic pathways to produce vit-A2 shift to produce vit-A_1, a character of terrestrial vertebrates. As a result, the visual pigment of adult frog's eye is rhodopsin instead of porphyropsin.

Tadpoles like other aquatic animals, are ammonotelic, i.e., their excretory end product is ammonia. Ammonia, though toxic, is easily dissolved, diluted and excreted by the aquatic animals. During metamorphosis, enzymatic machinery of tadpole liver changes to produce urea as major excretory end product. Synthesis of urea from ammonia requires four urea-cycle enzymes. Changes in visual pigments and urea cycle are triggered by an increase in T3 concentration.

Some experiments are dramatical to demonstrate the organ-specific responses to thyroid hormones. A tail tip is transplanted to trunk region and an eye cup is placed in the tail. The tail in the trunk undergoes degeneration but the eye cup retains its progressive development though placed in degenerating tail. Thus, programmed-cell-death is organ-specific and

specific organ/tissue will die when it receives the signal. Such programmed-cell-death is crucial in morphogenesis.

The response of each tissue to thyroid hormone is specific to that particular tissue. Isolated tadpole tail survives in simple culture medium for long time. Addition ofvery low levels of T3 in the medium induces macroscopic changes, characteristic tail resorption during metamorphosis. The process includes failure of epithelial stem cells to replace epidermis, increased accumulation of lysosomal enzymes and-apoptosis of muscle cells. When prolactin is added to the medium with Ty, these changes do not occur; thus, both thyroid hormones and prolactin act locally and specifically. Though it is said that every tissue or organ is affected by thyroid, at least one tissue seems to be unresponsive. Ventral retina is activated by thyroid hormones, in which neurons proliferate to form ipsilateral axons. Dorsal retina is not responsive to thyroid hormone and does not generate new neurons. Dorsal retina expresses Hormone receptors are also essential in metamorphosis.

12.4 HARMONE RECEPTORS ARE ALSO ESSENTIAL IN METAMORPHOSIS:

In metamorphosis of frog, thyroxine (T4) is released into blood by thyroid glands, which is then converted by target tissues into T3 by an enzyme - deiodinase. T3 is a more active hormone that binds with Thyroid-Hormone-Receptors (TR) with much higher affinity than T4 resulting gene activation. Thus, threshold model is restated that levels of both thyroid hormones and their receptors (TRs) are essential for producing response during metamorphosis.

There are at least two types of TRs - TRa and TRß. TRa is present widely in all tissues even the animal does not develop a thyroid gland. TRß is directly activated by thyroid hormones. Before metamorphosis, TRß level is very low; during metamorphosis, intracellular level of TRß increases as the levels of T3 and T_1 increase.

Each TR joins with a molecule called retinoic acid-receptor (RXR). This heterodimer (TR-RXR) binds with thyroid hormone and influence transcription. The TR-RXR complex is a transcriptional repressor but when T3 is added to this complex, T3-TR-RXR activates some genes and their transcription. **Thus,** it can be concluded that local tissue-specific responses and the regulation of hormone sensitivity are controlled by TR levels. Again, prolactin seems to decrease TR expression, which support that prolactin counteracts, at least, some actions of thyroid hormones.

12.5 SUMMERY:

Hormonal regulation plays a crucial role in driving metamorphosis in both insects and amphibians, orchestrating the transition between distinct life stages through precise endocrine control. In insects, ecdysteroids and juvenile hormones regulate molting and the progression to adult stages, while in amphibians, thyroid hormones are pivotal in initiating and controlling the transition from larval to adult forms. Hormone receptors are essential in mediating these processes, enabling the hormonal signals to influence tissue remodeling, organ development, and overall morphological changes. The interplay between hormonal pathways and environmental cues ensures the synchronization of metamorphosis with external and internal conditions. Understanding these mechanisms highlights the molecular basis of development and provides insights into the consequences of hormonal disruption on both individual organisms and ecosystems.

12.6 TECHINICAL TERMS:

hormonal regulation, insects, amphibians, metamorphosis, ecdysteroids, juvenile hormones, thyroid hormones, hormonal pathways, hormone receptors, tissue remodeling.

12.7 SELF-ASSESSMENT QUESTIONS:

1. What is metamorphosis? Describe the main hormones and their involvement in insect metamorphosis.
2. What are the important metamorphic changes in amphibians? Discuss an idea of morphological and biochemical changes during amphibian metamorphosis.
3. Write an essay on the role of hormones in metamorphosis in amphibians.
4. Write short note on: Holometabolous development, Imaginal disc, Development of wing disc in insects, Development of leg disc in insects, Retrogressive metamorphosis.

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LESSON - 13

APOPTOSIS

AIMS AND OBJECTIVES

- To define apoptosis and understand its mechanisms.
- To explain the process of programmed cell death.
- To explore the role of apoptosis in embryonic and postnatal development.
- To examine the importance of apoptosis in maintaining cellular homeostasis.

STRUCTURE

13.1 INTRODUCTION:

Programmed cell death is a fundamental biological process essential for the survival, development, and maintenance of multicellular organisms. Apoptosis, the most well-characterized form of programmed cell death, plays a pivotal role in eliminating damaged, unnecessary, or potentially harmful cells in a controlled and non-inflammatory manner. This process ensures proper development, tissue remodeling, and cellular homeostasis while preventing disease. Understanding apoptosis and its significance in development provides insight into how organisms maintain balance and adapt to changing conditions.

13.2 APOPTOSIS:

Programmed Cell Death (PCD) refers to a biological process where cells undergo a controlled and intentional self-destruction. This process is essential for the proper development, maintenance, and functioning of multicellular organisms. PCD is a key mechanism for maintaining cellular homeostasis and eliminating damaged or unnecessary cells. Balancing cell survival and cell death is fundamental to development and homeostasis.
Cell death is regulated by multiple interconnected signaling pathways and molecular mechanisms this is referred to as regulated cell death. Classically, cell death is categorized into three different types, based on morphological changes, triggers, and the biochemical pathways involved.

