

UNIT 10

PRINCIPLES OF DEVELOPMENT|

Structure

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10.1 INTRODUCTION

In the last two blocks of this course you learnt about the diversity in the anatomy of vertebrates. In the following units of this course we will learn about the progressive period of animal development during which the various organ systems develop in vertebrates. In order to achieve this you will study how various processes occur in the development of mature male and female gametes, called sperms and ova (or eggs) respectively in vertebrates. You will also become familiar with the events that occur prior to and during the period of fertilization (union of sperms and ova) which results in the formation of a single celled zygote that consists of genetic material from both parents. This zygote through various stages and cell processes develops into a multicellular organism that is capable of functioning, growing, reproducing and completing its life cycle. The process of development of the zygote from a single celled entity to a multicellular embryo is long. In humans as you may be aware the duration of development of the zygote into a fully developed foetus, ready to be born is approximately nine months.

In the present unit you will study about the principles of development which are common in all organisms both nonchordates and chordates. You will also come to know about the emergence of the field of embryology and how it progressed into the modern and vast discipline of Animal Developmental Biology, due to newer and better biological techniques and the advent of newer biological sciences like molecular biology, genetics etc.

This unit will also focus on the various nonchordate and chordate animal models that have been used in the past and are being used in the present study of embryology and animal developmental biology. These animal models have been used in order to understand how animals develop both at the observable, morphological, experimental level and at the level of underlying molecular biology and genetics. You will be able to understand the reasons for choosing a particular animal model in order to study a particular process of development

Developmental biology addresses some of the big questions that arise from the study of embryological processes such as: how does a single cell-the fertilized egg- give rise to a multicellular organism in which there are a multitude of different cells that give it form? How do the various cell types – muscle cells, blood cells, skin cells, neurons etc., - form and get differentiated from one another? How do these cells then get organized into functional organs in the animal body and what can influence these pathways of development? Along with this you will learn about some crucial experiments that gave the developmental biologists insight into these processes.

Objectives

After studying this unit you should be able to:

- ❖ differentiate between Embryology and Developmental Biology, stating the progressive stages in development of a multicellular organism;
- ❖ describe the features of the various nonchordate and chordate animals used as model organisms;
- ❖ explain the role of fate maps and patterns of development;
- ❖ state the concepts of cell specification, determination and differentiation;
- ❖ explain the concept of genomic equivalence, totipotency and pluripotency through classic experiments of Briggs and King;
- ❖ discuss the mechanism of differential gene expression.

10.2 DEVELOPMENT STAGES COMMON TO ALL ANIMALS

The blueprint of development of an organism from a fertilized egg to an adult is encoded in (i) genes present in the zygote and (ii) some special clues in the form of cytoplasmic determinants present in the cytoplasm of the zygote. During the course of development, the developing cells of the zygote

differentiate into many cell types that communicate and coordinate the various developmental activities and then subsequently get organized to form an integrated functional organism.

A development principle common to all higher organisms is that, the fertilized egg or zygote will develop progressively during several stages that last for different periods in order to form an integrated functional organism.

The stages of development that occur between fertilization and the birth of an organism (Fig.10.1) are collectively known as embryogenesis. It is during embryogenesis that the genotype (genes of the developing organism) of the organism determines the morphological appearance (phenotype) of the organism. Each animal whether it is a fruit fly or earthworm or frog, or bird or a mammal undergoes the same basic stages of development which include:

- i) **Fertilisation** – involves the fusion of mature male and female sex cells or gametes. Each gamete has only half the complements (set) of the chromosomes of the adult organism and union of the male and the female gamete to form the zygote restores the full genetic complement. The full genetic complement of the zygote instructs the zygote to develop in a similar manner to the parents and to produce an organism similar to the parents.
- ii) **Cleavage or rapid cell division** – is the stage in which the zygote is divided into numerous small cells known as blastomeres. During cleavage cells do not grow between each division and so with successive cleavage cells the **blastomeres become** smaller. These smaller blastomeres develop into early stages of development called **morula and blastula** stages.
- iii) **Gastrulation** – is the stage during which the cell division slows down and cells of the blastula undergo dramatic movement and rearrangement causing **cellular diversity** and formation of the three germ layers namely, ectoderm, mesoderm and endoderm. These three layer interact to form the organs of the body.
- iv) **Morphogenesis** – is the process of **cellular differentiation in the embryo**. Morphogenesis gives the embryo its shape.
- v) **Organogenesis** – is the process in which organ formation is completed so that the embryo becomes functional. A fully developed individual organism can then be distinctly seen as a member of a particular species at this stage. The embryo then, takes birth as a formed individual that lives on, till its death.

In many species a group of cells are set aside and do not participate in the formation of the embryo. These are known as germ cells and are used to produce the next generation. All other cells of the body are known as somatic cells. The germ cells migrate in the embryo to form the gonads and give rise to gametes in the adult organism. However, the process of development does not stop at birth, it is seen as metamorphosis and regeneration in some animal groups and finally as aging or senescence.

In Figure 10.1 we have shown the life cycle of frog as an example depicting all the above mentioned stages of development.

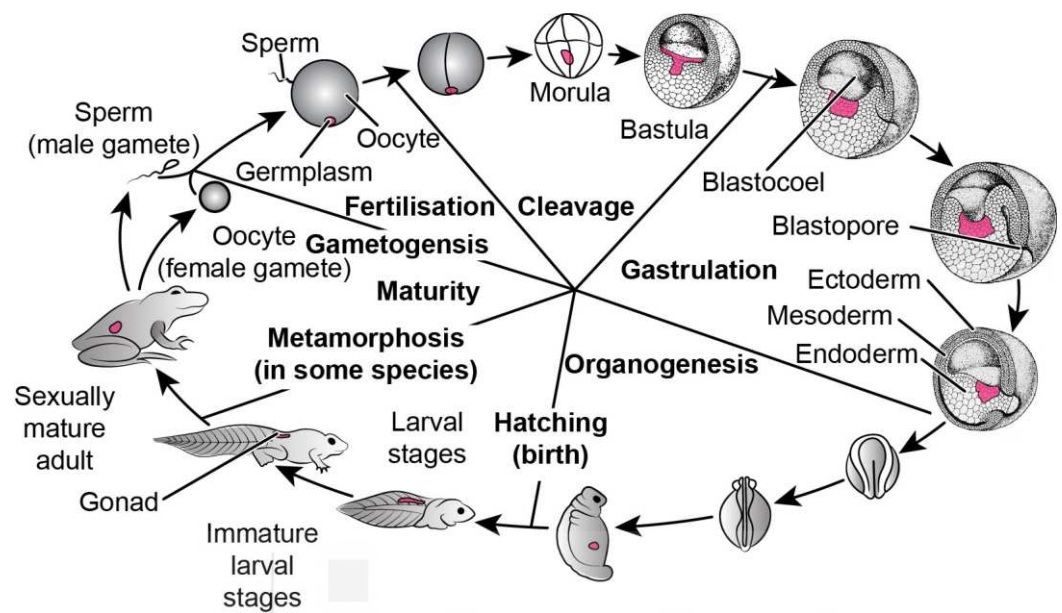


Fig 10.1: Life cycle of a frog, showing, the various stages from fertilisation and embryogenesis. In the frog the egg hatches as a larva (tadpole) and completes the rest of development through steps of metamorphosis; finally emerging as the adult frog capable of starting another generation.

10.3 HISTORICAL BACKGROUND OF DEVELOPMENTAL BIOLOGY

History of animal development is believed to have begun in the 4th century BC with simple observations of egg and embryos that could be seen with the naked eye. Aristotle was the first to record variations in life cycles of animals. He noted that some animals are born from eggs (**oviparity**) as seen in most invertebrates and some vertebrate groups like frogs and birds. He also noted that in some animals, embryos were born directly as young ones (**viviparity**) as seen in all mammals except for monotremes. He furthermore, observed that some animals were born from eggs that hatched within the body (**ovoviviparity**) as seen in some snakes and sharks. Aristotle also observed two types of division patterns in the fertilized egg as the embryos undergo cleavage namely: 1) **holoblastic** division where the entire embryo divides to form smaller cells as seen in frog and mammals, and 2) **meroblastic** division pattern of division where only a part of the fertilized egg divides and the rest provides nutrition for the embryo as seen in the chick.

Aristotle, on the basis of his studies on the development of chick embryo, for the first time advanced the **theory of epigenesis** (meaning: 'determination/ upon formation') for the development of organisms. According to the epigenesis theory, new structures develop progressively in the embryo during development. Thus, according to this theory there was no preformed tissue or organ in the embryo at the beginning of development.

However, the theory of epigenesis was not the only theory of animal development. The more popular theory that emerged later was that of preformation of embryo in development.

According to **the preformation theory**, all the parts in the embryo were preformed and they just got bigger with time. Preformationists believed that a preformed miniature, that is a fully formed infant, called the **homunculus** (Fig.10.2), existed within the germ cell of one of its parents, prior to fertilization and this would grow and enlarge into its full form during gestation until ready to be born. The Preformationists were thus either spermists or ovists. Spermists believed that the **homunculus** existed in the sperms while the ovists believed they were contained in the eggs.

Very little progress was made in the field of embryology and it was only in 1651 that William Harvey concluded that all animals arise out of eggs. He was the first to see the blastoderm of the chick which is the clear, yolk free germinal disc, also called the blastodisc and is in the form of a single layer of embryonic epithelial tissue and gives rise to the chick embryo. William Harvey saw the red dots of pooled blood that arose before the formation of the heart or vessels.

The two theories preformation and epigenesis were debated till the 17th and early 18th century. Kasper Freidrich Wolff, extended Aristotle's observations and supported epigenesis which disputed the idea of preformation. He observed the developing embryo of chick and demonstrated that the embryo takes its form from tissues that are not seen in the adult organism. The heart, intestine and blood cells all could be seen forming as new in each embryo and there were no preformed miniature organs. The final end to the preformation theory came only in the 1820s when the cell theory came in existence.

The cell theory in combination with newer staining techniques and improved microscopy contributed to the development of the discipline of descriptive embryology. Christian Pander (1794-1865) first recognized the existence of three germ layers (ectoderm, mesoderm, and endoderm) in the chick embryo. He also wrote about the interdependence of these layers in forming the embryo. A few years later Martin Rathke (1793-1860), another embryologist discovered layers of cells similar to what Pander had described in the crayfish and put forth the idea that three germ layers were not only found in vertebrates but also in invertebrates.

While studying embryos of vertebrates, embryologists discovered that there were many embryonic similarities between vertebrates belonging to different groups. Karl Ernst Von Baer, in 1828 proposed this to be an evidence of evolution and put forth his four laws of animal development; Von Baer described his laws of embryology in his book *Über Entwicklungsgeschichte der Thiere* [On the Development of Animals, published in 1828 and 1837]. In his work, von Baer reviewed existing information on the development of vertebrates. He used the information in this review to extrapolate his laws. These laws, translated by Thomas Henry Huxley in *Scientific Memoirs* are as follows:

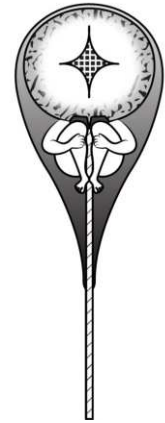


Fig.10.2: Pre formation theory depicted by homunculus.

- 1) The more general characters of a large group appear earlier in the embryo than the more special characters
- 2) From the most general forms the less general are developed, and so on, until finally the most special arise
- 3) Every embryo of an animal form, instead of passing through the other form, rather becomes separated from them
- 4) Fundamentally therefore the embryo of the higher form never resembles any other form but only its embryo

The explanations of the four laws given by Von Baer are as follows:

- i) The first law meant that in embryos of an animal group, the general characters develop first before the specialized characters develop. **His first law thus contradicted the preformationist theories.**
- ii) The explanation of his second law is that, embryos develop from a uniform and noncomplex structure into an increasingly complicated and diverse organism. For example, a defining and general feature of vertebrates is the vertebral column. This structure thus, appears early in the embryonic development of vertebrates. Other features those which are more specific to a group within the vertebrates, such as hair on mammals or scales on reptiles however, form later during development. **Von Baer thus, concluded from the first two laws that development occurs through epigenesis, and the complex form of an animal arises gradually from unformed material during development and not from preformed structures.**
- iii) Von Baer's third law referred to the fact that animals from different species start to develop in a similar manner but become more dissimilar from one another as ontogeny (the origination and development of an organism, usually from the time of fertilization of the egg to the organism's mature form—although the term can be used to refer to the study of the entirety of an organism's lifespan) proceeds. As an example, von Baer discussed the embryos of humans, fish, and chicks, all of which appear similar to each other in the early stages of their development. As they grow, however, they look increasingly different from one another. The embryo of one species never resembles the adult of another species. **Von Baer's third law thus, theorized that animal embryos diverge from one or a few shared embryonic forms.**
- iv) The fourth law meant that the stages of development in more complex animals never represent the adult stages of less complex animals.

In the second half of the 19th century another professor in Germany Ernst Haeckel extended the theory of recapitulation which was stated as the 'biogenetic law'. He proposed that **"Ontogeny repeats Phylogeny"**. This means that the stages of ontogeny (development) of the organism replay that organism's evolutionary history. The biogenetic law has been widely disputed, though Von Baer's laws are generally believed to have been responsible for the progress of developmental biology in the twentieth century.

The tools used by early embryologists were simple. Embryological studies involved the use of moulds made of wax or glass strips, direct observation of embryos of various animals, and their manipulation with the help of glass needles which were also used to move and pick embryos. Glass pipettes were also used to pull the embryo by narrow tubes for transfer. Embryologists of that time faced a difficult task in manipulating the embryos and ensuring their survival without them being infected, before they could be isolated, removed and transplanted. Later on with the development of better tools and microscopes with better resolution the “Era of Experimental Embryology began”.

By the 1880s, some embryologists began focusing on various experimental methods in order to understand embryogenesis. For this purpose the embryologists used physical manipulation of the embryo rather than just observing and describing them. The German evolutionary biologist, August Friedrich Leopold Weismann (1834-1914) put forward a model of development in which he assumed that the nucleus and cytoplasm of the zygote contained some special factors or determinants. He proposed that during cleavage these determinants would be unevenly distributed in the dividing cells and so could control future development. The fate of each cell according to him was, therefore, predetermined in the egg by these factors. He called this type of development mosaic. His theory was supported by the experiments conducted by the German Zoologist, Wilhelm Roux (1850-1924), in late 1880s on frogs. Wilhelm Roux in his experiments destroyed one of the blastomeres of the frog embryo after the first cleavage by using a hot needle. He found that the other blastomere formed half of the embryo (Fig.10.3). He thus, concluded that development was based on mosaic mechanism and the cells have their characteristic fate determined at each cleavage.

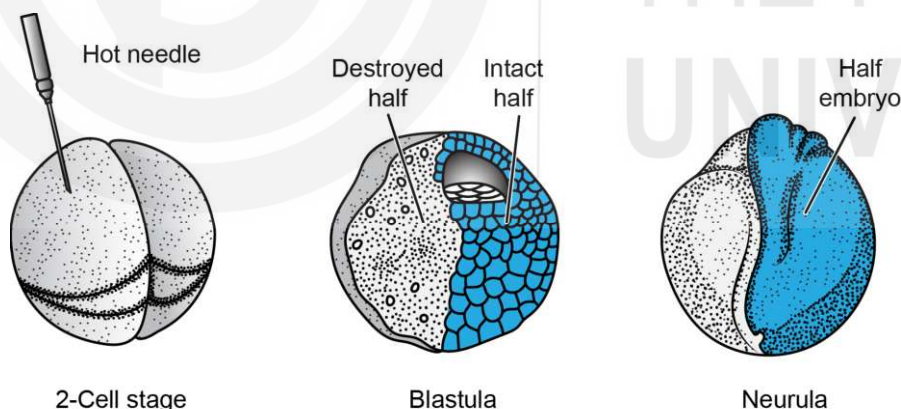


Fig.10.3: Experiment done by Roux that supported Weismann's theory of mosaic development.

Another German biologist Hans Adolf Eduard Driesch (1867-1941) found something quite opposite; he used sea urchin eggs and separated them after the first cleavage. He found that one blastomere died and the other formed a smaller but complete larva (Fig.10.4). He called this type of development **regulative**, referring to the ability of the embryo to restore normal development even though one cell had died. We will learn more about determinants and these two types of development namely mosaic and regulative when we discuss specification in cells.

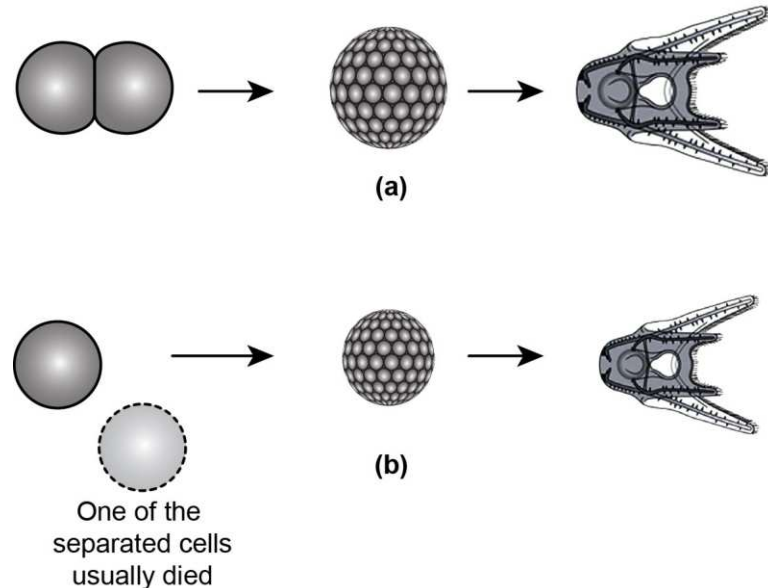


Fig.10.4: Driesch's Experiment with sea urchin blastula that demonstrated regulative development for the first time. a) normal development of sea urchin larva from two cell stage; b) separation of cells at two cell stage resulted in the death of one cell but the other survived to develop into a fully formed smaller larva.