1. **Apoptosis** is a tightly regulated form of programmed cell death (PCD), that triggers cells to self-destruct without any external influence. It is an essential part of life, particularly for multicellular organisms that must control the growth, development, and turnover of cells in order to maintain homeostasis.Apoptosis is critical for proper embryonic development, and a classic example of this process can be observed when cells between the digits on a hand apoptose in order to separate the fingers.
2. **Autophagy** is also often categorized as a type of programmed cell death.Autophagy is a process by which cellular organelles and other contents are devoured by lysosomes to clear away unnecessary or dysfunctional components. This critical mechanism allows for the systematic degradation and recycling of cellular materials.
3. **Necrosis** has historically been thought of as a process that occurs accidentally due to extreme external physiological stress or ACD. However, in recent years, researchers have shed light on specific regulated molecular mechanisms that can be triggered when a cell is under stress and alternative methods of cell death are not available,Some of these regulated necrotic cell death pathways include necroptosis, pyroptosis, ferroptosis, and necrosis
4. **Necroptosis** is a programmed form of necrosis, or inflammatory cell death but necrosis is associated with unprogrammed cell death resulting from cellular damage or infiltration by pathogens.
5. **Pyroptosis** is a highly inflammatory form of lytic programmed cell death that occurs most frequently upon infection with to form part of the antimicrobial response. This process promotes the rapid clearance of variousintracellular pathogens and is likely bacterial, viral, fungal and protozoan infections by removing intracellular replication niches and enhancing the host's defensive responses. Pyroptosis can take place in immune cells and is also reported to occur in keratinocytes and some epithelial cells.
6. **Anoikis** is a programmed cell death occurring upon cell detachment from the correct extracellular matrix, thus disrupting integrin ligation. It is a critical mechanism in preventing dysplastic cell growth or attachment to an inappropriate matrix.Apoptotic cells usually present characteristic morphological changes, including chromatin condensationand DNA laddering, loss of mitochondrial-membrane potential and of plasma-membrane phospholipid asymmetry, and detachment from the cellular matrix.

Apoptosis, a form of programmed cell death, is a coordinated and step-wise series of biochemical reactions resulting in the ordered disassembly of a cell from an organism. This normal biological process is required for proper organ development during embryogenesis and the removal of abnormal cells, such as the cells that are damaged by exposure to pathogens or undergo oncogenic transformation. The switch between cell survival and apoptosis is tightly regulated and critical to the development and well-being of an organism. For instance, defects in the apoptotic pathway that prevent cell death may lead to developmental abnormalities or unregulated tissue growth, as occurs in cancer. In contrast, unscheduled or premature apoptosis can result in the loss of functional cells, contributing to autoimmune, neurological and cardiovascular disorders. The term apoptosis was first introduced in a paper in 1972 by Kerr, Wyllie, and Currie to describe a morphologically distinct type of cell death. It consists of aseries of biochemical changes that lead to changes in the cell’s morphology or death. It results in the death of 50 to 70 billion cells per day in an average adult human being. It is also termed as ‘cellular suicide’ as cells undergo a highly regulated process for the programmed removal of cells from the body.

13.3 PROCESS OF PROGRAMED CELL DEATH:

Consequently, manipulation of the apoptotic process is essential to better understand the development of various diseases and to discover potential therapeutic targets. Induction of apoptosis evokes several significant bimolecular and morphological changes, some of which are commonly used as markers of apoptosis:

- Activation of apoptotic signaling cascades
- Phosphatidylserine exposure on the outer leaflet of the plasma membrane
- Release of cytochrome c (Cyt c) from mitochondria
- Activation of caspases
- Cleavage of specific caspase substrates
- DNA fragmentation

These events lead to major phenotypic alterations, highlighted in the figure below. Apoptosis concludes with the formation of apoptotic bodies, which are cleared by phagocytes or neighboring cells.

Apoptosis is executed by the extrinsic or intrinsic death signaling pathways, or in some cases by the perforin/granzyme B pathway and results in the activation of the caspase cascade. Multiple protein families are involved in the signaling pathways that promote or inhibit caspase activation.

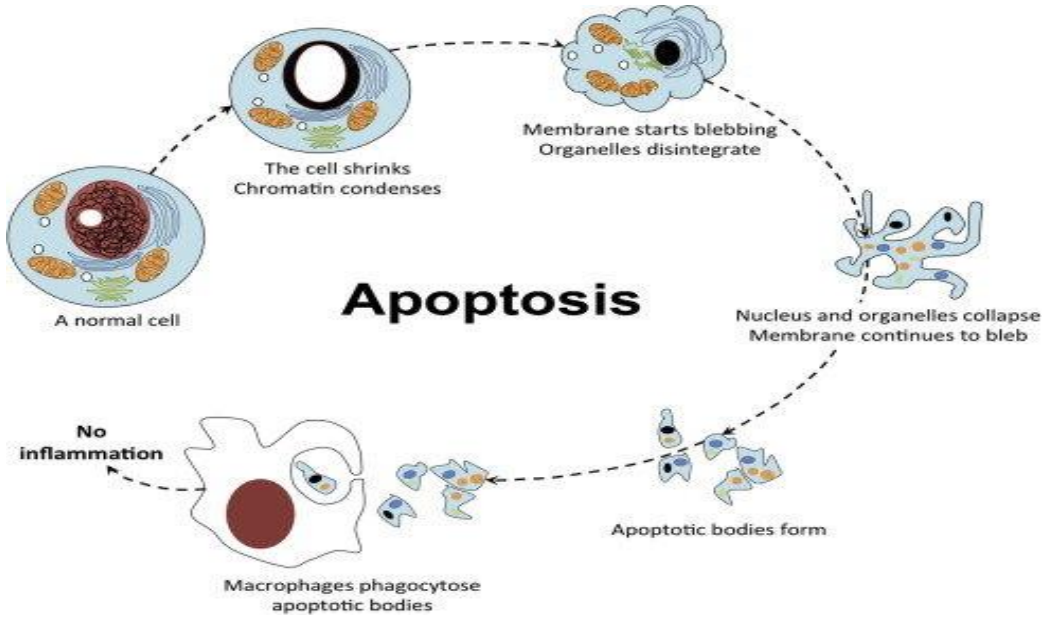

Figure 1: Apoptosis

- Most cells are provided with an in-built mechanism of apoptosis as a part of the cell cycle.
- This mechanism allows the body to get rid of unnecessary cells or infected cells.
- Apoptosis is considered a vital part of various processes including normal cell cycle, proper development and functioning of the immune system, embryonic development, and chemical-induced cell death.
- Apoptosis is a part of development as it is essential in the differentiation of a mass of tissue into various groups.