The next major step in experimental development biology came in 1924 when German embryologist, Hans Spemann (1869-1941), and his graduate student Hilde Mangold proposed the concept of '**organizer**' and the principle of embryonic induction during development as seen in the development of amphibian embryos. Their work (Fig.10.5) showed that, in the earliest stages, the fate of the embryonic parts is not determined and if a piece of presumptive skin tissue is excised and transplanted into an area of presumptive nervous tissue, it will form nervous tissue, not skin. It provided the first unambiguous evidence that cell and tissue fates can be determined by signals received from other cells. This experiment is probably the best known in embryology.

The groundbreaking and technically demanding experiment was performed in newt embryos at the gastrulation stage, the period during which the three primary germ layers the ectoderm, mesoderm and endoderm become established. The experiment involved transplantation of a structure present on the dorsal side of the blastopore stage of the embryo, called the dorsal lip, to the ventral side of another embryo. By grafting tissue between differently pigmented *Triton* species, the fates of the graft and host tissues could be distinguished. This graft, nowadays referred to as the **Spemann organizer** (also the Spemann–Mangold organizer) had two effects. It induced the formation of neural tissues (neuralization), from the ectoderm that would have generally formed the skin. Furthermore it caused dorsalization of the ventral mesoderm, leading to the formation of somites.

This experiment therefore, demonstrated the existence of an organizer that instructs both neuralization and dorsalization, and showed that cells can adopt their developmental fate according to their position when instructed by other cells. The molecular nature of this signal remained elusive until 65 years later and is still not completely understood. Hans Spemann was awarded the Nobel

Prize, in 1935, for his discovery of the effect now known as **embryonic induction**

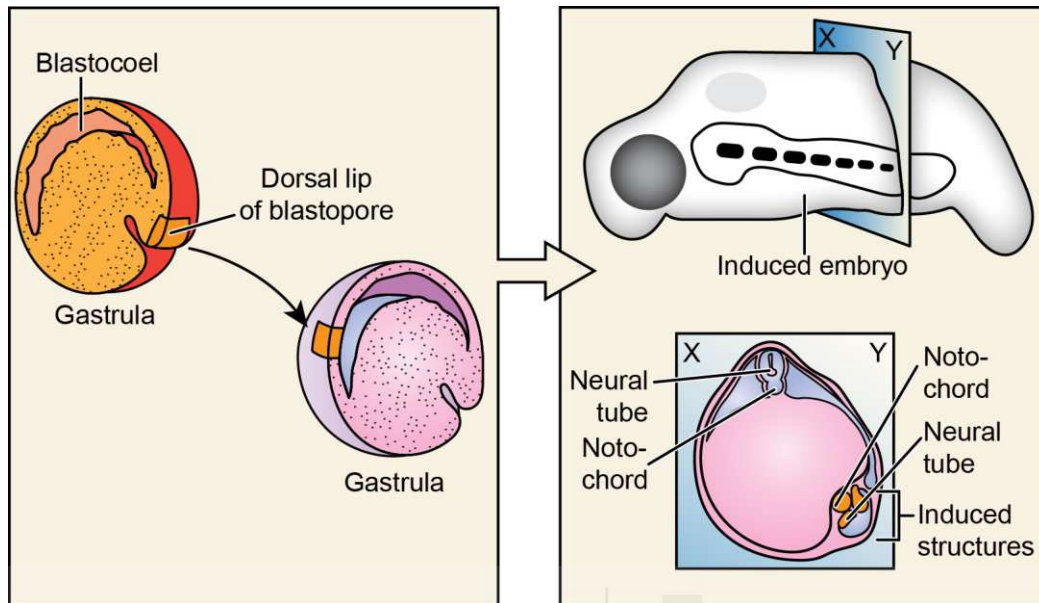


Fig.10.5: Experiment conducted by Spemann and Mangold that demonstrated induction of a new main body axis by the organizer region in early amphibian gastrula. a) dorsal lip of blastopore from unpigmented species grafted on the blastocoels roof of a pigmented species; b) a secondary embryo is induced.

Towards the middle of the 20th century, the scientific discipline of embryology began to emerge as the modern discipline of developmental biology. As is true for other disciplines in biology, knowledge of animal development advanced: (i) as new techniques for experimentation were invented and (ii) with the progress in knowledge of other branches of biology such as cell biology genetics, and especially molecular biology with the discovery of DNA, and the processes of transcription, translation and gene regulation.

With the increase in the knowledge of molecular biology, development biology emerged as a field of study which attempted to correlate genes with the morphological changes that were observed in embryos undergoing development. Which genes were causing which morphological changes and how these genes were controlled to express at certain stages of development became an exciting subject for research for many biologists. It thus became clear that genes which all embryonic cells contained, switched on and switched off as and when required in the developing embryo.

More recently, experiments on gene expression have demonstrated clearly that even in widely different animals, body plans share many basic features and mechanism of development. They are controlled by a common set of regulatory genes which direct cells to become capable of distinct functions. For example, the position for formation of eyes in a vertebrate like the mouse has a close counterpart with a nearly identical function in the fruit fly *Drosophila* which is an invertebrate.

Research in animal embryology and subsequently in animal developmental biology advanced with the use of a number of nonchordate and chordate

model organisms for studying the development in laboratories. These models were chosen because: 1) They had short life cycles, 2) were easily available and 3) and were easy to manipulate. Studies using different animal models clearly demonstrated that there are many similarities between the invertebrates and vertebrates during their development.

Animal Developmental Biology has now jumped far ahead of where it began in the early 20th century. The tools have changed, certain concepts continue to hold good and new concepts have gained ground. Embryology has travelled far ahead to get the new name Developmental Biology. If a difference between the two was to be sought then it can be conveniently said that “all embryology is developmental biology but some developmental biology is not embryology”

SAQ 1

- a) Give the difference between embryology and developmental Biology.
- b) What were the two theories of development? Which theory describes our current view of development?

10.4 ANIMAL MODELS IN DEVELOPMENTAL STUDIES

As experimental embryology advanced in the 20th century, scientists observed that there are remarkable similarities in the developmental mechanisms of all animals. Not only genes and proteins but entire signaling pathways and their response in developing embryos appear highly conserved throughout evolution. More recently, experiments on gene expression during embryogenesis have demonstrated that even in widely different body plans of various kinds of animals many basic mechanisms of development are controlled by a common set of regulatory genes. They direct the cells to become capable for performing distinct functions in the developing embryos. For example, gene specifying position for formation of eyes in the mouse (vertebrate) has a close counterpart with a nearly identical function in the fruit fly *Drosophila* (invertebrate).

Scientists, however, found that only a limited number of animal species could be utilized for investigating the developmental processes. These animal species were chosen as model organisms for experimentation earlier in the field of embryology and animal developmental biology. We can define animal model organisms as non-human species that have been widely studied, mostly because:

- i) of the ease with which the different experimental techniques can be conducted,
- ii) of the ease with which they can be maintained,
- iii) of the ease of breeding them in the laboratory
- iv) of having a short life cycle,

- v) of having rapid development,
- vi) of the fact that their robust embryos are easy to manipulate
- vii) these models may be representative, for answering common and specific questions of development across animal groups. For example, they have been used to obtain answers about whether the mechanism of eye formation in *Drosophila* maybe considered representative of most vertebrates.

Though, the ability to manipulate the model organism is a critical criterion for its selection, it also has to be combined with other considerations such as how easy it is to initiate an experimental set up with an animal model, in terms of cost and availability of the animal models and of availability and suitability of the infrastructure. For example, chick embryo is an established model organism that is easy to manipulate but the inability to apply genetical methods to the chick embryo has limited its use in recent times.

The development of newer physical tools and techniques has ensured that organisms like sea urchin and amphibians (in the form of different species of frogs and salamanders) and still later the fruit fly, *Drosophila* and relatively recently the round worm *Caenorhabditis elegans* and the zebra fish *Danio* have become popular experimental models for the study of the various processes and mechanisms of development. Recent developmental studies have moved from the overall, gross study of the embryonic development to studies of the cellular and molecular aspects of animal development. In other words animal development studies have moved from macro level to micro level. Let us examine the characteristics of some of the model organisms that are particularly useful for experimentation in developmental biology.

10.4.1 Classical Models: Sea Urchin and Frog

The earliest animals used for study of development were sea urchins, and frogs because of their large eggs which could be handled and observed under the microscope. Their suitability depended on i) easy accessibility; ii) ease of manipulation and iii) easy visualization with the available tools and techniques

Sea urchin

Historically sea urchins have been a key model system for finding out answers in developmental biology. Sea urchin is an echinoderm and is found in the shallow tide pools in oceans of the world. The features that make it suitable for study are:

1. Gametes can be obtained easily and artificial spawning and fertilization and rearing is possible.
2. Lots of large eggs are produced which are easily visible (Fig.10.6).
3. Early development is highly synchronous. Large numbers of synchronously developing embryos can be obtained quickly and easily.
4. Low maintenance costs due to high fecundity. The gravid adults produce enormous numbers of gametes that are easy to collect in the laboratory.

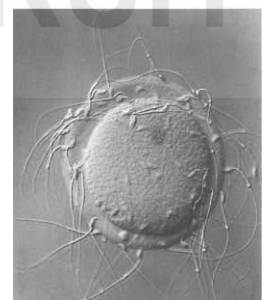


Fig. 10.6: Sea urchin egg seen under the microscope with sperms surrounding it, for fertilization.

5. A single female can provide many millions of oocytes and a single male many billions of sperm. Both types of gametes are fully competent to carry out fertilization without additional maturation.
6. Fertilization and embryonic development occur externally, both in nature and in the laboratory. Development is initiated synchronously by simply mixing sperm and eggs.
7. Embryonic development is rapid. In many warm-water species, only 1–2 days are required for the fertilized egg to develop into the pluteus larva. One practical consequence of this rapid development is that the time between experimental manipulations and analysis of their developmental effects is very short.
8. The embryo has a simple structure. At the late blastula stage, the embryo is a simple, hollow ball composed of about 800 cells surrounding a central cavity (the blastocoel). The number of cells approximately double by the end of gastrulation but do not exceed 2000–3000 cells during pre-feeding development. The embryo and pluteus larva have relatively simple morphologies and only 15–20 distinct cell types arise during embryogenesis.
9. The embryo is highly transparent. The embryos of many species of sea urchins are almost as clear as glass. This transparency, coupled with the anatomical simplicity of the embryo, helps in light microscopic observations of development. Thus biologists have constructed a very detailed picture of sea urchin embryogenesis at the cellular level.
10. After microscopic studies to see fertilization and early stages of embryo, the bio chemical and molecular events have also been studied. Small molecules can be used to perturb development.
11. Diverse tools are available for analyzing and manipulating gene expression. Sea urchins are well suited to all modern approaches for analyzing gene expression. Recent sequencing of its genome has made it a model organism for study of developmental genetics. Though it is an echinoderm (invertebrate) it encodes many vertebrate immune system related genes.
12. Maternal molecules are specially restricted and are involved in determining cell fate, apoptosis, autophagy and recently are seen to have a role in cellular and molecular mechanisms involved in human health and disease.
13. Over a century, sea urchin has been a prototype for investigation of development of body plan, cell adhesion, cell signaling and age related pathways and mechanisms of cell death.

Frog

As animal model organisms amphibians offer several advantages because they have a well understood morphology and physiology. The taxonomical position of amphibians is well suited for the comparative developmental studies of reptiles and birds as well as mammals. Developmental studies of

frogs and salamanders have made possible important insights into molecular mechanisms that control embryonic cell division, differentiation and animal development. Salamanders serve as important vertebrate models for the study of regeneration and tissue repair. However, the most important models for studies of early vertebrate development have been the African clawed frog *Xenopus levis* and *Rana pipens* due to the following practical advantages:

- 1) Eggs of *Xenopus* and *Rana* are large sized and the eggs develop outside the body of the mother and are laid in water. This makes it easy for researchers to collect them from water and carry them to the laboratory so that microscopic studies become possible.
- 2) They can be easily bred in captivity and be induced to spawn artificially by chorionic gonadotropin. Furthermore, the large number of eggs produced at a time, facilitate biochemical analysis.
- 3) Their embryos can be observed easily. *Xenopus* has a short time of development (4 days) before it reaches the free swimming larval stages.

10.4.2 A Later Model: *Drosophila*

Since the middle of the 20th century, the fruit fly *Drosophila melanogaster* (Fig.10.7) has been a favourite model for research in genetics. After the extensive use of *Drosophila* for genetic analysis, developmental biologists began to use it as an experimental model for development which led to advances in understanding the process and mechanism of animal development. *Drosophila* has today at the level of genes become a crucial model organism in developmental biology especially in order to understand gene activation, gene regulation, differential gene expression, genomic equivalence, genetic control in the process of animal development. The advantages of *Drosophila* as a key model for laboratory studies for genetic and developmental biology are as follows:

- 1) *Drosophila* can be easily cultured, maintained and bred in the laboratory.
- 2) It has a short reproductive cycle (Fig.10.7) of approximately of two weeks and produces many offsprings. The embryo develops outside the mother's body and so can be easily observed under the microscope
- 3) *Drosophila* genome contains only about 15,500 genes.
- 4) Genetic analysis of the fruit fly has unraveled many genes that control development and differentiation. There is a close relationship between *Drosophila* and vertebrate genes especially human genes that operate during development. For example, it has been found that the homeotic genes that are master regulators as they direct the development of particular body segments are highly conserved in animals and have corresponding genes in fruit flies, mice and humans.
- 5) *Drosophila* as a model organism has been very important in research work for information on genetic and molecular basis of animal development as **mutants** are readily available. Mutants can also be

induced to be formed. The mutants have been helpful in studying the powerful role of the genes in directing normal and abnormal animal development.

- 6) Even more detailed genetic analysis of the development processes has been possible due to current techniques of molecular biology.

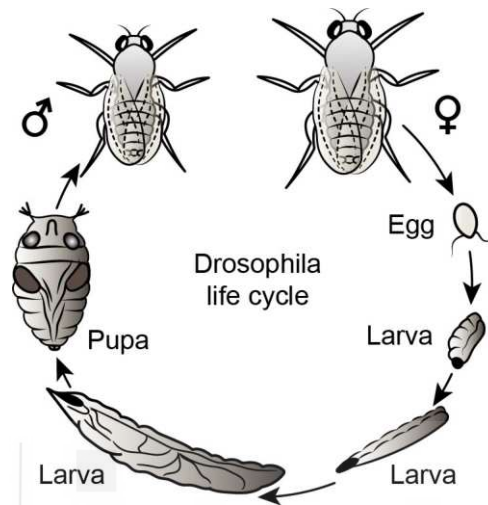


Fig.10.7: *Drosophila* life cycle.

10.4.3 Newer Models: *C elegans* and *Danio rerio*

Two other organisms now being used by developmental biologists are the round worm *Caenorhabditis elegans* and the zebra fish *Danio rerio*.

***Caenorhabditis elegans*:**

- 1) *Caenorhabditis elegans* is a free living nematode that lives in soil but is easily grown in petri-dishes (10.8a).
- 2) It is only a millimeter long transparent worm with limited cell types (only 959 somatic cells in the body).
- 3) The adult and embryo are transparent. It requires only 3.5 days to grow from zygote to mature adult and has a large brood size.
- 4) Embryonic development takes place within 12 hours.
- 5) Its genomes have been completely sequenced.
- 6) *C elegans* was the first metazoan genome to be sequenced.
- 7) *C elegans* has a simple system, and so by following the division of its cells, scientists have worked out the complete cell lineage which is invariant. Invariant means that all individuals of the species have the same anatomy.

Zebrafish (*Danio rerio*)

- 1) The zebrafish is small and robust and cheaper to maintain than mice.
- 2) *Danio rerio*, has been chosen for its unique combination of large, easily

accessible eggs and embryos that are transparent which allows researchers to easily examine the development of internal structures. Every blood vessel in a living zebrafish embryo can be seen using just a low-power microscope. Thus, changes can actually be observed in the living organism (Fig.10.8 b).