- Apoptosis occurs in cells that might have been infected with viruses or might even be cancerous. This process usually takes place when the cell detects defects in the DNA and is not able to repair it.
- Apoptosis is also an essential part of the immune system as it clears the pathogen-specific immune cells once the foreign particle is removed from the body.
- This also helps to remove the immune cells that might react against the body's cells and cause autoimmune diseases.
- Another reason for apoptosis is to maintain homeostasis in the body by removing old cells to make space for the new ones.

The process of apoptosis is highly complex and sophisticated, involving an energy-dependent series of molecular events.

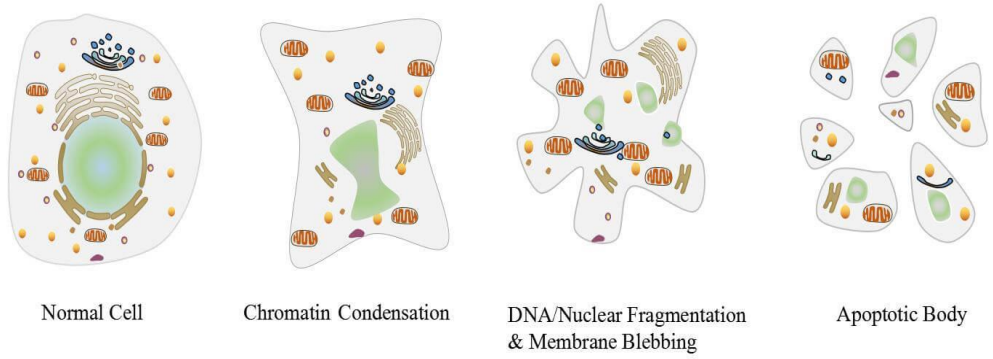

Figure 2 : Process of apoptosis

Three different pathways work on different mechanisms to achieve apoptosis. All three of these pathways converge at the same terminal pathway, which results in the sequential degradation of cellular organelles.

1. Extrinsic or death receptor pathway:

- The extrinsic pathway that initiates apoptosis involves transmembrane receptor-mediated interactions.
- These interactions take place between ligands and their corresponding death receptors that are all part of the tumor necrosis factor (TNF) family.
- All members of the TNF receptor family share a common cysteine-rich extracellular domain with about 80 amino acids called the "death domain".
- The death domain plays a vital role in transmitting the death signal from the cell surface to the intracellular signaling pathways.
- The events or interactions that take place in the extrinsic phase of apoptosis involve two models; FasL/FasR and TNF-α/TNFR1 models, both of which include the clustering and binding of receptors and their ligands.
- Upon ligand binding, cytoplasmic adapter proteins are activated, which causes the receptors to exhibit death domains.
- The binding of FasL to FasR results in the activation of the adapter protein FADD whereas the binding of TNF ligand (TNF α) to TNF receptor (TNFR1) results in the binding of the adapter protein TRADD with activation of FADD and RIP.
- These events cause the dimerization of the death effector domain, causing FADD to bind with procaspase-8.
- As a result of the binding, a death-inducing signaling complex (DISC) is formed, resulting in the auto-catalytic activation of procaspase-8.

- Once caspase-8 is activated, the terminal phase or execution phase of apoptosis is triggered.

2. The intrinsic or mitochondrial pathway:

- The intrinsic pathway that initiates apoptosis involves a series of non-receptor-mediated processes that produce intracellular signals and act directly on targets within the cell.
- This pathway involves mitochondrial-initiated events.
- The factors that initiate the intrinsic pathway produce intracellular signals that might act in either a positive or negative fashion.
- Negative signals include the absence of certain growth factors, cytokines, and hormones that can lead to failure of inhibition of death programs, thereby triggering apoptosis. In simple words, the withdrawal of factors causes loss of apoptotic suppression and subsequent activation of apoptosis.
- The factors that act positively include, radiation, toxins, hypoxia, hyperthermia, viral infections, free radicals, among others.
- All of these factors cause changes in the inner mitochondrial membrane that causes the opening of the mitochondrial permeability transition (MPT) pore and release of two main groups of pro-apoptotic proteins from the intermembrane space into the cytosol.
- The first group consists of cytochrome *c that* binds and activates Apoptotic protease-activating factor – 1(Apaf-1) as well as procaspase-9, forming a protein complex termed, apoptosome.
- The apoptosome cleaves the procaspase into the active form, caspase 9, which further cleaves and activates procaspase into the effector caspase 3.
- The first group also has other proteins like SMACs (second mitochondria-derived activator of caspases) and HtrA2/Omi that promote apoptosis by inhibiting the activity of IAPs (inhibitors of apoptosis proteins).
- The second group of pro-apoptotic proteins is released from the mitochondria during apoptosis, but this occurs as a part of the terminal phase after the cell has committed to die.
- These proteins translocate to the nucleus and cause DNA fragmentation and condensation of peripheral nuclear chromatin.**(Fig.4.3)**

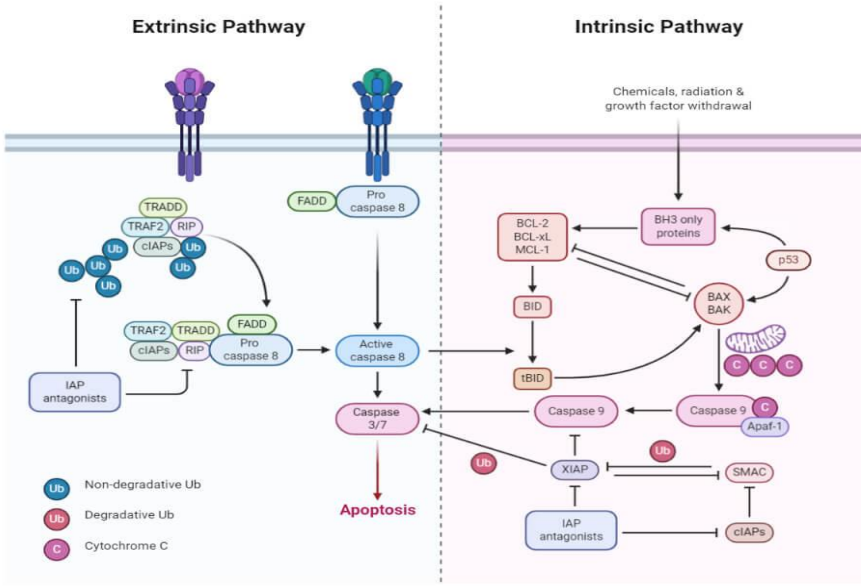

Figure 3: Extrinsic pathway & Intrinsic pathway

13.4 ROLE OF APOPTOSIS IN DEVELOPMENT:

Since 1974, when Sydney Brenner first introduced Caenorhabditis elegans in the scientific community as a model organism, the nematode has been extensively used as a model system to study cellular biology.

In C. elegans, apoptosis is a normal component of growth.

During development 1090 somatic cells are generated for each hermaphrodite, of which 131 invariantly undergo apoptosis. Most of the cells that die during early development in C. elegans are neurons, although several hypodermal, muscle and pharyngeal cells also suffer the same fate. The first one occurs between 250 and 450 minutes after fertilization and removes almost a fifth (113/628) of the cells that are generated during embryonic development17. The second wave of death is observed in the larval stage L2 and removes some of the newly generated neurons. No apoptotic cell death occurs in the soma after the L2 stage. The third wave of death is observed in the adult hermaphrodite germ line, where about half of all potential oocytes are removed by apoptosis.

Over the past 25 years, the genetic dissection of programmed cell death in C. elegans has led to the identification of >20 cell-death genes. These genes have been placed into a genetic pathway, which includes four distinct steps.

- First, a cell decides to die.
- In a second step, it kills itself in a cell-autonomous fashion.
- The dead cell is subsequently recognized and engulfed by a neighbouring cell.
- Finally, the engulfed cell is degraded.

Surprisingly, genes that are involved in engulfment and apoptotic DNA degradation have been shown to also contribute to the killing process.

The essential four genes [cell death abnormal (ced)-3, ced-4, ced-9 and egg-laying defective (egl)-1]

Genetic screens for mutants with abnormal cell-death patterns identified four genes that regulate all somatic cell deaths in C. elegans: cell death abnormal (ced)-3, ced-4, ced-9 and egg-laying defective (egl)-1.

Loss-of-function mutations in ced-3, ced-4 and egl-1 result in the survival of almost all (~131) apoptic cells, which indicates that these three genes have pro-apoptotic functions.

By contrast, ced-9 has anti-apoptotic activity because a ced-9 gain-of-function mutation blocks apoptosis, whereas ced-9 mutants die during early development due to excessive cell death.

Studies have allowed the ordering of these genes in a pathway, with egl-1 acting as a negative regulator of ced-9, and ced-9, in turn, negatively regulating ced-4 and ced-3.
ced-3 encodes a protease of the caspase family - a group of enzymes pivotal for their role in the execution of apoptosis.

CED-4 is an adaptor protein that is similar to mammalian apoptotic protease-activating factor-1 (APAF-1).

ced-9 and egl-1 encode members of the Bcl-2 family of mammals.

13.5 SUMMARY:

Apoptosis, or programmed cell death, is a tightly regulated biological process critical for development and cellular balance in multicellular organisms. This mechanism involves well-defined pathways, including intrinsic and extrinsic signaling, leading to the activation of caspases that dismantle the cell without causing inflammation. Apoptosis is crucial for processes like shaping tissues, removing unnecessary or damaged cells, and ensuring proper immune system development. Its role in embryonic development is particularly significant, as it helps sculpt structures, refine neural connections, and maintain balance in cell populations. By understanding apoptosis, we gain deeper insights into its importance in health, disease prevention, and development.

13.6 TECHINICAL TERMS:

Apoptosis, programmed cell death, intrinsic pathway, extrinsic pathway, caspases, phagocytosis, tissue sculpting, cellular homeostasis, development, cell signaling.

13.7 SELF-ASSESSMENT QUESTIONS:

1. What is apoptosis, and how does it differ from other forms of cell death?
2. Describe the process of programmed cell death and its key stages.
3. What role does apoptosis play in embryonic development?
4. Why is apoptosis essential for maintaining cellular homeostasis?

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LESSON - 14
AGING AND SENESCENCE

AIMS AND OBJECTIVES

- What are the biological mechanisms underlying aging and senescence?
- How do anti-aging actions counteract the effects of aging?
- What are the major disorders and diseases associated with aging in humans?
- How can age-related conditions be effectively prevented or managed?

STRUCTURE

14.1 INTRODUCTION

Aging is a universal biological process marked by the progressive decline of physiological functions, ultimately leading to diminished adaptability and increased susceptibility to diseases. Senescence, a hallmark of aging, refers to the cessation of cell division and functional decline at the cellular level. While aging is inevitable, understanding its mechanisms provides opportunities for intervention. Anti-aging actions aim to mitigate or reverse these effects, while the study of age-related disorders and diseases highlights the importance of proactive health strategies to enhance longevity and quality of life.

14.2 AGING AND SENESCENCE

All higher organisms exist in life cycles. Particularly, animals including man begin their life as fertilized eggs that develop into adult through embryonic and juvenilr stages. The adult reproduces by producing eggs or sperms, which unite to form a new zygote-The adult reproduces by pro the beginning of a new individual. After reproduction, adults deteriorate and eventually die. Thus, organisms bound to perish and life cycles, apparently, are tremendous wasteful.

Organisms on this planet are mortal, death is inevitable and a general feature of living beings. Unless there is disease or accident, an organism lives for considerable period and then become old, which means to approach a state of infirmity. At this phase of life, deterioration prevails over synthesis and the organism ages. The simple chronological progress of an individual's life along time axis is called aging. This process of deterioration of physiological functions necessary for survival associated with aging is called senescence. In simple words,

senescence characterizes aging, though aging and senescence are often taken as integrated. In recent times, many studies have been made on human aging. These studies reveal that body functions reach a peak in a certain age, then decline gradually until death. In humans, peak performance of body functions reaches at the age of 30 years. Taking the performance at 30 years as 100%, at 55-year age.

- Pumping efficiency of heart is reduced about 20%;
- Kidney function is reduced about 25%;
- Maximum breathing capacity is reduced to 40%, and
- Basal metabolism rate goes down about 10%.

Normal aging also includes decrease in bone mass, muscle strength and decreased immune responses to infections. General consequences of aging in man can be applied to other animals as well.

14.2.1 SENESCENCE DEFINED MATHEMATICALLY:

Senescence is apparent from many signs posture, limited physical activity, reproductive fitness, slow wound healing, less immune response, reduced skill to fight against predators etc. But any one of these manifestations cannot be used in defining senescence. Such as, a man having limited physical ability may father a child; a menopausal woman may participate in marathon race. So there must be some parameters to calculate the biological age of individual. But at present, there is no such parameter, and we have to rely one or more physiological criteria to define senescence.