- 3) Zebrafish produce hundreds of offspring at weekly intervals, providing scientists with an ample supply of embryos to study. The eggs are fertilised and develop outside the mother's body and also serve as an ideal model organism for studying early development
- 4) Embryos of zebra fish grow at an extremely fast rate, developing as much in a day as a human embryo develops in one month. Although generation time in zebra fish is 2-4 months, the early stages of development take place quickly. By 24 hours after fertilization most tissues and early versions of the organs are formed. After 2 days the fish hatches out of the egg.
- 5) Zebrafish share 70 per cent of genes with humans. The zebrafish genome has been fully sequenced and the model is also useful for molecular studies

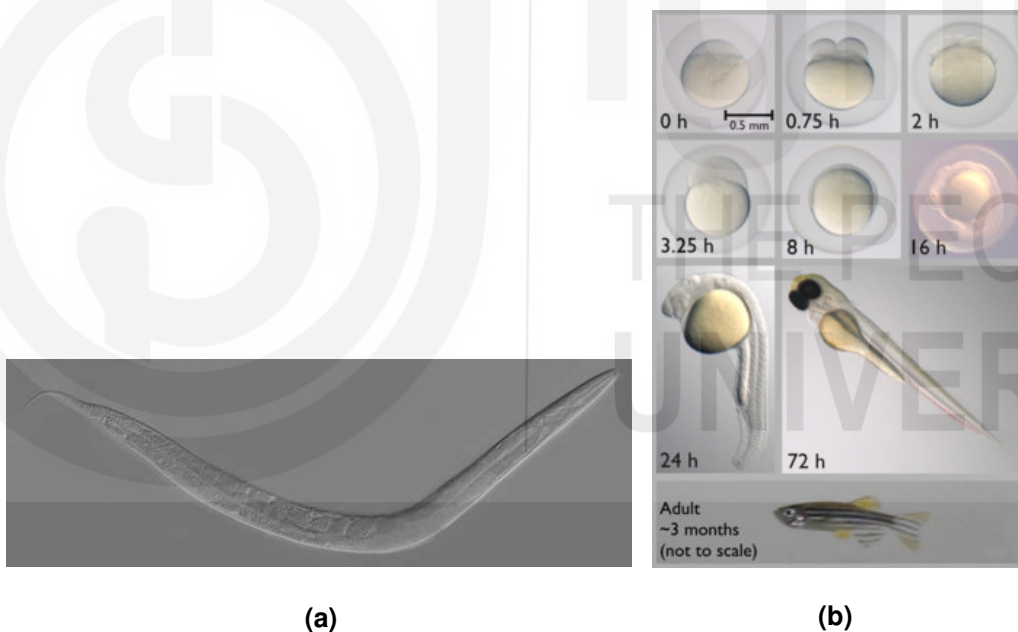


Fig.10.8: a) The free living nematode *C. elegans*; b) early embryonic development of zebrafish eggs and embryos.

SAQ 2

- a) List three classical animal model used by investigators to study development in animals. What are the common features in them that make them good animal models for studying animal development.
- b) Which model organism has provided information of genetic control of development through the study of its mutants?

10.5 EMERGENCE OF PATTERNS

In the beginning of this unit we had written that one of the most fascinating questions in animal development is the generation of a complex organism from a single fertilised cell the zygote. As embryonic development proceeds it involves the emergence of a pattern which shows the overall position of cells in the embryo so that the body's shape and form begins to emerge. This is followed by cell differentiation and growth.

Development is a gradual process by which a complex multicellular organism arises from a single cell (the zygote). It involves 5 major overlapping processes:

- 1) growth = increase in size
- 2) cell division= increase in number
- 3) differentiation = diversification of cell types
- 4) pattern formation = organization
- 5) morphogenesis = generation of shapes and structures

10.5.1 Pattern Formation

As the zygote divides it undergoes a process by which the initially equivalent (similar) cells acquire different identities which depend on their relative positions in the embryo at different times during development. As a result a well ordered structure of the developing embryo emerges. This process is known as pattern formation. **In other words pattern formation in developmental biology, is the mechanism by which initially equivalent cells located at a particular position in a developing tissue of the embryo assume complex forms and functions. Thus, what a cell will become later in the embryo depends on its position within the developing embryo.** The final fate of development of a single cell takes place in several progressive steps. For example, during development the pattern formation of the face enables the cells to know where the nose, ears, eyes have to be formed and what muscles are to be placed in the space to support the structure of the face. In animal development, pattern formation is established early in embryogenesis. This patterning during animal development in different organisms is achieved at different times by cellular and molecular mechanisms.

- The first step in pattern formation is the laying down of the body axes: i) that run from head (anterior) to tail (posterior) and ii) from back (dorsal) to the underside (ventral). Most of the animals that we are taking as examples to discuss development in this course are externally observable as bilaterally symmetrical. This means, that the left and right side of their body are mirror images of each other. The body of such types of animals have an antero-posterior axis and a dorso- ventral axis. Both these axes are always at right angles to each other and make a coordinate on which the form of the animal is specified (see Fig.10.9).

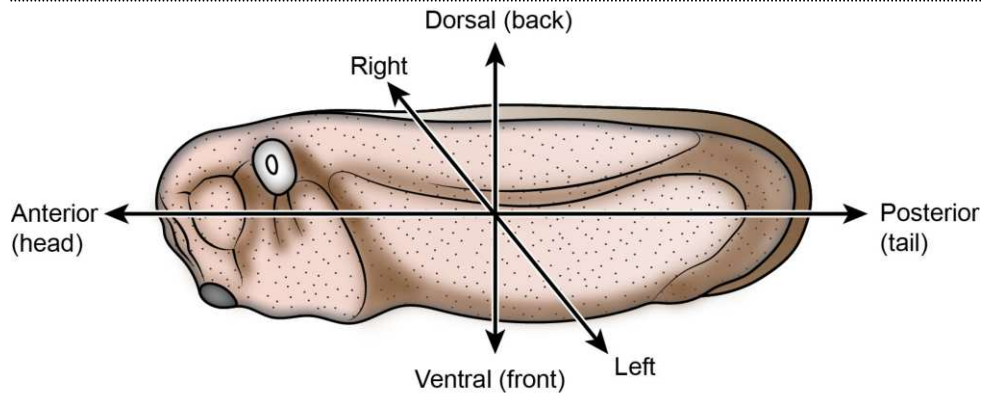


Fig.10.9: The main axes in a developing embryo of *Xenopus laevis*.

Vertebrate animal bodies have externally visible symmetry but the internal organs are not symmetrical for instance the heart is on the left side and liver on the right. These axes in the embryo are established very early in development. Several genes are involved in the establishment of axes in the embryo. Before the externally visible body axes get specified in the embryo the egg also always has a distinct polarity or orientation. At the same time that the axes are being formed in the embryo, the cells in the embryo of triploblastic animals get organized into the three different germ layers namely ectoderm (external layer), mesoderm (middle layer) and endoderm (internal layer). In the case of diploblastic animals the cells in the embryo get organized into the two different germ layers namely ectoderm (external) and endoderm (internal). The positions of cells in the germinal layers of both diploblastic and triploblastic embryos of the animals which are laid down during gastrula stage show their prospective fate by the end of development. During further emergence of pattern, the cells of these layers acquire different identities (become different) so that they form the different tissues and organs of the body like skin, muscle, blood cells, neural cells etc. Figure 10.10 gives a diagram of the fate of the three germ layers in animals.

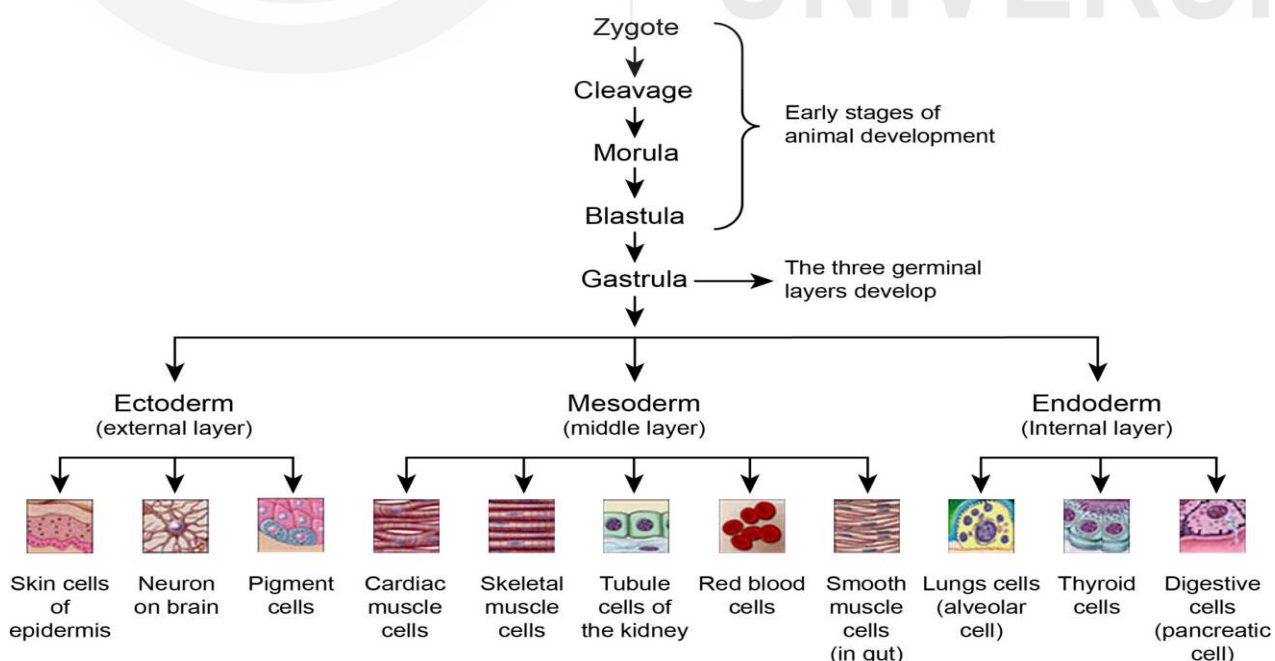


Fig 10.10: Generalised cell fates of the three germ layers of triploblastic animals.

Pattern formation can take place by two mechanisms: (i) **cell to cell interactions** also known as **inductive mechanisms** in which one type of cells induces or instructs other cells to change their behaviour and develop in a different way or to become different types of cell; (ii) **morphogenetic mechanisms** that act on previously established patterns in order to form the three dimensional tissues and organs. This process involves changes in the location of the cells, without changes in their behaviour. For instance, during gastrulation in frogs, the endoderm and mesoderm move inside the embryo from the surface. This results in the formation of the gut which is a tube within a tube structure. The embryo thus, during development undergoes remarkable changes in appearance or morphology. These changes occur due to extensive cell migration which will also cause changes in the shape of the embryo. In this process some cells undergo programmed cell death as well. We will be studying in greater detail about the mechanisms involved in pattern formation in unit 11 and unit 13.

10.5.2 Fate Maps

Since the early 1900s, as you will recall, experimental embryologists became interested to find out the fate of each cell in the developing embryo and what it would give rise to in the adult animal. Initially it was not easy to trace the cell lineage (which structures will be formed from the cell and its descendants at a later stage of normal development) or cell fate of individual embryonic cells. Thus, embryologists used different techniques to label groups of cells in the early stages of embryonic development and followed their progressive development until the stage of a fully formed embryo. By using various labelling techniques of groups of cells from early stages to end of the embryo formation, embryologists were able to construct **fate maps** or diagrams of areas of the embryo for depicting the fate of the various cells during normal development. Most embryonic cells have predictable fates. Cells in different embryos of same species will develop same structures or will have the same fate. In fact, most early vertebrate embryos show remarkable similarities in their fate maps as can be seen in Figure 10.11 Fate maps can be constructed using different methods which are becoming more sophisticated with increasing technological advances. Each has its advantages and disadvantages.

TECHNIQUES USED FOR PREPARATION OF FATE MAPS

- 1) **Observing living embryos:** some species have embryos that have relatively small number of cells that are transparent and the development of blastomeres can be observed directly under the microscope or the cells may be naturally coloured (pigmented) so that the development can be traced following the coloured cells. E. G. Conklin in early 1900s was able to follow the development of early cells in a tunicate *Styela partita*. From his studies it was found that only one pair of blastomeres at the 8 cell stage (the posterior vegetal blastomeres) were capable of forming tail muscles. These had yellow pigment hence it was easy to follow their development. Removal of one such blastomere (B4-1) resulted in an embryo with no tail confirming Conklin's fate map of the tunicate *Styela partita*.

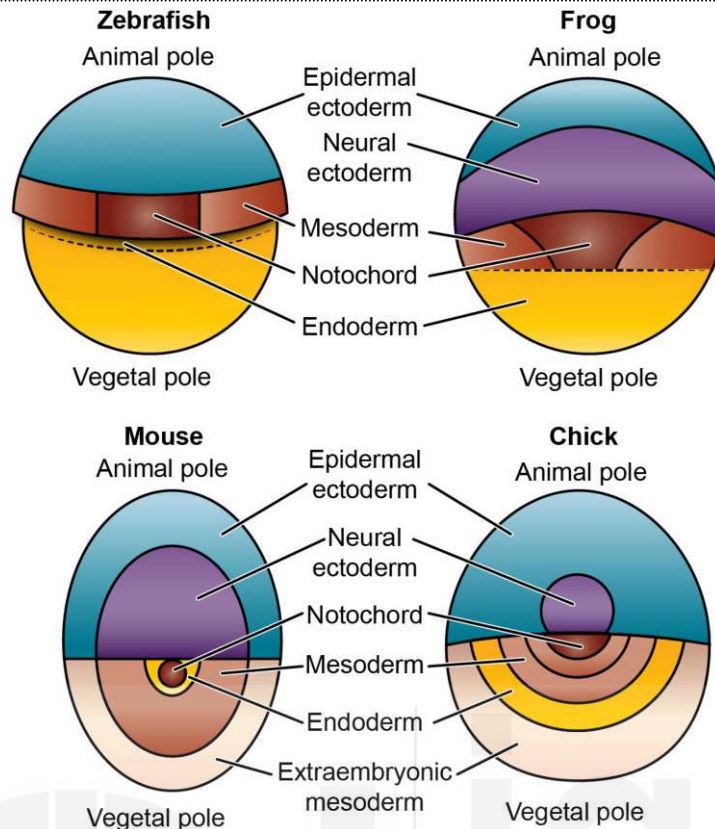


Fig.10.11: Fate maps of some vertebrate embryos in dorsal view (that is looking down on the embryo to see its back view). You can observe that there are general similarities in all. The cells that will form notochord are in the central position. Ectoderm precursors are anterior, mesoderm lateral and endoderm precursors lie posterior to the cells that make the notochord. The dashed lines show the path where the cells will move inside the embryo.

- 2) **Use of Dye marking:** vital dyes (dyes that do not damage the embryo but just stain the cells) are often used to trace cell lineages. Walter Vogt in 1929 used such a dye to follow the development of stained cells in newt embryos. However vital dyes get diluted over the period of cell division and become difficult to trace.
- 3) **Fluorescent dyes and radioactive marking:** are used to overcome the problem seen with vital dyes. Fluorescent dyes can be used that are so intense that they can be traced for a large number of cell divisions. Similarly radioactive labelling of dividing cells can also help to trace the path of the dividing embryonic cells.
- 3) **Genetic labelling:** This is a method of permanently labelling cells in order to follow their path in development. The best method is the formation of chimeras that are embryos created by fusion of cells from two different, but closely related species. The best example seen is of a chick quail chimera made by grafting quail embryonic cells in a chick embryo while it is still in the egg (Fig.10.12). When the chick hatches it also has quail cells in the tissue which develops from the grafted quail embryonic cells. Another method is by using genetic markers. In this case a marker gene is inserted in the nucleus. Each time the cell divides the tagged gene will also duplicate and in this way these cells that express the genetically modified gene can be traced to the cells of the embryo where the gene was inserted.

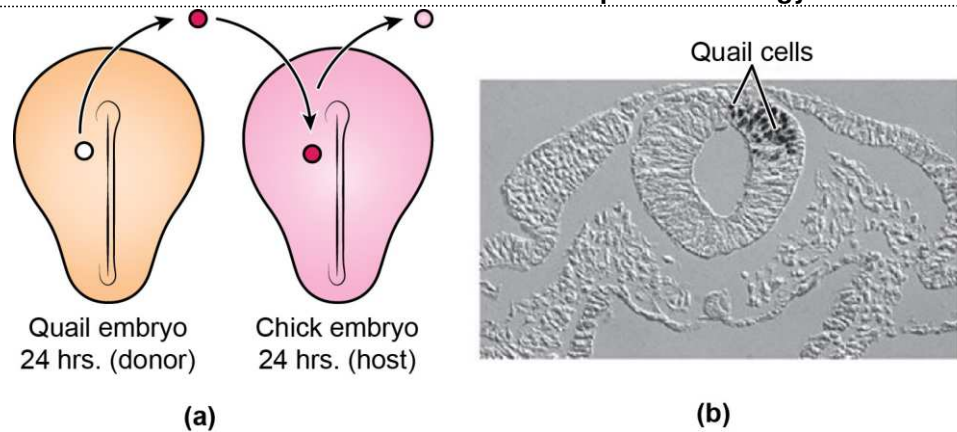


Fig.10.12: Genetic labelling of cells of the living embryos for determining their fate and for constructing the fate map. Cells from the quail embryo are transplanted on chick embryo. As the chick embryo develops the quail cells can be traced in it.

Fluorescent antibody: have been used to study development in the transparent, free living nematode (roundworm) *Caenorhabditis elegans*. *C. elegans* has been used as a model organism to follow cell lineage from zygote to the adult form under the light microscope by using a fluorescent antibody which is specific for the nematode. The intestine develops from the cells that arise exclusively from one of the four cells that are formed from the first cleavage furrow in the zygote. Other methods that have been used for constructing fate maps, was by destroying particular cells or cell groups by means of a laser beam. Subsequently, the complete fate map and cell lineage of *C. elegans* has been worked out.