But using physiological criteria in defining senescence is arbitrary; because it does not consider that individual senesces at different age. Thus, a mathematical definition of senescence develops that depends on survival data. The survival data is based on life tables, which shows how many individuals of a group born in 1900 in a certain country are alive in 1901, 1902, 1903 and so forth. From life table data, a useful parameter has been formulated called mortality rate.

$$\text{Morality rate } [m(t)] = \frac{\text{No. of individual s died at t years age}}{\text{No. of individual s alive at the begining of t year}}$$

The mortality rate increases with time and that we call senescence. Senescence can be gradual, typical for large animals like humans, who produce fewer young and provide considerable parental care. Again, senescence can be rapid in semelparousspecies, which reproduce only once in its life cycle, such as flies or salmon.

Animals have definite maximum life span that is characteristics of the species. Maximum life spanmeans the maximum number of years a member of a certain species has been known to survive. Such as maximum life span of humans recorded to be 122 years –Jeanne Calment (Fig.1) of Arles, France, who died on August 4th, 1997. But that does not mean most people are expected to live up to 121 years. So, the length of time an individual of a given species expected to live is known as lifeexpectancy**.** Life expectancy is not the characteristics of a species, but of a population.

Figure 1: Life span of more than hundred years is not uncommon in man. From top left, clockwise - Jeanne Calment, 21st February 1875-4th August 1997; Shigechiyo Izumi, 29th June 1865-21st February 1986; Sarah Knauss, 24th September 1880- 30th December 1999; Elizabeth Bolden, 15 August 1890-11th December 2006.

It is determined as the age to which half of individuals of a given population usually survive. Surely it varies in humans from country to country; such as in England, life expectance was 35 years in 1780, but 28 years in USA. For different reasons, life expectancy increases in developed countries, such as 74-80 years in USA today, while around 40 years in some areas of world.

14.2.2 CAUSES OF SENESCENCE:

Like many other biological processes, causes of senescence can be identified as two types proximate causes and ultimate causes. Proximate causes are direct mechanical and/or physiological causes. Ultimate causes refer to the adaptive functions of senescence.

1. **Proximate causes of Senescence:**

a. Oxidative Damage

All higher organisms require oxygen to produce metabolic energy through oxidative phosphorylation. The process reduces molecular oxygen to water and the liberated energy is used to synthesize our energy currency the ATP. Oxidative phosphorylation is essential for survival for organisms but produce oxidative stress to cells a driving force in organismic senescence. During oxidative phosphorylation, an oxygen molecule (O2) is reduced to two molecules of water (H_2O) in four steps and three intermediates are generated (Fig.22.5). These intermediates, popularly known as reactive oxygen species (ROS) include superoxide ion (O2), hydroxyl ion (-OH) and hydrogen peroxide (H_2O_2). These ROS molecules are highly reactive and also called oxidants. Oxidants aggressively react with other molecules in cells to produce various reactive oxygen metabolites (ROMs) and cause extensive damage to biologically important macromolecules. ROS and ROMs cause damage to major molecules in cells like DNA, lipid and protein. Damage to DNA often includes oxidation products of nucleotides, which impair DNA replicatiorr and transcription. Lipids react with ROS to form lipid peroxides, which alter the properties of biological membranes. Biomembranes with

lipid-peroxide increase uptake of low-density lipoproteins by vascular endothelial cells. It invites early atherosclerosis. In different mouse strains, life span is negatively correlated with lipid peroxide level in serum.

Cellular proteins are much damaged by oxidants. The oxidants cause conformational changes and covalent modifications of proteins, thus damage their biological activities. One very common covalent modification is formation of carbonyl group C=O), a result of oxidative damage. Carbonyl content in the proteins increases with age in many vertebrates and invertebrates studied and much significant in second half of life span.

It is to be mentioned here that cells produce a set of enzymes to convert dangerous oxidants into water and oxygen. They include superoxide dismutase, catalase and peroxidase. Since the enzymes are products of DNA and oxidative damage to DNA accumulates with age, it is likely that these useful enzymes become deficient with age. Certain molecules reduce oxidants into harmless molecules, called antioxidants. Ascorbic acid (Vitamin C), tocopherol (Vitamin E), beta-carotene, glutathione is some of natural antioxidants. Much of deterioration in association with senescence is caused by oxidative damage. This is known as oxidative stress hypothesis, which led to two predictions

(i) Oxidative damage increases with age, and

(ii) High antioxidant activities are correlated with long life span and slow down senescence.
There is no doubt that oxidative damage to DNA, lipid and protein increase with age. Life spans of animals are correlated negatively with peroxide levels but positively with antioxidant level. It is also found that experimental enhancement of antioxidant activities delayed senescence.

b. Aging is Programmed Process:

Discoveries in genetics of aging have raised the debate that aging is genetically-determined and programmed process. Finding that a single gene can modulate longevity and, to some extent, regulates aging supports the idea that aging is programmed.

Endocrine system seems to play a role of pacemaker in aging. Levels of growth hormone (GH) and insulin-like growth factors 1 (IGF-1) decline with age, and probably cause onset of aging. Because brain is the top regulator of endocrine changes, brain is supposed to act as master clock for aging process. Hormonal changes play a role in Caloric Restriction (CR), which in turn, has a life-extending effect. Though, exact mechanism, how hormones impact on aging is still unknown, they indirectly affect growth and maturation. Some hormones like GH probably regulate growth and development in early life and later contribute to aging.

Genes influencing life span is some model organisms confirm that there is a link between the timing of development and the timing of aging. In salmon, it is seen that development programs can cause aging or age-resembling phenotypes. But there are no details about how developmental mechanisms influence age-related changes. It is likely that some age-related changes are result of accumulation of toxic byproducts of metabolism. So, there seems to be overlapping of causes for aging.