The fate map of *Drosophila melanogaster* has also been studied extensively and has revealed the molecular and genetic mechanisms involved in body segmentation and axis formation (Fig.10.13)

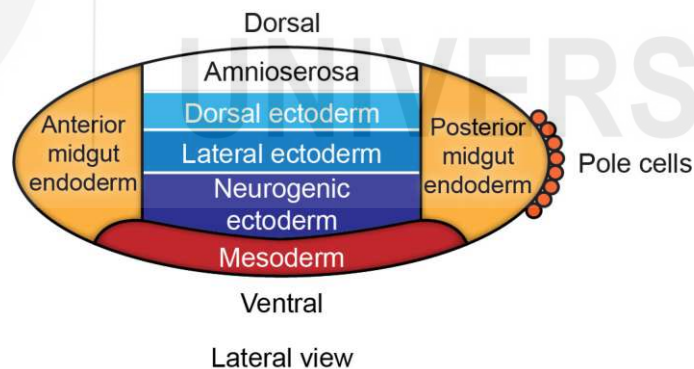


Fig.10.13: Fate map of *Drosophila melanogaster* showing the regions from where the future body tissues and organs will arise. (modified from <https://bastiani.biology.utah.edu/courses/3230/db%20lecture/lectures/b11flydv.html>)

Thus, we find that fate maps are essential for understanding the development and formation of structures in the embryo. As newer techniques develop, the accuracy of fate map improved and embryologists have been able to follow individual cells to see what they eventually give rise to.

SAQ 3

What is a fate map? Write a few sentences on the usefulness of fate mapping.

10.6 CELL DETERMINATION AND DIFFERENTIATION

In the earlier section we have seen that fate maps show the areas of embryo that will produce different tissues and organs in the adult and cell fate describes what a cell will become in the future during the normal course of development. We have also learnt that the fate of a particular cell can be traced by labelling it and subsequently observing what structure it will become a part of. The **developmental potential** or the **potency** of a cell describes the range of different cell types that it can give rise to. The zygote and very early blastomeres formed in it by cleavage are **totipotent**. This means that each of the very early blastomere of the zygote has the potential to develop into a complete organism as was demonstrated by experiments conducted by Dreisch (Refer again to Fig 10.4). Totipotency is not seen commonly after the first few divisions in the blastula. As development proceeds, the developmental potential of individual cells decreases until their fate is "**determined**". This means that each cell becomes committed to form a part of a specific structure and so its fate is determined for following a particular path of development. **Differentiation** on the other hand, is the process during which the cells stop dividing and acquire the unique structure and functional properties of a particular cell type. This is thus, the last stage in the process when an undifferentiated cell undergoes a series of events to become a specialized cell type like a muscle cell or nerve cell or skin cell etc.

10.6.1 Cell Determination

You are aware that blood cells differ vastly in morphology and function from each other and from other specialised cells like the muscle cells, though all of them arise from the same germ layer namely, mesoderm (refer to Fig 10.10 again). However, before these differences arise there is a period when these cells in the mesoderm do not look different from their neighbouring cells despite the fact that their fate has been already determined. This means that they are **committed** to follow a specific path of development to form particular types of cells

The process of commitment takes place in two steps:

- a) **Specification:** The fate of the cell is known to have been specified when it is capable of differentiating autonomously even if it is placed in an environment that is neutral like a culture medium in a petri dish (Fig.10.14 a). However, at this stage the commitment of the cell is flexible and it can be influenced by its environment to become another type of cell rather than what it **was specified** or fated to be (see Fig.10.14 b).
- b) **Determination:** this step is after **specification** of the cell to form a particular type of cell. In the stage of determination of the cell the fate of the cell becomes inflexibly or irreversibly specified or determined to form a particular type of cell. As a result, the cell will still autonomously differentiate into its original specified fate and its differentiation into a

particular type of cell will be independent of its environment or its position in the embryo it (see Fig.10.14 c).

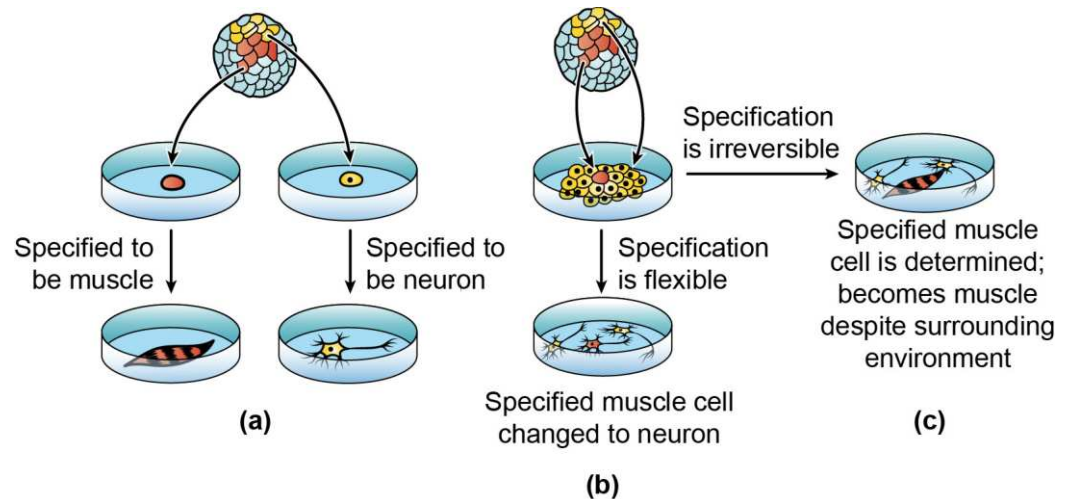


Fig.10.14: Two differently positioned cells that are fated to form muscle and neuron cells have been taken and isolated from the blastula and grown in a petri dish. a) blastomeres that are specified for muscle cells differentiate into muscle cells and those specified for neuronal cells differentiate into neurons; b) If the blastomere specified but not determined for forming muscle cells is placed in a cluster of neuronal tissue it differentiates into neural cells; c) if the blastomere is already in the determined phase then it will differentiate into muscle cells even if it is placed with neuronal tissue.

Specification

Embryos of different animal species show different strategies of specification

- A. **Autonomous specification:** in this type of specification the blastomeres of the early embryo have a set of critical determination factors called **cytoplasmic determinants** that they get from the egg cytoplasm. The cytoplasm is not homogenous but has different regions containing cytoplasmic determinants that are often transcription factors or mRNA encoding factors that will influence the cell's development. There is asymmetric segregation of cellular determinants during cell division. Thus, some cells will have more or less of the transcription factors and some may have none. This will result in daughter cells with different cell fates. These cells already "know" what they are fated to become. Some of the best examples of autonomous specification are seen in the development of molluscs, annelids and tunicate embryos (Fig.10.15). In the tunicate embryo experimental results showed that when the blastomeres were separated at the 8 cell stage each blastomere gave rise to the respective cell type it was fated to make. Moreover, if some of the cytoplasmic determinants like those for muscle development were removed and placed in another cell that cell began to differentiate into muscle cells. Such embryos that have their fates specified early in cleavage are known as mosaic embryos and their development is known as **mosaic development**.

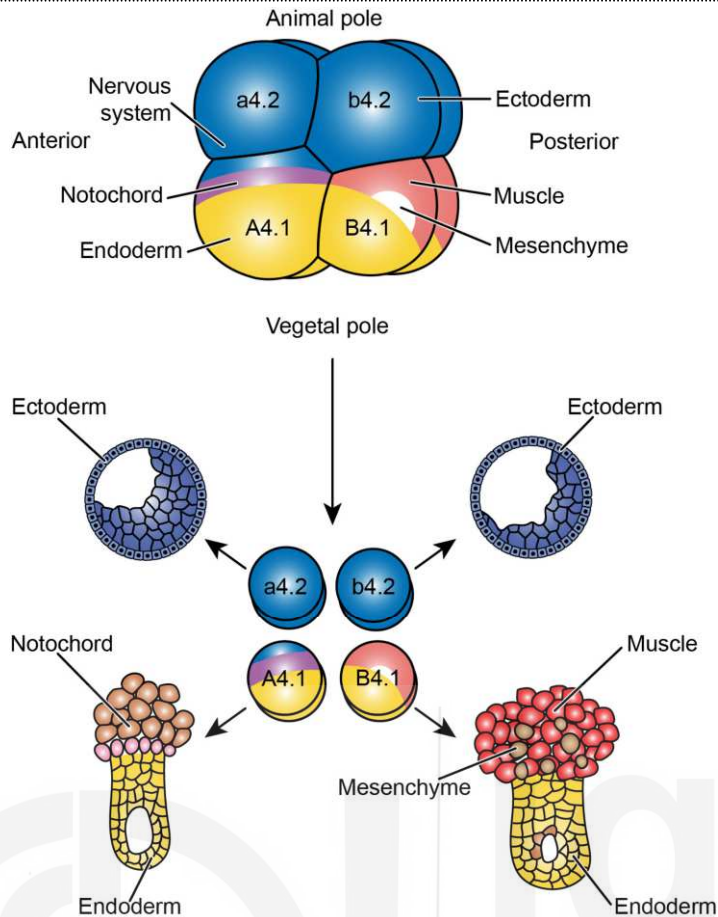


Fig.10.15: Autonomous specification seen in tunicate embryo. The cells were separated at the 8 cell stage and each cell gave rise to only those structures that it would have made in the entire embryo.

- B. **Conditional specification:** in this process the cells achieve their final fate by interacting with other cells or their local environment. These cells – to cell interactions may be of various kinds (which we will discuss in greater detail in the next unit). **The fate of a cell in this type of specification depends on its position in the embryo.** For example, if the cells from a region of a vertebrate blastula 'A' that are known to give rise to the dorsal region of the embryo are taken out and transplanted to the region of the blastula 'B' that gives rise to ventral region in the embryo, then **A type of cells** will change their fate and differentiate into **B type** cells. Moreover the region from where A type cells were taken will also continue to develop normally (Fig.10.16).

Various experiments conducted by embryologists since the 1890s that dealt with the separation of early blastomeres in sea urchin which resulted in each blastomere giving rise to a separate embryo (recall both Roux's and Dreisch's experiments). Thus, it was seen that each blastomere or cell acquires its identity based on its position and its interaction with its neighbouring cells and molecules with which it comes in contact (Roux's experiment) and when the early blastomeres were separated they lost that interaction and were able to develop into separate embryos (Dreisch's experiment). Embryos, especially vertebrate embryos in which the early blastomeres are conditionally specified were traditionally called **regulative embryos**. With further use of molecular biology in the process of development it has been

concluded that both autonomous and conditional specification is seen to occur in the development of most embryos.

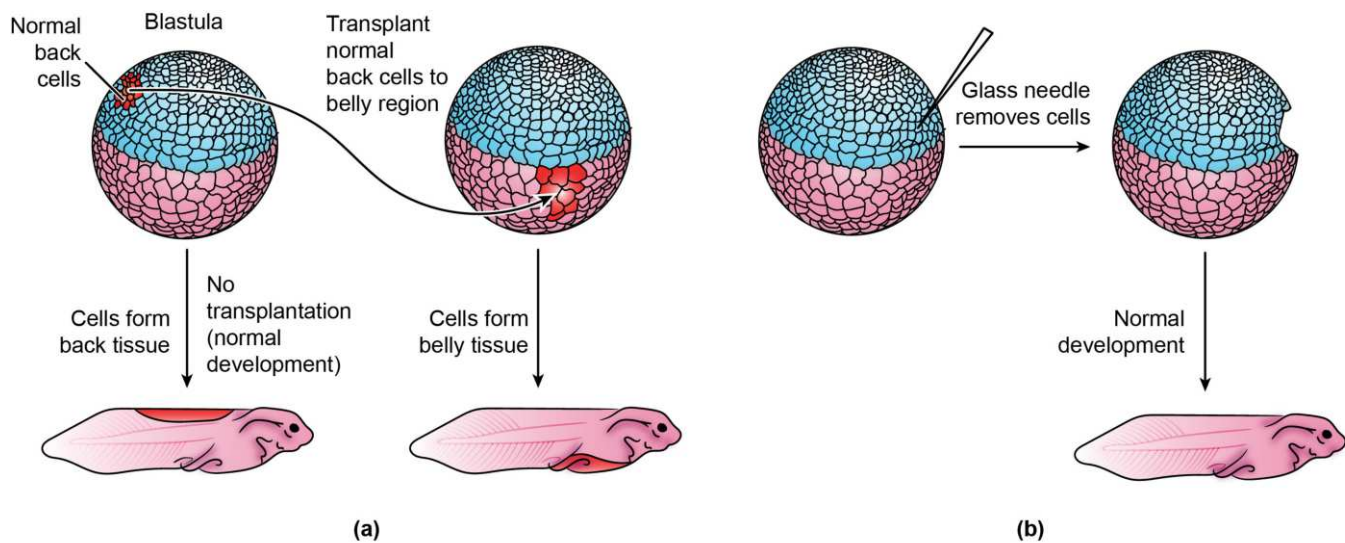


Fig.10.16: Conditional specification. a) The fate of a cell depends on its position in the embryo; b) the region from where the cell had been removed compensates for the loss and the embryo develops normally.

Syncytial specification: is the third strategy which is seen mostly during embryo development of insects and has been demonstrated best in the *Drosophila* embryo. The cytoplasm in *Drosophila* egg already has certain factors or maternal determinants distributed in it unequally. For instance, the anterior most part of the egg contains an mRNA called Bicoid mRNA that encodes a protein called **Bicoid**. The posterior most part of the egg contains an mRNA called Nanos mRNA that encodes a protein called **Nanos**. After fertilization of the egg, these two mRNAs are translated into their respective proteins in the cytoplasm, contributed by the ovum. During the early cleavage stage the nucleus alone divides and the cytoplasm does not separate or undergo cellularization to form individual cells. This results in formation of the blastoderm (a blastula having the form of a disc of cells on top of the yolk) which in *Drosophila* appears in the form of a large cell with many nuclei and a common plasma membrane (Fig.10.17). **A cytoplasm that contains many nuclei is called a syncytium** as you may recall. It is in this syncytial blastoderm that the cell identities are determined even before individual cells are formed. Research has shown that the concentration of Bicoid protein is highest in the anterior part of the cytoplasm of the embryo and declines toward the posterior end. While the concentration of the Nanos protein is maximum in the posterior part of cytoplasm of embryo and declines as it diffuses anteriorly. Thus, the long axis of the *Drosophila* egg is spanned by two opposing gradients—one of Bicoid protein coming from the anterior side, and one of Nanos protein coming from the posterior side. The Bicoid and Nanos proteins form a coordinate system based on their ratios, such that each region of the embryo will be distinguished by a different ratio or concentration of the two proteins. As the nuclei divide and enter different regions of the egg cytoplasm, they will be instructed by these ratios as to which position should they occupy along the anterior-posterior axis of the animal. Those nuclei in regions containing high amounts of Bicoid and little Nanos will be instructed to activate those genes necessary for producing the head. Those nuclei in regions with slightly less Bicoid but with a small amount of Nanos will be instructed to activate those genes that generate the thorax. Those nuclei in regions that

have little or no Bicoid and plenty of Nanos will be instructed to form the abdominal structures. After the 13th cleavage division just before gastrulation the cell membranes are laid down to separate the nuclei. The nucleus is able to keep its position in the embryo because of its exposure to the different concentrations of the cytoplasmic determinants and each cell or blastomere after cellularisation would know what part of the embryo it will become. Thus, the specific fate of each cell is determined both autonomously (from cytoplasmic determinants) and conditionally (by interaction with other neighboring cells).

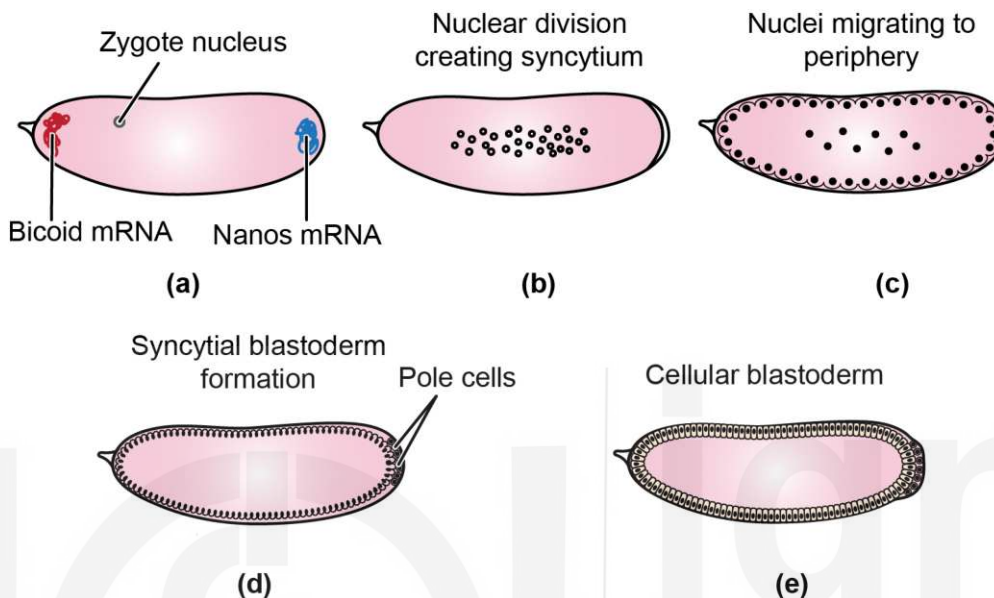


Fig.10.17: Syncytial specification in *Drosophila melanogaster*. The egg already has the *Bicoid* mRNA concentrated in the anterior part (head region) and the *Nanos* mRNA in the posterior region (tail part). After fertilisation the cytoplasm of the fertilized egg would contain these two mRNAs which would encode their respective proteins namely Bicoid and Nanos proteins. The noncellularized nuclei present in the embryo are influenced by the ratio of the opposing concentration gradients of Bicoid and Nanos proteins.