C. Cellular Senescence:

Fundamental building blocks of every organism are cells, so it is assumed that cellular changes may contribute to aging. Many eukaryotic cells divide only a limited number of times though they remain viable without further proliferation. Replicative arrest is surely a powerful mechanism to prevent tumour formation, but there is evidence suggesting replicative arrest contribute to senescence of organisms. Earlier scientists thought the senescence is a character of organisms, not of cells. Hayflick and Moorhead (1961) discovered that embryonic mammalian cells could divide a finite number of times in culture. The phenomenon of division arrest after a period of normal cells proliferation is known as replicative limit or Hayflick limit. Though initial discovery has been made on fibroblast, replicative limit has been found in other cell types like keranocytes, endothelial cells, lymphocytes, adrenocortical cells, chondrocytes etc. Replicative limit is also observed in adult cells of many other animals including birds and mammals. Replicative limit changes from species to species and is correlated with the maximum life span of species. For example, cells from Galapagos tortoise divide about 110 times, whereas mouse cells divide roughly 15 times. There are exceptions of course, some cells can divide indefinitely without reaching replicative limit; they include embryonic germ cells and most cell lines from tumours. But it is no doubt, that replicative limit is an indication of cellular senescence (CS) and is a cause or contribute to the process of senescence in organisms.

After the knowledge of ES, the question arises about the features of CS, called biomarkers. One obvious biomarker is that the cells enter into progressive morphological changes; senescence cells are bigger and aging cell population has more diverse morphotypes than normal cells. One widely used marker of cell senescence is senescence-associated ß-galactosidase (SA B-gal) activity. B-galactosidase is a lysosomal enzyme and found to increase in cells with age. It is known that lysosomes increase in number and size in senescent cells and increase in SA ß-gal is due to increase lysosomal activity. In vitro studies suggest that autophagy (digestion of cell's own organelles) increases with age and may be associated with increased lysosomal mass and SA B-gal activity. Expression levels of some genes change during cellular aging. Interleukin 6 (IE6) is an inflammatory regulator gene that over-expressed in senescent cells. Senescent cells also secrete proinflammatory protein, which induce normal cells to enter into senescence. Increased metalloproteinase activity is displayed in senescent cells, which degrade extracellular matrix.

- **Telomere dysfunction induces senescence:**

It is well known that chromosome ends are protected by telomere-like plastic caps at the end of shoeless. Telomere capping is important to prevent chromosome fusion Telomeric DNA has short tandem repeats that contain a block of many G nucleotides, such as the repeat sequence in human telomere is GGGITA. Telomeric DNA may vary in length but generally contain several thousand base pairs. The telomeric DNA and associated proteins prevent the degradation of chromosome ends and also attachment to other chromosomes.As the mechanisms of DNA replication are better understood, it is clear that DNA polymerase cannot fully replicate the 3' end of linear (non-circular) DNA - it is known as end-replication problem. Therefore, without additional mechanisms eukaryotic chromosomal DNA (linear) becomes a little shorter after each round of DNA replication. If the DNA lost due to end-replication problem contains functional genes, those genes are either lost or damaged. But telomeric DNA is non-coding, so the loss at the chromosomal end has little consequence as long as enough telomeric DNA is present to form a functional telomere. When the loss is

significant after a number of cell division, and telomere approach a minimal length, the cell stops dividing. Telomere shortening is now regarded as one crucial proximate cause of cellular senescence. Telomere length probably acts as the molecular clock that counts the cumulative population doubling (CPD) a cell can endure.

Whether changes occur during telomere dysfunction, the mechanisms inducing replication arrest appear to involve DNA damage pathway. Precisely, telomere dysfunctions are recognized as DNA damages, and p53 and pkb genes are involved to stop cellular proliferation due to telomere shortening. An enzyme known as telomerase can replicate the end of telomere and thus corrects the normal telomere erosion. The activity of telomerase in cells is correlated with their proliferation capacity. Telomerase activity is high in normal germ line cells, low in somatic stem cells and undetectable in other somatic cells. The loss of telomerase activity limits the number of cell divisions in somatic cells. At the same time, persistence telomerase activity in germ line cells and somatic stem cells allow them the ability to divide.

Switching off the telomerase genes in our somatic cells not only limit their capability to divide, but also help us to avoid most tumours and cancers. 95% of tumour cell lines show telomerase activity, which indicate that most tumour cells have the ability to overcome the normal inhibition of telomerase gene expression. So, knowledge about telomerase activity seems to have a promising application in preventing tumours and cancers.

- **Aging is not simple wear and tear:**

All materials and objects are subject to natural decay. So wear-and-tear is among the oldest hypothesis for our aging. But second law of thermodynamics is not applicable to aging and genes can regulate the process of aging. Apparently, like many other age-related changes like skin aging, bone aging etc., erosion of teeth appears to a result of wear-and-tear. But senescence of our teeth is not only due to wearing but also lack replacement. Similarly, female reproductive senescence or menopause is failure of ovum production. Hence, above wear-and-tear, aging is a consequence of lack of replacement. All molecules and cells in organisms', wear-and-tear, but it is the lack of or insufficient replacement of those components, that leads to aging.

Teeth of shark suffer wear-and-tear, but they have the ability to replace throughout their life. In mammals, teeth of rabbits and many rodents grow continuously to compensate their increased wear-and-tear. So, wear-and-tear does contribute to the erosion of body parts, but it is genetic program of the species that determines the limitation of replacement or repair. Complex biological systems are dynamic and have the ability to repair and regenerate the damaged components. Even for those components, which cannot be replaced, their degeneration is seen as limitation of the genetic program, not as mechanical senescence. Interpretation of aging differ among authors; some believe aging is genetic, some see aging as build-up of damages counter-acted by genetically-regulated mechanisms. In either case, aging has a strong genetic component, not merely wear-and-tear.

- **Multiple versus unifying mechanism of aging**

It is generally argued that aging has multiple origins, a combination of age-related changes. But some authors defend that aging is genetically programmed. Some species surely have a precise and uniform genetic clock. For example, aging and death of salmons follow a very

specific and well-timed program analogous to development. But aging in humans is a gradual process, most age-related changes do not happen suddenly. So mechanism of aging may not be same in all species. Usually, a mouse ages 25-30 times faster than a human being. A species age at different pace is probably regulated by its genome. Proper nutrition and exercise may make us to live longer than life expectancy, but not as long as Galapagos tortoise, because there is genetically determined blueprint of aging. In simple word, aging is programmed in our genes. There is probably a molecular clock regulating the aging processes.

of such a clock. No doubt, organismal aging is more complex and under regulation of many, extrinsic and intrinsic factors. But aging is not merely random deterioration and must entail mechanisms under regulation of genome. So we can close this discussion with a conclusion that aging is a multifactorial process but regulated by a unified molecular clock.

- **Age-related changes**

Aging can be simply defined as progressive functional decline. There is gradual deterioration of physiological functions associated with aging, with decrease in fecundity. Aging is intrinsic, inevitable and irreversible process with increasing vulnerability and loss of viability. Aging is associated with a wide range of physiological changes that limit normal functions of body and make one organism more susceptible to diseases and death.

Some functions in humans like hearing and flexibility begin to deteriorate early, after sexual peak roughly at the age of 19. Aging is characterized by changes in appearance, a gradual reduction in height and weight due to loss of muscle and bone mass, and lower metabolic rate. Associated changes are decline in memory functions; decline in sexual activity including menopause in women; functional decline in audition, olfaction and vision; decline in functions of kidneys, lungs and immune system, and multiple endocrine secretion. Although immune system deteriorates with age known as immunosenescence, there is an increase in inflammation levels, parallel with higher levels of circulating pro-inflammatory cytokines. Presbyopia or farsightedness is also universally associated with aging.

- **Genetic regulation of aging**

As we grow older, traumas to the body and genome are increasingly accumulated. At molecular level, number of point mutations increase and efficiency of enzymes synthesized by our genes decrease. Mutations in protein synthetic systems result production of faulty proteins; faulty DNA polymerases are documented in senescence cells which in turn increase the overall rate of mutations in the organisms. DNA repair may be crucial in preventing senescence. The individual of a species, whose cells have more efficient DNA repair enzymes have longer life. Mutations in such DNA repair enzymes are known to cause certain premature aging (progeria) syndromes like Werner's syndrome and Hutchinson-Gilford progeria. Patients with Werner's syndrome appear normal in childhood exhibit features resembling accelerated aging like looseness of skin, grey hair, muscular dystrophy, baldness etc. at the age 20 years. They also show early onset of age-related diseases like cataract, osteoporosis, atherosclerosis and a tendency towards diabetes. Average age of death in Werner's syndrome is 47 years and they do not show all symptoms of normal human senescence. For example, patients with Werner's syndrome do not suffer from high blood pressure and Alzheimer disease.

1. Ultimate causes of Senescence:

Because aging increases the vulnerability of an organism leading to death, it is apparently in contradiction with natural selection theory. How evolution favours a process that gradually decreases reproductive capacity and increases mortality? Again, how the genes causing aging are evolved? There are several explanations about how senescence might have evolved, and those are outlined as ultimate causes of senescence.

A. Mutation Accumulation Hypothesis:

Mutation accumulation hypothesis is based on two observations, first, random mutation occurs in all cells including germ line cells and majority mutations reduce reproductive fitness. Second, in all populations, old individuals are less in number because of senescence and accidental death. In wild populations, most individuals do not live up to senescence; they die due to accident, fighting with rivals, predation or natural disasters. Thus overall presence of older individuals in population is minimum and their coritribution to reproduction is almost zero. Therefore, force of natural selection against random mutation having harmful effect in late life is minimal. Mutation accumulation hypothesis postulates a decreasing force of natural selection on the older individuals, who are smaller segment of population.

There are two predictions of mutation accumulation hypothesis. First, semelparous species, who breed only once in life should display immediate senescence after reproduction, because there is no question of natural selection afterwards. This pattem is well-known in salmon, mayflies etc. Second, organisms whose fertility increases with age should show delayed senescence and long-life span. They include fishes, reptiles, birds and mammals. Experiments confirm the predictions of mutation accumulation hypothesis, which ascribes senescence to harmful effects of late-acting mutations, against which no natural selection force operates.

B. Antagonistic Pleiotropy Hypothesis:

According to this hypothesis, there are several genes with two or more alleles (multiple alleles). For each of these genes, one allele promote rapid rate of reproduction in early life, but accelerated senescence in later life. Other allele(s) of same gene limits rate of reproduction, but promotes a longer life. Combination of these alleles is best for a population, hence favoured by natural selection. The alleles are showing antagonistic pleiotropy.

We may take the example of sex hormone production in humans. Increased production of androgens after puberty is better for production of sperm as well as development of strong bones and muscles. But them, who live a longer life, will develop hyperplasia/neoplasia of prostate gland. Thus any allele that would enhance androgen production or slow down androgen turnover will have an effect of antagonistic pleiotropy. Such an androgen production system will be favoured automatically.

Another example of antagonistic pleiotropy comes from the genes, which encode for signals and receptors to set appetite level. High appetite favours increased deposition of fat stores in body, beneficial for children and pregnant women. But in later life, the problem arises in the form of atherosclerosis, diabetes and overweight. In each human population, a trade-off evolved to fit the level of physical activity and food supply.

This model targets those genetic alleles, which are relatively adaptive and provide with increased fitness in early life for the price of an accelerated senescence. Such alleles are positively selected when benefits of trade-off are received by all individuals in a population. Surely, a large portion of the population dies due to various reasons before the onset of accelerated senescence. Data from wide range of experiments support that antagonistic pleiotropy is a driving force in the evolution of senescence.

C. Disposable Soma Hypothesis:

According to this hypothesis, there is a separation between germ line and soma (somatic cells) and every individual is a temporary carrier of its germ line. In a young soma (individual), cost of maintenance and repairing is low. Protein repairing mechanism is efficient, wound healing and recovery from physical illness is fast. When the soma gets older, protein repairing mechanisms are disturbed by mutations, injury and illness require more time and energy. Then it is better to dispose of the old soma and transfer the germ line to the somatic carrier (young) of next generation.

The findings in support of this hypothesis are:

- Organisms with distinct germ lines show senescence while organisms without germ line do not.
- Maintenance and repair mechanisms operate in germ line in highest level than the somatic cells. In fact, germ line cells divide slowly than somatic cells, thus repair mechanisms receive more time to correct the damage.
- Cost of maintenance and repair increase with age. Molecular damage to the repairing tools makes them workless efficiently and requires more energy to keep the pace of repair
- If the maintenance cost is high in old soma, it is better to pass the germ line to young soma of next generation.

Available data support the notion that one of the ultimate causes, of senescence is to cut the cost of maintaining germ line through a series of young soma carrier, which are ultimately disposable.

14.3 ANTI-AGING ACTIONS:

Aging is a natural process that involves the progressive decline of cellular and physiological functions, ultimately leading to reduced resilience, increased vulnerability to diseases, and visible signs of aging such as wrinkles and loss of vitality. Anti-aging actions aim to delay, prevent, or even reverse the effects of aging by targeting its underlying biological mechanisms. Below is a comprehensive explanation of the key mechanisms, lifestyle strategies, and emerging therapies involved in anti-aging interventions.