The three processes of specification of undifferentiated cells in the embryo lead them to different pathways of cell differentiation. The fate of determined cells becomes self-perpetuating which means that their progeny will have the same fate. In the next sub-section we shall look at the mechanisms involved in differentiation of determined cells.

SAQ 4

Fill in the blanks

- Differential acquisition of certain cytoplasmic molecules present in the egg result in specification.
- In development cells cannot change their fate if a blastomere is lost.
- Capacity for development results in the change in a cell's fate if it is transplanted elsewhere in the embryo.
- Specification by interaction of cells is known as

- v) specification is seen predominantly in insects where after cellularisation both autonomous and.....specification are involved in of cell fate.
- vi) As embryonic development proceeds, cells become restricted to a particular pathway they are said to be
- vii) Most cells physically change once they take their mature form, they are

10.6.2 Cell Differentiation

Cytological studies in the early 20th century established the fact that all the somatic cell arising from the fertilized egg contained the same chromosomal complement. This fundamental concept is known as **genomic equivalence**. In this section we will learn how this concept has been proved to be true by the help of some path breaking experiments. This concept of genomic equivalence has raised several questions. For instance if all the cells contained the same genomic complement then how do cells become different from each other? Furthermore, why do only some cells like the red blood cells (RBC) make haemoglobin proteins which are never produced in other cells of the body? Or why insulin hormone is produced and secreted only from certain cells of the pancreas and never in the kidneys or in the brain? By 1960s, based on experimental evidence, the concept of **differential gene expression** came into existence to provide answer to these and several other questions and to explain how similar looking cells with the same type of genetic complement differentiate into different types of cells.

Genomic Equivalence

The best test of whether all somatic cells contain the same complement of genes as the fertilized egg from which they have arisen is to check if the nuclei of the differentiated somatic cells still have the ability or potency to generate all types of cells. If indeed this is the case then a nucleus taken from one type of cell in the body should be totipotent (having the ability to produce all types of cells) and if transplanted into an activated enucleated (nucleus removed) cell should be able to give rise to all the cells of the body.

In the 1950s, Robert Briggs and Thomas King conceptualized and conducted the experiments for determining the totipotency of the nucleus of early embryonic cells. In their experiment they first combined the technique of enucleation and activation of an oocyte of the leopard frog *Rana pipens*. The oocyte was activated by pricking it with a sterile needle which provided it with the necessary stimulus to undergo all the cytoplasmic and biochemical rearrangements associated with fertilization, including the completion of second meiotic division at the animal pole of the cell. Puncturing the oocyte at this point let the spindle and chromosomes flow out of the cell (enucleation). The nucleus from a donor cell was then removed and by using a micropipette

was inserted into this activated, enucleated cell. Briggs and King demonstrated that blastula (early stage embryo) nuclei when transferred into activated enucleated oocytes could direct the development of a completely formed tadpole (Fig.10.18). They also found that while the nuclei at the blastula stage were totipotent, there was a dramatic decrease in the nuclear potency of cells at later stages. For instance, when nuclei from somatic cells of the tail bud region of tadpoles were used in a similar experiment, normal development did not occur. Thus it was concluded that nucleus from developing embryonic cells appeared to lose the ability to direct development as they underwent determination and differentiation. Further work continued using nuclear transplantation studies and in 1962 John Gurdon a PhD student in Cambridge University showed that if the nucleus of a fertilised frog egg was replaced with a nucleus from the cell of the tadpole intestine, the egg could develop into a new frog. Though the success rate was low, it proved that the nucleus of a mature cell still contained the genetic information needed to build all cell types. This was a major landmark in animal development though the acknowledgment of the importance of his work came much later. It was 40 years after his first experiments with nuclear transformation that the Nobel Prize was awarded in 2012 to Gurdon along with Shinya Yamanake, whose lab induced pluripotent cells.

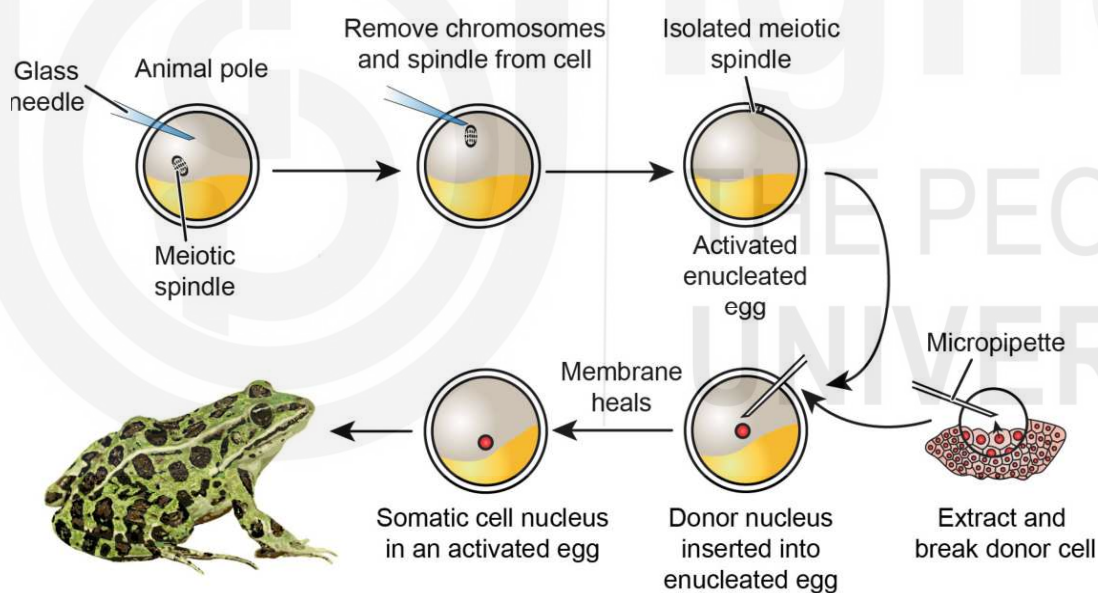


Fig.10.18: Procedure used in enucleation and transplantation of a nucleus from a somatic cell in *Rana pipens*.

Nuclear transplantation techniques were used by experimental embryologists to create clones of several species over the years. In 1997 Ian Wilmut and his colleagues demonstrated that somatic cells still had the potency to produce all the cells of the body. The result was Dolly (Fig.10.19), a sheep that was cloned by using the nucleus taken from the mammary gland of an adult sheep and transplanting it into the egg taken from another strain of sheep. This was done in a culture and later the eggs containing the transplanted nucleus were implanted into the uteri of pregnant sheep. Of the 434 sheep oocytes only one survived to become Dolly. DNA analysis however, confirmed that Dolly's cells had indeed been derived from the donor nucleus. This was the final proof of



Fig.10.19: Cloned Sheep Dolly

genomic equivalence of somatic cells and that the genome is conserved during differentiation of cells. Since the first cloning experiments, cloning of adult mammals has been done in mice, rats, guinea pigs, rabbits, dog, cat, cows and horses. Figure 10.20 shows the cloning of rats using two different types of rats by using similar techniques that were used for cloning Dolly

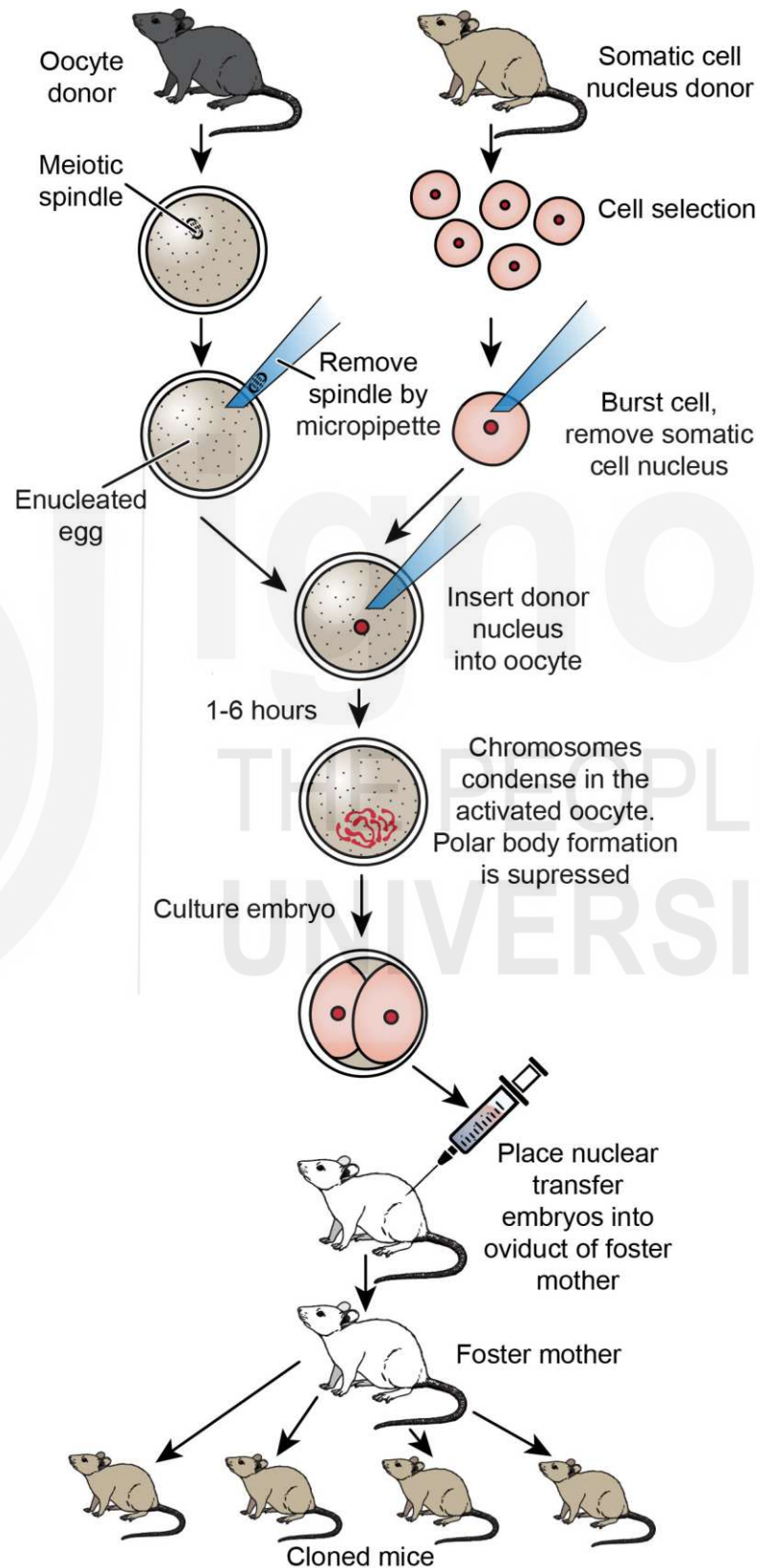


Fig.10.20: Cloning of mice by employing similar techniques that were used to clone the sheep of Dolly.

BOX 10.1

You would certainly have heard about stem cells and their use in medical research. Cell mechanisms of determination and differentiation are the basis of this research. Stem cells refer to cells present in embryos and adult that retain their ability to differentiate into many kinds of cells. In humans, stem cells have been found in bone marrow, brain, in some muscles, skin and liver. These cells can normally differentiate into a limited number of cell types. Because of this they are referred to as multipotent cells and not totipotent. Under normal conditions our bodies use the stem cells to regenerate and replace tissue such as skin, blood, liver etc. Stem cell research hopes to exploit the multipotent characteristics of cell in order to regenerate damaged and diseased tissue and organs. Research is going on with some success in stem cell therapy to regrow damaged spinal cord tissue, replace diseased heart muscle tissue and skin tissue and in search of cures for cancer which is really a disease of abnormal cell division and differentiation patterns.

10.6.3 Differential Gene Expression

Genomic equivalence established the general principle that somatic cell nucleus of an organism contains the complete genome established at the time of fertilization and there is no change in the genetic content after differentiation of cells. If all cells in the developing embryo have the same genetic content, differences between cells must be due to **different gene activity in each cell type**. This means that only a small portion of the genome is expressed or “turned on” in each cell. As a result only those RNAs are synthesized in the cell that will translate (make) only those proteins that will provide structural information to these cells to help them differentiate and ultimately develop into very different cell types. It is important to understand how various signals cause cells to express different portions of their genetic information. Nuclear transplantation and cell fusion experiments reveal that gene activity is controlled by the **cytoplasmic environment**. New cytoplasmic signals can activate previously silent genes and silence previously active genes. Such genetic control continues throughout embryonic development and results in the generation of a variety of cell types with different gene activities. As the zygote cleaves, its cytoplasmic contents contributed by the egg do not pass uniformly into different blastomeres. The descendent cells at cleavage, therefore, have different cytoplasmic environments. This initiates a pattern of embryonic cells with a distinct programme for gene expression. Later in embryonic development, interactions between blastomeres release new signals, which then determine additional group of cells to activate new sets of genes.

Gene expression is the process by which the genetic code - the nucleotide sequence - of a gene is used to direct protein synthesis and produce the structures of the cell. Genes that code for amino acid sequences are known as 'structural genes'.

The first evidence for **differential gene expression** came from the study of polytene chromosomes of *Drosophila* larvae (Fig.10.21 a). In polytene chromosomes DNA duplication occurs in many rounds without any division and separation of the cell. The cell size increases and so as a result, the many stranded DNA is easy to observe. It was seen that the banding pattern of the chromosomes was identical throughout the various cells of the larva. No loss or addition of any chromosome portion was seen in different cell types in the larva. However, different parts of the chromosome were ‘puffed up’ in different

cell types suggesting that different RNA was being synthesized in different cells and while one part was puffed up in one cell type the other genes were silent and so not puffed up in that cell (Fig.10.21 b).

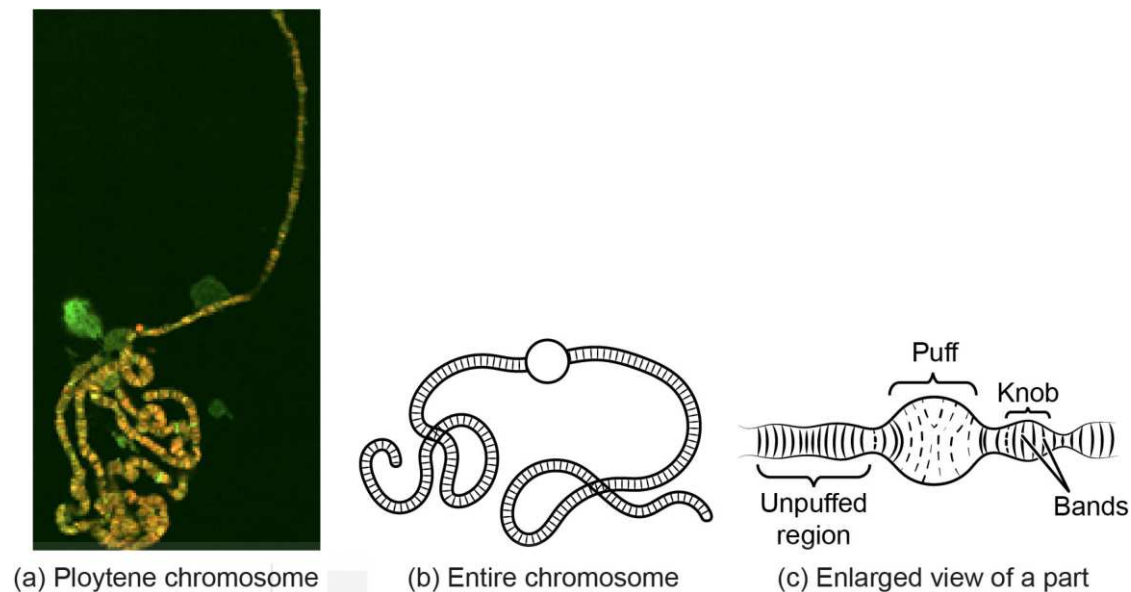


Fig 10.21: Polytene chromosome from salivary gland of *Drosophila*

***malanogaster*.** <http://www.biologydiscussion.com/chromosomes/useful-notes-on-chromosomes/34691>

By the late 1980s it was understood that gene expression could be regulated basically at 4 steps and this could explain the mechanism of differential gene expression.

- i) At the stage of **gene transcription** so that only those genes that are required for that particular cell type are expressed in the form of nuclear RNA. Transcription is the first step of gene expression and its control involves general and tissue specific transcriptional regulators.
- ii) At the nuclear RNA **processing** stage, by regulating which part of the RNA or which of the transcribed RNAs are allowed to leave the nucleus.
- iii) At the RNA **translation** stages **by regulating** which of the mRNAs are to be translated into proteins.
- iv) At the **protein modification** stage, so that only those proteins are modified that can provide the structure and function to the specific cell type.

In order to understand the way genes are expressed it would help if you were to read Box 10.2 so that you can quickly recapitulate the principles of the **central dogma of biology**. Before proceeding further let us look at the structure of a gene. You know that a gene is a distinct sequence of the DNA molecule that has the information to make a polypeptide or a nucleotide. Figure 10.22 shows the gene responsible for making the β -globin in the haemoglobin molecule. The β -globin is made up of different components. Only the parts labelled exons will provide the information for the appropriate amino acid sequences for translating the appropriate protein and not the parts labelled intron. On this portion of DNA the transcription will start at the **transcription initiation site** and end at transcription termination site. This

segment will form the hnRNA (heterogeneous n RNA) and will undergo processing in which the transcribed introns will be spliced or removed. This would result in putting together the consecutive exons and forming the mRNA. In further modification, 7-methylguanosine (G) cap and a poly (A) tail will be added to the 5-prime end and 3-prime end of this mRNA respectively. This modified mRNA will then leave the nucleus to move into the cytoplasm where it will be translated into a protein of interest. Look at the gene again in the figure 10.22. You will see that the DNA has a portion marked promoter which is not transcribed. The promoter is very important as it is at this location where the proteins and factors bind which can thus regulate or enable the process of transcription. Furthermore in the upstream (anterior end) region or in some cases even in downstream (posterior end) region and also in the introns, there will be sequences that will be able to influence the promoter to initiate transcription. These sequences are regulatory factors also known as enhancers (that stimulate), repressors or silencers (that inhibit transcription).

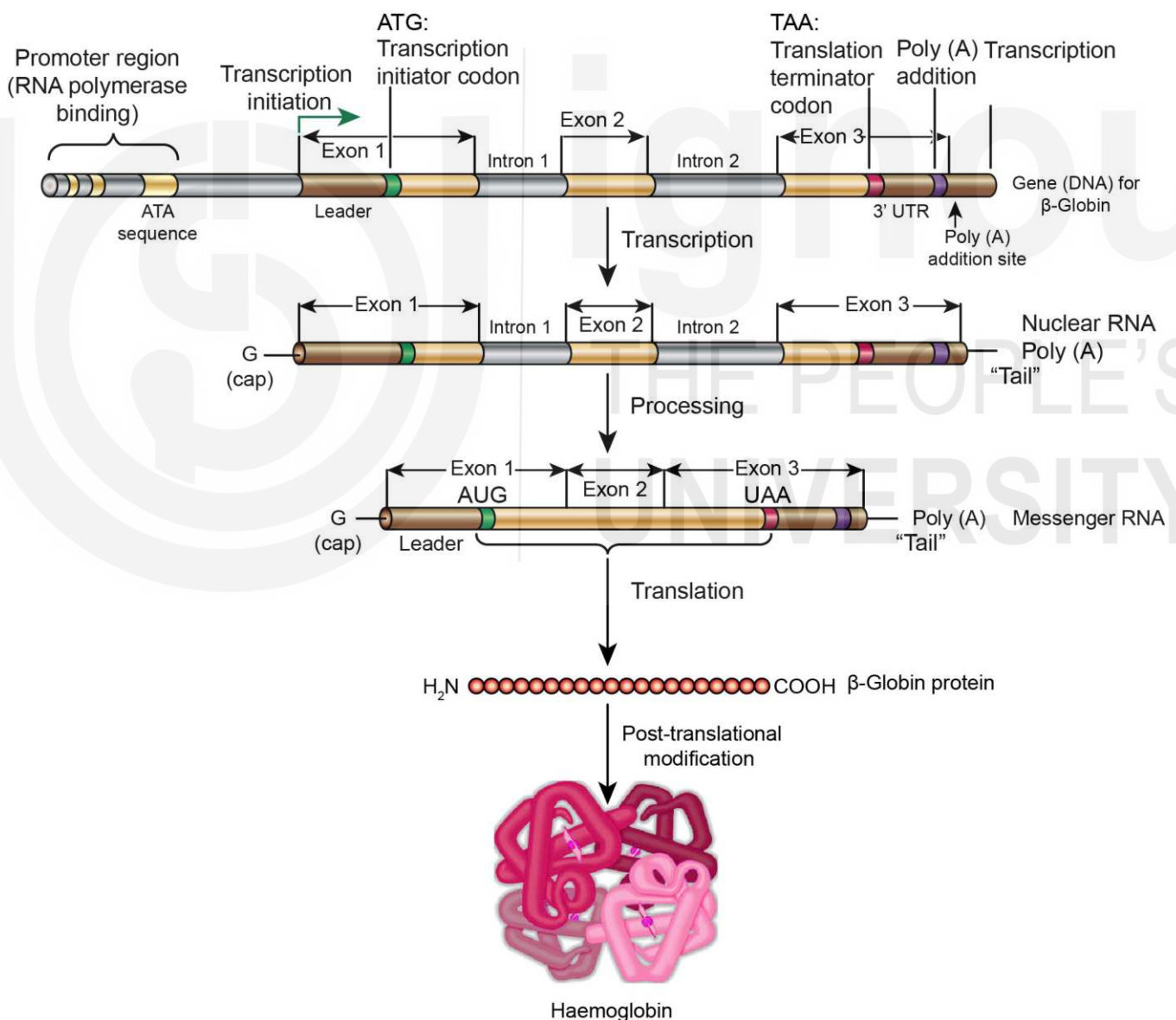


Fig.10.22: The making of beta-globin for the haemoglobin molecule. Beta-globin is inactive unless it is modified and combined with alpha globin to make the complete haemoglobin molecule.

Let us now see how regulation of gene expression can occur. Earlier in the section we had said that gene expression can be regulated at all stages from transcription to the final making of the gene product, namely protein. We will discuss the regulation at the transcription level in a little more detail so that you can understand this important mechanism. If a gene is to be regulated then first it should be accessible to a variety of factors that can bind to the DNA and regulate its expression.

- 1) The first step would be the loosening of the chromosome so that it can change from heterochromatin state (when the DNA is packaged and coiled around the histones) to the euchromatin state (when the uncoiling of the DNA occurs) so that the gene becomes accessible to a variety of factors. Therefore, regulating the chromatin to uncoil or coil will influence where and when in the genome certain genes can be expressed. For this process small organic molecules can be added or removed from the histones around which the DNA remains coiled. DNA remains coiled if the histones have methyl groups attached to their tails and uncoiling occurs when the methyl groups are replaced by acetyl groups on the histone tails. It is a general rule that acetylation will give access to the promoter region and initiate active transcription, while methylation will repress transcription. Experiments have shown that this is one way of regulating gene expression.
- 2) Another way of regulation is by transcription factors that bind to the promoter region and can turn genes on or off. If even one transcription factor is changed in the embryo it can have dramatic effects. For example, the loss of a gene *ultrabithorax* can have drastic effects in insects. This gene is a homeobox gene which contains a transcription factor, whose absence changes the segmentation pattern in the fly. As a result the fly forms two pairs of wings instead of the usual one pair due to loss of a gene *ultrabithorax*. There are different transcription factors which bind to their affiliated DNA binding domains and these are very specific. Let us take the example of an enhancer region on a gene. A transcription factor specific to that enhancer forms a complex with it and can change the shape of DNA so that the promoter and transcription factor- enhancer complex come near the promoter. This causes a very important enzyme DNA polymerase to bind to the promoter which causes the transcription of a particular RNA. If transcription factor binds to the repressor region it will stop or slow down the gene from expressing. For example, the human foetal embryo liver cells synthesize serum albumin but only after a certain stage of development. Till then the gene that encodes for serum albumin is silent or repressed as a transcription factor is bound to the repressor domain. Thus, again it is the specific combination of the transcription factor with the enhancer or repressor that will regulate the rate of transcription in specific cells and cause differential gene expression. Often there is a cascade of reactions in which the gene for making a transcription factor is regulated by another transcription factor.

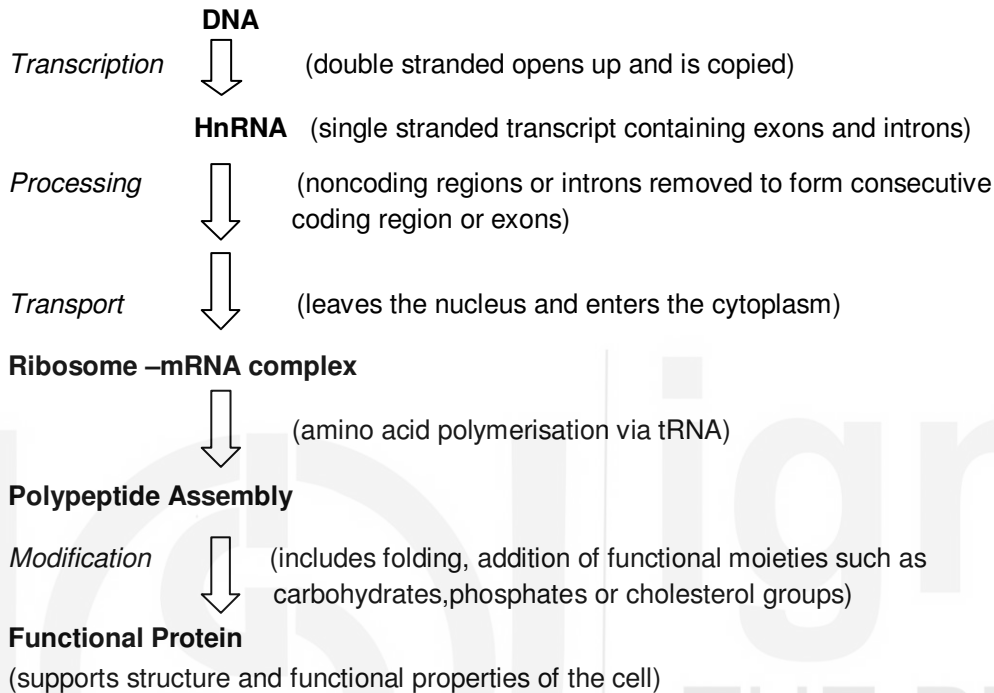
Therefore, it is important for you to understand that there are a variety of ways to regulate the gene and the protein it forms, which in turn

influence the differentiation of the incredible varieties of cells seen.

Scientists have still a lot to learn and discover about these mechanisms.

BOX 10.2: Central Dogma of Biology

Central dogma is the sequence of events that enable the information stored in the DNA of the nucleus to be copied and transcribed into instructions for translating proteins in the cytoplasm of the cell. These proteins decide the ultimate structure and function of the differentiated cell. The sequence is given below in the chart.



SAQ 5

- a) Fill in the blanks with appropriate word/words in the following sentences
 - i) Cell differentiation is the result of expression.
 - ii) During gene expression methylation of histone tails results in while acetylation causes
 - iii) bind to enhancer regions of the gene to form a complex that binds to to initiate transcription.
- b) Define genomic equivalence.
- c) Give one word for each statement given below:
 - i) The zygote cell has the potential to form any cell in the body and so it is
 - ii) Stem cells have the ability to form a limited variety of cells and so they are

10.7 SUMMARY

After studying this unit you have learnt:

- The study of embryonic development started more than 2000 years ago. Aristotle put forward the idea of epigenesis which states that embryos were not completely preformed in the egg but structures emerged gradually as the embryo developed. This idea was questioned in the 17th and 18th century and the idea of preformation of the embryo in the eggs or sperm was advocated. However, with the emergence of the cell theory the idea of preformation was laid to rest. As more experimental methods and sophisticated microscopes came into use experimental embryology developed further.
- Some early experiments in embryology showed that in sea urchin embryos if one of the blastomeres is destroyed the other blastomere develops in half of the embryo and if the blastomeres are separated at 2 cell or even 4 cell stage, each can develop into a full but small embryo. This was evidence that showed that cells are able to regulate development. Direct evidence of cells being regulated by other cells in the embryo came in 1924 by the experiment of Spemann and Mangold that showed the existence of an organiser region in the early embryo.
- The role of genes in controlling the development of embryos has only been established in the last 50 years and developmental biology as a discipline was established through inputs from other disciplines such as cell biology, genetics, biochemistry and foremost molecular biology.
- Development starts with the fertilisation of the egg by a sperm and the resultant zygote undergoes cleavage; followed by emergence of pattern; change in cell form and determination of cell fate; cell differentiation and growth.
- Pattern formation involves laying down of the overall body plan around the body axes and establishment of germ layers. The concept of germ layers and fate maps of the embryo are useful to distinguish between regions of early embryo that will give rise to distinct cell types and the future tissues and organs of the organism.
- The developmental potential of early embryo cells is greater than their general fate and as development progresses cells lose their potency and cell fate becomes specified and determined. This means that the internal state of cells changes and they become committed to a specific cell fate. During specification embryos of different species can show any or a combination of three strategies-autonomous (mosaic), conditional (regulative) and syncytical. At this stage the cell's fate is still flexible and it can be induced to differentiate into another cell type. However once determined the cell has a stable internal change and its fate becomes fixed.
- All cells of the embryo and the adult organism have the same genome (genomic equivalence) as the zygote. This genomic equivalence was proved by nuclear transplantation experiments involving enucleated cells

and transplanting nucleus from other early embryonic cells and later from differentiated somatic cells. The first successful experiment in a mammal was the creation of a sheep named Dolly.

- Cell differentiation involves the gradual emergence of cell types that have a clear cut identity in the adult, such as nerve cells, red blood cell, muscle cells etc. Initially the embryonic cells fated to become different cell types, differ from each other in the pattern of gene activity that forms different proteins. Over successive cell generation these cell acquire their new structural features and their potential fate becomes more restricted. The central feature of cell differentiation is differential gene expression. This is regulated primarily at the first step of gene expression by transcription factors that bind to enhancers or repressors of gene expression. Transcription factors can be general or tissue specific. However, protein synthesis can be regulated even after the transcription at the successive stages of gene expression.

10.8 TERMINAL QUESTIONS

1. What were the three classical experiments that laid the foundation of experimental embryology?
2. What characteristics should a good model organism have to be useful in developmental biology studies?
3. What is the difference between cell specification, determination and differentiation? Explain with the help of an example in each case.
4. Describe how developmental fate maps can be made?
5. List at least three stages in gene expression that can be regulated to result in differentiated cell types? Explain any one of them with the help of an example.

10.9 ANSWERS

Self-Assessment Questions

1. a) Embryology is the science which studies the stages from fertilisation to birth of an organism while developmental biology includes stages of embryogenesis, and growth after birth as well as regeneration and senescence.
b) The two proposed views on development – 1. epigenesis which says that development happens progressively at every stage and 2. preformation which states that the entire organism is already pre formed in miniture state in the egg or sperm. He emphasized epigenesis a view which is also subscribed by modern animal developmental biology.
2. a) Chick, sea urchin and frog because of the ease of manipulation, large size of eggs, abundance of eggs and ease of rearing.
b) *Drosophila*

3. Fate maps are formed or drawn of those regions in the early embryo that will form the future tissue and organs during normal development. They are significant because they give general information of the cell fates or what cell types they will differentiate into. They are true for all members of the species for which they are drawn.
4.
 - i) autonomous
 - ii) mosaic
 - iii) regulative
 - iv) conditional specification
 - v) syncytial; conditional; determination
 - vi) determined
 - v) differentiated
5.
 - a)
 - i) differential gene.
 - ii) coiling of DNA around nucleosomes; uncoiling /opening of the gene.
 - iii) Transcription factors; promoter region
 - b) Every blastomere, of blastula and every cell of the body, possesses identical genes both qualitatively and quantitatively.
 - c)
 - i) Totipotent
 - ii) multipotent

Terminal Questions

1. Refer to Section 10.3.
2. Refer to Section 10.4.
3. Cell specification is the stage when the undifferentiated cells are not committed to their fate and are still flexible to take the fate of another cell type if induced to do so.

Determination is the final fate of the cell when it can no longer be induced to take the path of development of surrounding cells.

Differentiation means that the cells actually become what they are fated to be e.g., muscle cells neurons Also see section 10.4.
4. Refer to Section 10.3.
5. Refer to Box 10.2 and Section 10.5.

UNIT 11

CELL TO CELL COMMUNICATION

Structure

11.1	Introduction	11.3	Cell-Cell Interactions
	Objectives		Morphogen Gradients
11.2	Morphogenetic Processes		Embryonic Induction
	Cell Movements	11.4	Cell Death
	Cell Adhesion		Apoptosis
	Cell Signalling		Necrosis
	Epithelial-Mesenchyme		Autophagy
	Transition	11.5	Summary
		11.6	Terminal Questions
		11.7	Answers

11.1 INTRODUCTION

In Unit 10 we had discussed how descriptive embryology evolved into the discipline of developmental biology and how fate maps in early animal development help to trace the development of differentiated cells. We learnt that genes control development by controlling when and where proteins will be synthesized. This differential gene expression is the cause of determination of different cell fates and cell differentiation. In this unit we will look at early development processes through which the embryo acquires its shape and structure. The differentiated cell types are not placed in the embryo in a random manner but are arranged in organized structures for example limbs, heart, lungs, eyes wings and other internal organs. This formation of organized structures from simple epithelial sheets and mesenchymal masses is termed **morphogenesis**. The early germ layers- ectoderm, endoderm and mesoderm undergo extensive rearrangement, through regional specification and directed movement of cells from one location to another in the embryo to form the three dimensional animal body. These morphogenetic processes involve cell shape changes, cell migrations and cell to cell interactions which will determine how the embryo will get its shape. We will learn more about cell to cell interaction and patterning with the example of the development of a

vertebrate eye and the influence of chemical signals called morphogens in the process. Finally we will look at the different processes of cell death which are important in morphogenesis for giving rise to the shape and contours of organs in the embryo.

Objectives

After studying this unit you should be able to:

- ❖ discuss the mechanism of differential gene expression;
- ❖ describe the basic mechanism of cell movements and cell migrations in morphogenesis;
- ❖ explain cells adherence and its role in morphogenesis;
- ❖ describe the different types of cell signalling and their role in morphogenesis;
- ❖ explain how pattern formation occurs through morphogens and inductive signal; and
- ❖ discuss the different mechanisms of cell death and their need in development.