1. Mechanisms of Aging and Anti-Aging Actions

a. Reducing Oxidative Stress

A state where the production of reactive oxygen species (ROS) exceeds the body’s ability to neutralize them using antioxidants.

ROS damage DNA, proteins, and lipids, leading to cellular dysfunction and aging.

Anti-Aging Action

- **Antioxidants**: Molecules like vitamins C, E, glutathione, and polyphenols neutralize ROS.
- **Dietary Sources**: Fruits (e.g., berries), vegetables, green tea, and nuts are rich in antioxidants.

b. Promoting DNA Repair

DNA damage accumulates over time due to environmental factors (e.g., UV radiation, toxins) and replication errors.

Unrepaired DNA can lead to mutations and cellular aging.

Anti-Aging Action:

- Activating DNA repair enzymes (e.g., PARPs) can restore genomic stability.
- Lifestyle measures such as reducing UV exposure and avoiding smoking help minimize DNA damage.

c. Telomere Maintenance

Protective caps at the ends of chromosomes that shorten with each cell division.
When telomeres become too short, cells enter senescence or die.

Anti-Aging Action:

- **Telomerase Activation**: Telomerase is an enzyme that can rebuild telomeres.
- **Lifestyle Impact**: Regular exercise, stress reduction, and a healthy diet have been linked to longer telomeres.

d. Enhancing Mitochondrial Function

Mitochondria generate energy (ATP) for cellular functions. Aging impairs mitochondrial efficiency, increasing oxidative stress.

Anti-Aging Action:

- **Interventions**: Caloric restriction, regular exercise, and mitochondrial-targeted antioxidants (e.g., CoQ10) enhance mitochondrial function.

e. Regulating Cellular Senescence

A state where cells stop dividing but remain metabolically active, secreting inflammatory molecules.Senescent cells contribute to tissue dysfunction and chronic inflammation.

Anti-Aging Action:

- **Senolytics**: Compounds like quercetin and fisetin selectively eliminate senescent cells.

f. Stimulating Autophagy

A cellular process that removes damaged organelles and proteins, recycling them for new cell components.Declines with age, leading to accumulation of cellular waste.

Anti-Aging Action:

- Intermittent fasting, caloric restriction, and compounds like rapamycin stimulate autophagy.

g. Balancing Hormones

Hormone levels, including estrogen, testosterone, and growth hormone, decline with age. This contributes to decreased metabolism, energy, and vitality.

Anti-Aging Action:

- Hormone Replacement Therapy (HRT) can restore balance but should be used cautiously under medical supervision.

h. Controlling Inflammation (Inflammaging)

Chronic low-grade inflammation associated with aging.
Promotes age-related diseases such as arthritis, diabetes, and cardiovascular disorders.

Anti-Aging Action:

- Anti-inflammatory diets (e.g., Mediterranean diet), regular exercise, and medications like metformin reduce inflammation.

2. Lifestyle Strategies for Anti-Aging

a. Diet and Nutrition

- A nutrient-rich diet supports cellular health and reduces aging effects.
- **Antioxidants**: Found in fruits, vegetables, and green tea.
- **Omega-3 Fatty Acids**: Found in fish, flaxseeds, and walnuts, reduces inflammation
- **Polyphenols**: Found in dark chocolate, berries, and wine, supports longevity.
- **Caloric Restriction**:Reducing calorie intake without malnutrition has been shown to extend lifespan and enhance cellular repair mechanisms.

b. Physical Activity

- Regular exercise improves cardiovascular health, boosts mitochondrial function, and reduces inflammation.
- Activities like resistance training, yoga, and aerobic exercises promote anti-aging benefits.

c. Stress Management

- Chronic stress accelerates aging by increasing oxidative stress and inflammation.
- Techniques such as mindfulness, meditation, and yoga reduce stress levels and promote longevity.

d. Skin Care

- Protecting skin from UV damage prevents photoaging.
- **Topical Interventions**: Retinoids, peptides, and hyaluronic acid enhance skin regeneration and collagen production.

3. Emerging Therapies in Anti-Aging

a. Stem Cell Therapy

- Stem cells regenerate damaged tissues, potentially reversing aging effects.
- Promising in treating age-related diseases and restoring organ function.

b. Gene Editing

- Technologies like CRISPR can target genes associated with aging, offering potential for reversing cellular aging.

c. NAD+ Boosting Therapies

- NAD+ is a coenzyme essential for metabolism and DNA repair.
- Supplements like nicotinamide riboside (NR) and nicotinamide mononucleotide (NMN) restore NAD+ levels, improving energy and repair mechanisms.

d. Pharmacological Interventions

- **Rapamycin**: Inhibits mTOR signaling, enhancing autophagy and lifespan.
- **Metformin**: Improves insulin sensitivity and reduces inflammation, showing potential in extending lifespan.
- **Resveratrol**: Found in red wine, activates longevity-associated pathways.

14.4 DISORDERS AND DISEASES IN HUMANS RELATED TO AGING:

It is already mentioned that immune system deteriorates with age called immune senescence. A major hallmark of aging is an increase in age-related disorders like Alzheimer's disease, Parkinson's disease, atherosclerosis, arthritis, presbyopia, cataract, and of course, menopause in females. Incidence of a number of diseases also increase with age, like type 2 diabetes, heart diseases, renal diseases, respiratory diseases, cancer. But aging is not merely a collection of disorders and diseases; rather, it is a complex biological process with strong genetic control.

14.5 SUMMARY

Aging and senescence represent interconnected processes characterized by the progressive decline of cellular and physiological functions. These changes lead to increased vulnerability to diseases and decreased resilience over time. Anti-aging actions focus on countering the effects of aging through lifestyle modifications, dietary interventions, and emerging therapies like telomere maintenance, autophagy enhancement, and inflammation control. Disorders and diseases such as cardiovascular conditions, neurodegenerative diseases, and arthritis are prevalent in aging populations, underscoring the need for preventive healthcare strategies. By understanding and addressing the biological basis of aging and its associated conditions, individuals can achieve a longer, healthier, and more fulfilling life.

14.6 TECHINICAL TERMS

Aging, senescence, anti-aging, oxidative stress, telomeres, cellular repair, inflammation, age-related disorders, longevity, health span.

14.7 SELF-ASSESSMENT QUESTIONS:

1. What are the biological mechanisms underlying aging and senescence?
2. How do anti-aging actions counteract the effects of aging?
3. What are the major disorders and diseases associated with aging in humans?

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