11.2 MORPHOGENETIC PROCESSES

Before we start discussing the morphogenetic processes, it is important to know that all embryonic cells are basically of two types - **epithelial** and **mesenchymal** (Fig.11.1). This categorisation of embryonic cells relates to cell shapes and cell behaviour rather than to their embryonic origin. Epithelial cells can arise from all three germ layers and mesenchyme arises from mesoderm and ectoderm. An epithelium is a sheet of cells that rests on a basement membrane and each cell joins its neighbour by specialised junctions; the cells have a distinct apical – basal polarity. Epithelial cells form sheets, tubes and lining of organs. Mesenchyme is made up of loose cells embedded in the extracellular matrix lying between the ectoderm and endoderm of the developing embryo. It fills up much of the embryo and later forms the fibroblasts, adipose tissue, smooth muscle and skeletal tissues.

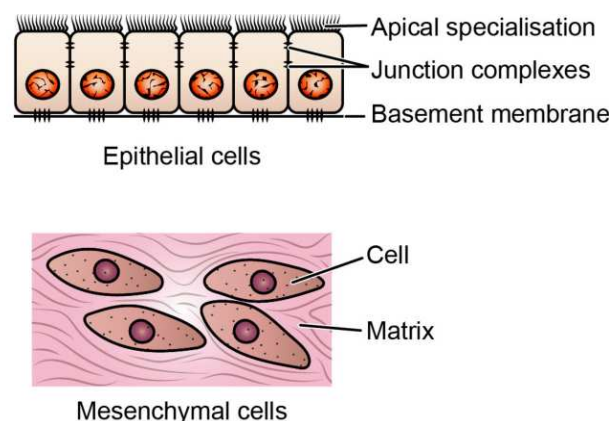


Fig.11.1: Epithelial cells and mesenchymal cells are the two basic cell types in the embryo.

Remember that for the embryo to form its structure from a single cell the zygote, the processes that take place broadly are cell division to make more cells; then these cells have to be differentiated as per cell fate; the cells have to move, rearrange themselves, change their shapes and aggregate to form different tissues and organs. Thus the embryo acquires a recognisable shape and structure of the particular organism. All this takes place because of communications that occur between the cells of the embryo. Till a little more than two decades ago, not much was understood regarding the manner in which cells communicate with each other to construct an organism from a single cell, the fertilized egg or zygote, through genetically controlled events. However, towards the late 20th century it became clear that molecules in or on cell membranes were involved in the ability of cells to adhere, migrate and influence other cells.

In this section we will discuss three basic processes that require cell to cell communication through the cell surfaces - cell movement, cell adhesion and cell signalling. These are the key properties of cells that are involved in changes in embryonic form. Let us look at the property of cell motility first.

11.2.1 Cell Movements

Cell movements or motility is an active phenomenon that is essential for many biological processes such as morphogenesis, wound healing, immune response and even cancer metastasis. In this unit our focus is on morphogenetic processes where cell movement is targeted to specific sites in the developing embryo to form tissues and organs. A good example of these cell movement or cell migrations is seen in the movement of neural crest cells (multipotent cells that arise from embryonic ectoderm and give rise to different types of cells) and germ cells in vertebrates. Short range movements are also important and cell motility is responsible for both movements of individual cells as well as change of shape while remaining part of a tissue. For example, the folding of epidermal sheets to make tubes is caused by changes in the shape of the cells.

All cells move and change shape by rearranging their internal cellular skeleton (cytoskeleton) or scaffolding by contraction of the cytoskeleton fibres made up of microtubules and microfilaments that are actin – myosin complexes also termed as actomyosin complexes. These **actomyosin** complexes are simpler version of those seen in muscles. The energy required to produce the movement comes from adenosine triphosphate (ATP). In non muscle cells these actomyosin complexes are concentrated in the region just below the cell membrane. Moving cells also have a polarity that is, a front and a back region. The mechanism of cell movement can best be seen in the movement or *crawling* of fibroblasts (a type of connective tissue that secretes collagen found in the extracellular matrix) on a substratum which is the extracellular matrix inside the embryo or glass surface of petri-plates under *in vitro* conditions (Fig.11.2). Fibroblasts extend a flat process called lamellipodium which is rich in microfilaments made up of a crisscross of actin. From the lamellipodium extend focal contacts that attach it to the substratum and these are connected to the microfilament bundles of the lamellipodium. During movement the microfilaments contract and the body of the cell is pulled forwards. Cells of the

embryo essentially move in a similar manner. Instead of the large lamellipodium they may have multiple thin filopodia that make the contact with the extracellular matrix as they move over it.

In the embryo the cell movement is directional towards a signal which is a chemoattractant that is detected by the proteins on the cell membrane. These chemoattractants are diffusible molecules and the cells move towards increasing concentrations of the diffusible molecules. You will learn more about this phenomenon in a later section.

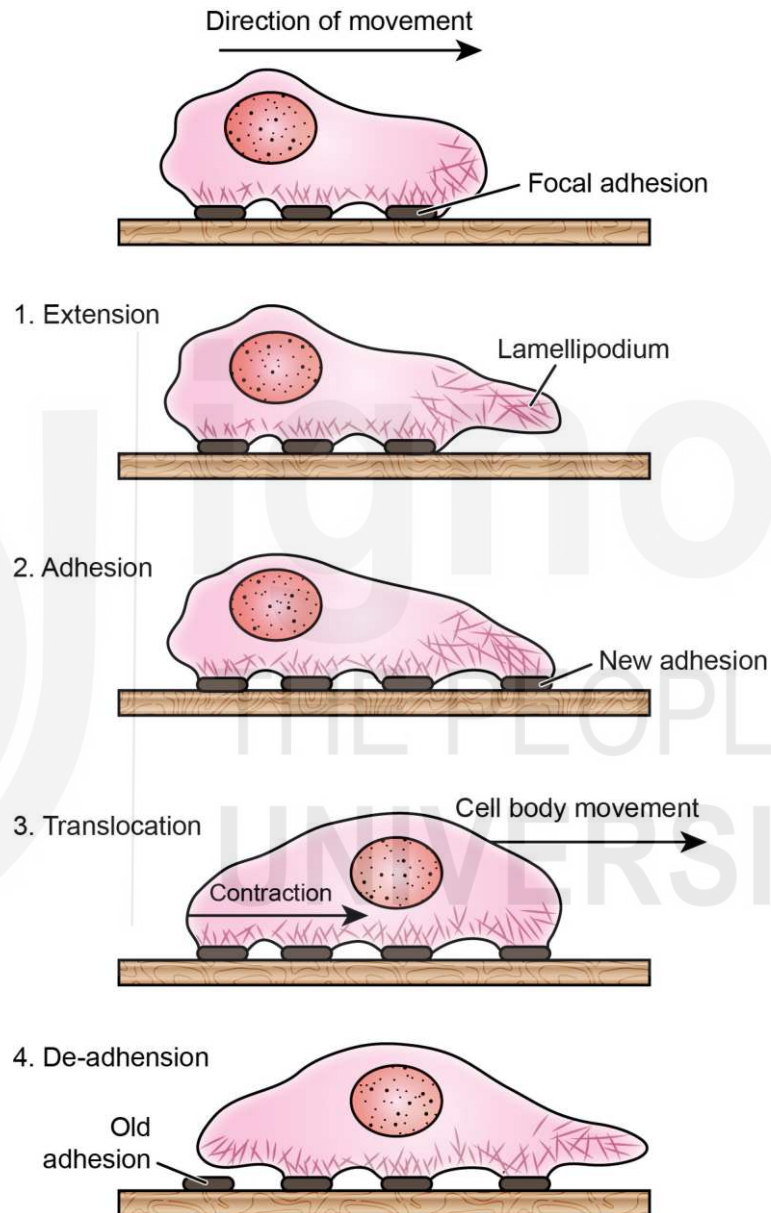


Fig.11.2: Fibroblast moves by extending the large flat lamellipodium that makes contact with the substratum.

Cell shape also changes by the contraction of microfilaments and the associated motor proteins actin and myosin. If the constriction happens in the apical region of epithelial cells it will reduce the apical surface area and elongate the cell (Fig.11.3). This happens initially during invagination during the process of gastrulation when the cells leave the epithelium to move inside the gastrula (you will learn more about the cell movements during gastrulation in Unit 12).

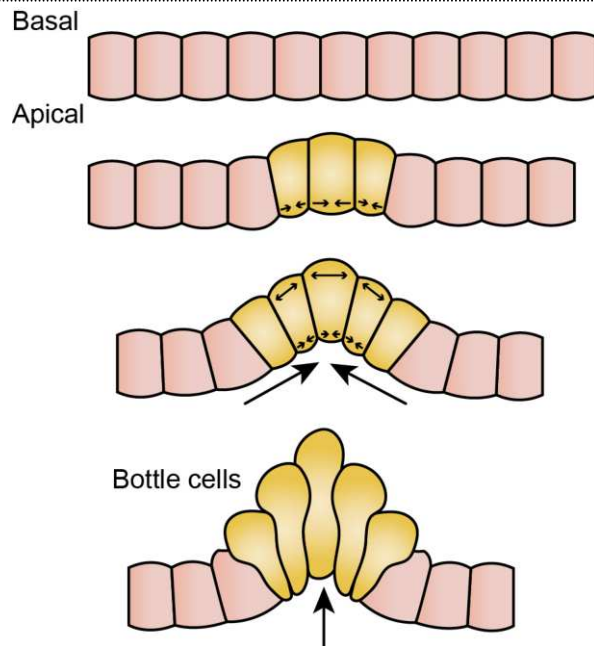


Fig. 11.3: Cell shape change in epithelial cells by apical constriction and result in elongation of the cell.

SAQ 1

Fill in the blanks:

- i) Cell movement takes place because of rearrangement of of the cell along with molecules.
- ii) Cells in the embryo move over
- iii) Embryonic cell movement is a response to from other cells.

11.2.2 Cell Adhesion

The other important property that is involved in changes in animal embryonic form is cell adhesiveness. Animal cells stick to one another and to intercellular matrix through interactions involving cell surface proteins. These cell surface proteins can determine how specifically and tightly the cells adhere to one another. These proteins can affect the cell surface tension and contribute to the arrangement of cells in the three germ layers and later in different tissues.

Differences in cell adhesiveness also help to maintain the boundaries between different cell types and tissues. Different cell types have both, different types and different amounts of cell adhesion molecules on cell surfaces thereby, having **selective affinity** for each other which is important for giving positional information to embryonic cells. Because of cell adhesion embryonic cells do not sort out randomly but can actively move to create tissue organisation. The differential adhesion interactions of cells form a certain hierarchy. If cell type A is situated internal to cell type B and the final position of cell type B is situated internal to C, then cell type A will always be internal to C. There are different

classes of cell adhesion molecules, but the major cell adhesion molecules appears to be **cadherins** (calcium dependent adhesion molecules). Cadherins are transmembrane proteins that interact with other cadherins present on adjacent cells. Cadherins are anchored to their cell by a complex of proteins called **catenins** (Fig.11.4). This cadherin- catenin complex is the classic adherin junction seen in epithelial cells. As the catenins bind to the cytoskeleton of the cell they integrate the epithelial cells and keep them together. Cadherins perform several related functions. (i) their external domains serve to adhere cells together. (ii) cadherins link to and help assemble the actin cytoskeleton, thereby, providing the mechanical forces for forming sheets and tubes of cells. (iii) cadherins can serve to initiate and convert signals that can lead to changes in a cell's gene expression

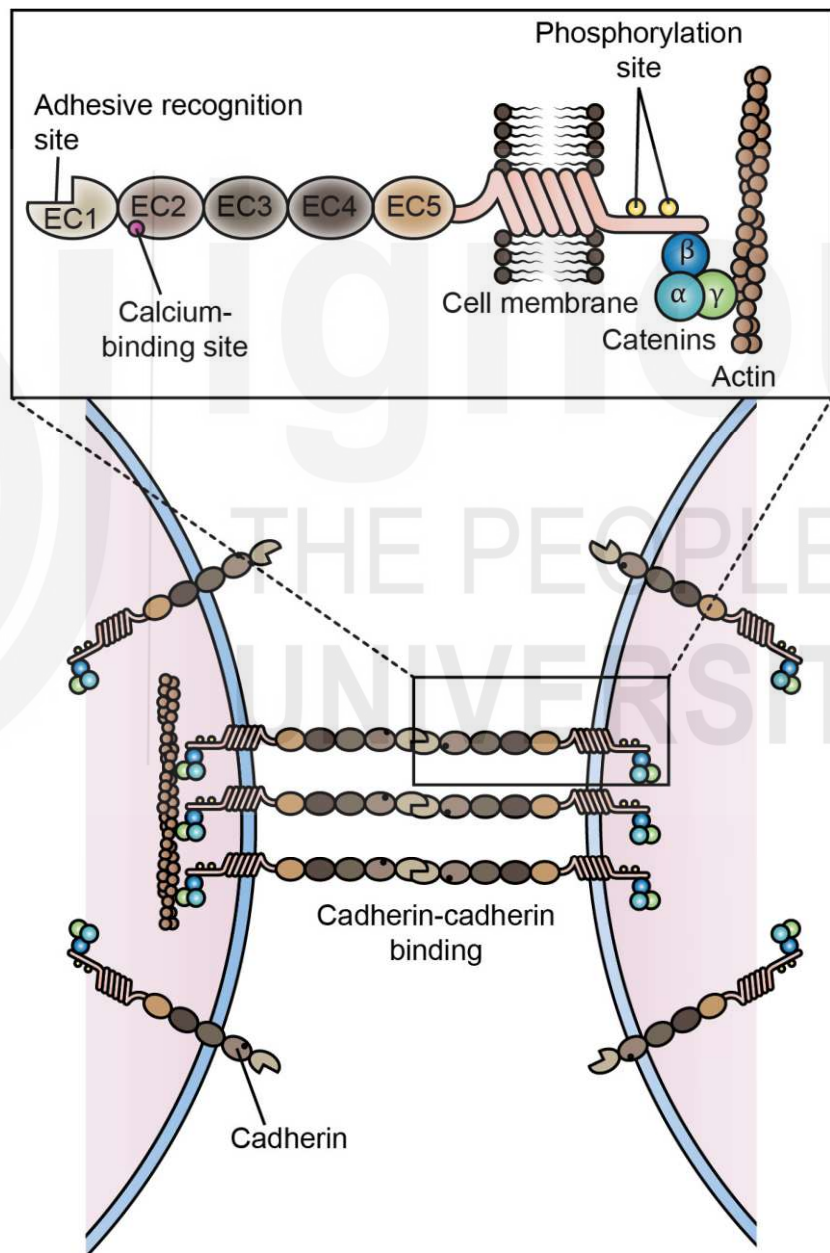


Fig.11.4: Diagram showing cadherin - cadherin cell adhesion. Inside the cell the cadherin molecule associates with a catenin molecule which itself is bound to the actin microfilament system of the cytoskeleton (adapted from Takeichi 1991).

Cadherins can be of different types (see Box 11.1) and cadherin of one cell binds to the other cells by the same type of cadherin. For example, cells with E-cadherin will stick to other cells with E-cadherin and sort out from cells with N-cadherin. This type of binding is known as **homophilic binding**. The sorting of cells was first demonstrated in the 1950s. Since it was known that amphibian tissue dissociate into single cells when placed in alkaline solutions, a single cell solution of the three germ layers of amphibian embryos was made and when the pH was restored these cells adhered to one another in the petri dishes. Thus the behaviour of recombined cells could be studied. The results of the experiment were striking, the cells sort out into their cell type and into their own regions (Fig.11.5).

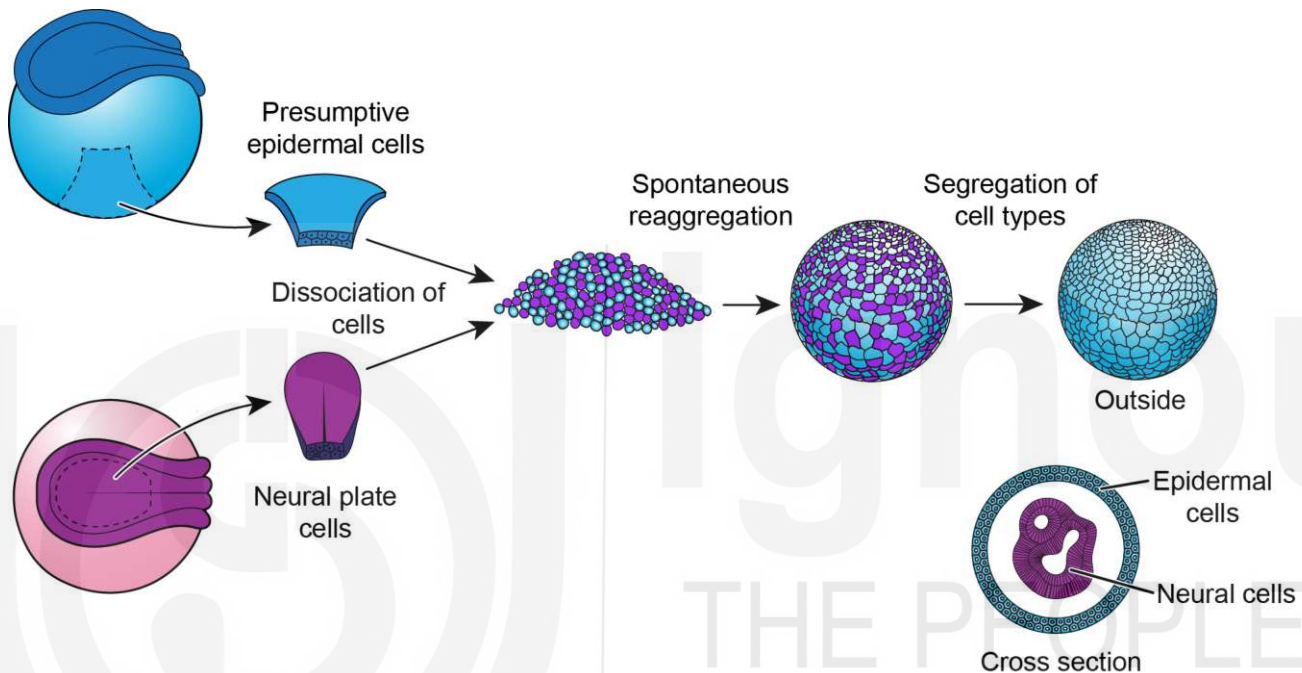


Fig 11.5: Reaggregation and sorting of cells from two different amphibian neurulae. Presumptive epidermal cells from a pigmented embryo and neural plate cells from a unpigmented embryo were dissociated and mixed together. The cells first aggregate together and then the cells segregated according to their cell type position. The presumptive epidermal cells cover the neural cells.

The sorting of cells is the combined effect of cell adhesiveness and cell movement. Initially the cells move randomly but then they seek similar cells to aggregate because they have stronger adhesive interactions. The ectoderm is the tissue with the strongest cohesive interaction among cells so it forms the outermost layer of the embryo. The less cohesive mesoderm and endoderm cells are arranged internal to the ectoderm. Cell sorting hierarchy is, therefore, strictly dependent on the amount of cadherin interactions amongst cells.

In vitro experiments with fibroblasts that are generally migratory cells and do not express E-cadherin, showed that when activated E-cadherin genes were added to the culture, the fibroblasts started expressing E-cadherin and they became tightly bound to each other and started behaving like epithelial cells. Blocking the function of cadherins by using antibodies that inactivate cadherin or by blocking its synthesis at translation stage can prevent epithelial tissue from forming and causes the cells to disaggregate.

Cell adhesion, cell movement and formation of epithelial sheets and tubes depend on the ability of cells to attach to extracellular matrix. When epithelia are to be made the attachment has to be very strong but when cells move or migrate, they have to break their attachment to other cells and reform them at another location. In some cases the extracellular matrix has to serve as a pathway for the moving cells, providing direction or the signal for a developmental event. Fibronectin a component of extracellular matrix in a way paves the roads on which the cells move, for example, fibronectin paths lead the germ cells to gonads and heart cells to the midline of the embryo. The binding of the cell to the extracellular matrix takes place through another family of adherin molecules called as **integrins** and the mechanism is **not dependent on calcium** unlike that in cadherins. Integrins are large protein molecules that span across the cell membrane. On the outside of the cell, it binds to the fibronectin of the extracellular matrix; on the inside of the cell, it serves as an anchorage site for the actin microfilaments that move the cell.

Box 11.1: Cadherin molecules.

There are many type of cadherin molecules and cadherin – cadherin attachments are the strongest when they are of the same kind.

- E-cadherin: epithelial cadherin is expressed on all early mammalian embryonic cells and later gets restricted to the epithelial cells of embryo and adults
- P –cadherin: this is placental cadherin expressed on the trophoblast of the placenta and on the uterine wall epithelium. It possibly facilitates the attachment of the placenta to the uterus
- N- cadherin: is neural cadherin, first seen on mesodermal cells in gastrula cells as they lose their E-cadherin expression. It is also seen on cells of the developing central nervous system.
- C-cadherin: is found to be critical for keeping the blastomeres together in *Xenopus* and for normal development for gastrulation cell movements.
- Protocadherins: are calcium dependent adhesion molecules like cadherins but lack the connection to cytoskeleton through catenins.

SAQ 2

- a) How are boundaries between different tissues maintained?
- b) What is the difference between cadherins and integrins?

11.2.3 Cell Signalling

Cells typically communicate with each other by use of chemical molecules or signals. These molecular signals are highly diverse and are responsible for specific protein-protein interactions, which can result in diverse cellular responses like changes in gene transcription, cell metabolism, cell migration and cell death. The signal molecules generally contact other proteins that may

be housed in or on the plasma membrane of the target cells. The signal protein molecules are called **ligands** (a general term for molecules that specifically bind to other molecules). The proteins that are attached to or embedded in the cell membrane of the target cells are known as **receptors**. A receptor in the membrane of one cell can bind a similar receptor in another cell in **homophilic** binding. In contrast, **heterophilic** binding occurs between different receptor types. Binding of a ligand to a receptor generally sets up a **signal transduction pathway**. The major signal transduction pathways all appear to have a common theme (see Fig.11.6). Each receptor spans the cell membrane and has an extracellular region, a transmembrane region, and an intracellular cytoplasmic region. When a ligand binds to its receptor's extracellular part, it induces a shape change in the receptor's structure. This shape change is transmitted through the membrane and alters the shape of the receptor's cytoplasmic part, giving that domain the ability to activate cytoplasmic proteins. Such a conformational change often makes the cytoplasmic part become enzymatically active, using ATP to phosphorylate specific tyrosine residues of particular proteins. Thus, this type of receptor is often called a **receptor tyrosine kinase (rTk)**. The active receptor can now catalyze reactions that phosphorylate other proteins, and this phosphorylation in turn activates their latent activities. Eventually, the cascade of phosphorylation activates a dormant transcription factor or a set of cytoskeletal proteins.

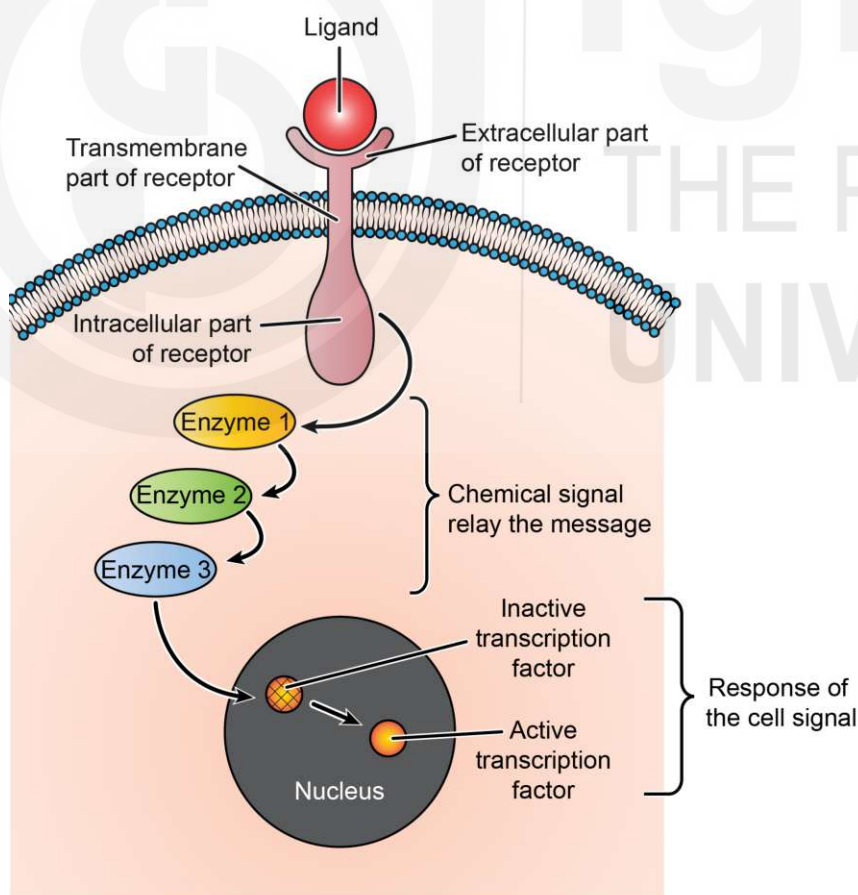


Fig. 11.6: Structure and function of a tyrosine kinase receptor.

Signal transduction pathways that end in expression of a gene in the target cell are generally slower than those that enzymatically activate biochemical pathways or regulate cytoskeletal elements for movements.

Cell signals can be categorised according to the distance travelled in the body as: autocrine, paracrine, direct contact and endocrine (see Table 11.1).

Table 11.1: Types of cell signalling

Paracrine signalling	Takes place over short distances between cells by diffusion
Juxtacrine signalling	Takes place between cells in direct contact
Autocrine signalling	Signals are received by the same cell that sent them or from adjacent cells of the same kind
Endocrine signalling	Signals are carried over long distances through the blood stream to the target cells
Direct signalling	Occurs through gap junctions between neighbouring cells. Allows flow of small molecules between cells

In an embryo, communication between cells can occur (i) across short distances, such as between two neighbouring cells through their cell membranes in direct contact, or between a cell membrane and extracellular matrix secreted by another cell called **juxtacrine signalling** (Fig.17.7 A&B), or (ii) between neighbouring cells through the secretion of proteins into the extracellular matrix, called **paracrine signalling** (Fig.11.7 C). In the next section we shall examine cell to cell interaction using these two types of communications.

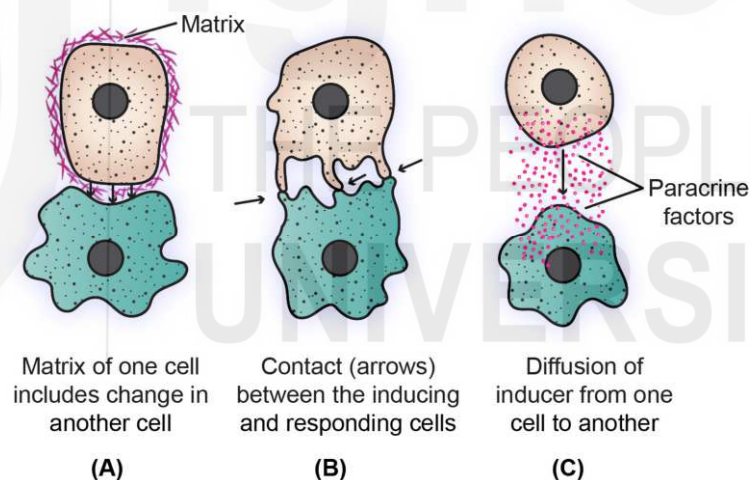


Fig.11.7: Modes of communication between cells of the embryo. A) Paracrine signalling in which one cell secretes a signalling protein or ligand into the environment and across some distance from many cells. Only those cells can respond that have receptors for that particular ligand. The receptor can respond, either rapidly through chemical reactions in the cytosol, or more slowly through the process of gene and protein expression; B) and C) Juxtacrine signalling is local cell signalling carried out via membrane receptors that bind to proteins in the extracellular matrix (ECM) or directly to receptors from a neighbouring cell.

11.2.4 Epithelial-Mesenchyme Transition

All the cell processes that we have learnt in the previous sub-sections are seen to be integrated in another morphogenetic process –the **epithelial-mesenchyme transition also known as EMT**. During this process the

stationary epithelial cells get detached from the basal lamina and change their identity to become migratory mesenchymal cells that can invade tissues and form organs in new places in the embryo. EMT is usually initiated by a paracrine signal from neighbouring cells that initiates gene expression in the target cells. This gene expression instructs the target cells to downgrade their cadherins, release their attachment from their basal laminin, rearrange their cytoskeleton components and secrete the extracellular matrix molecules that are characteristic of mesenchymal cells. EMT can take place involving individual cells or the collective epithelial cells (Fig.11.8)

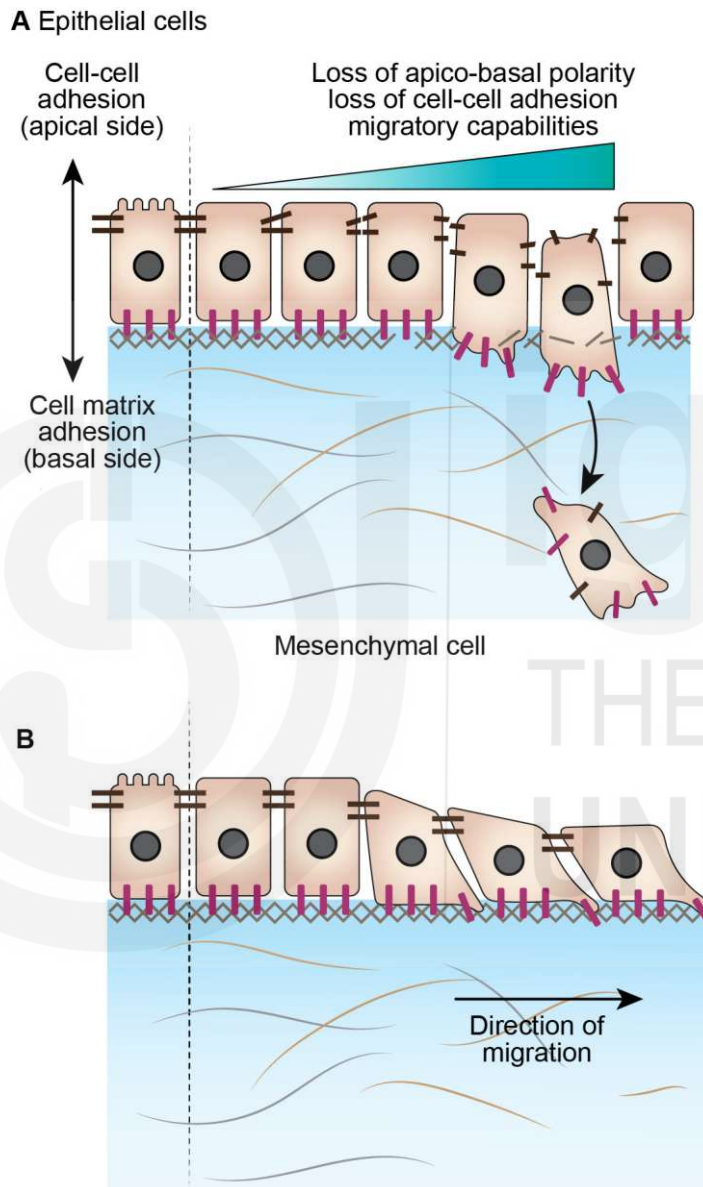


Fig.11.8: Epithelial –mesenchyme transition. A) individual epithelial cells can detach and change into mesenchymal cells; B) a sheet of epithelial cells that moves along the front end towards the direction of migration.

This EMT is very important in the developmental process. For example, it is seen in the formation of neural crest cells from the dorsal most region of the neural tube in fish, birds and mammals. It is also seen in the formation of chick and mouse mesoderm where cells that were part of the ectoderm detach and convert into mesodermal cells that migrate into the interior of the embryo; and in cells that detach from the somites and migrate around the developing spinal cord to ultimately form the vertebrae. In all these cases the epithelial cells lose

their “epithelialness” that is the breakdown of the basal cadherins-intercellular matrix adhesion and cadherin–cadherin junction at the apical region of the cells, thus loss of cell to cell adhesiveness before they begin to behave like mesenchymal cells and migrate.

In adults the process of EMT is needed for wound healing, regeneration of tissue and is the cause of metastasis of cancer cells, where the solid tumour cells detach from the tumour and migrate to other parts to form more solid tumours.

SAQ 3

- a) How would you define a ligand in cell- to cell signalling?
- b) What is the difference between juxtacrine and paracrine signalling?
- c) How is EMT used in the embryo and in the adult?

11.3 CELL–CELL INTERACTION

In the last section we discussed the role of cell adhesion, cell motility and cell signalling as crucial processes in morphogenesis. We should also realise by now that from the early stages of embryogenesis, cells do not function in isolation or in a random manner. All cell behaviours like cell adhesion, cell migration, differentiation and cell division are regulated by signals being passed from one set of cells to another. These cell to cell interactions allow the embryo to get its form and shape. But how do organs develop in their proper place in the embryo? And how do cells “know” that they have to migrate and position themselves? **Pattern formation** is the process by which the cells find their positional information. There are two general modes of pattern formation: (i) through use of **morphogen** gradients and (ii) by **sequential induction**. Let us first take a look at the role of morphogen gradients.

11.3.1 Morphogen Gradients

Pattern formation in the embryo can involve gradient of chemical signals known as morphogens. This term was coined by Allen Turing in 1952 for substances whose distribution through diffusion would determine the development of cells which would respond to different threshold concentrations of the morphogen. This is a type of paracrine signalling in which the concentration of morphogen is high near the source of release and becomes lower as the distance of the responding cells increases from the source. Morphogens can consist of cytoplasmic proteins such as transcription factors that can form a concentration gradient in a single cell or syncytium as seen in early embryo of *Drosophila*, or secreted as signalling molecules that travel from cell to cell. In most cases, the responding cells find their positional information because of the different concentrations of morphogens along its gradient. Thus morphogens guide the formation of different cell types in a specific positional order by inducing transcription of genes in a dose

dependent manner. Let us understand this with a hypothetical example. In Figure 11.9 we see a plane of unspecified cells in a region of the developing embryo. Out of these cells type **A** cell have matured enough may be due to maternal cytoplasmic determinants (refer to Unit-10) so that their function is specified. **A** type cells begin to express a signalling protein that functions in this case as a morphogen. The other cells seen in the figure (B, C, and D) are still unspecified in their cell fate but are **competent** to respond to this particular morphogen as they have the receptors to bind to the morphogen. As the morphogen is secreted the cells closest to the signalling cells come in contact with the signal first. Over time the morphogen diffuses and a concentration gradient is formed as the cells closest to the source experience more ligand-receptor binding and for longer duration than the cells located farther away from the source. Cells show a differential response to the morphogen in term of the duration and the concentration of the morphogen they are experiencing. As a result they differentiate and mature by different pathways.

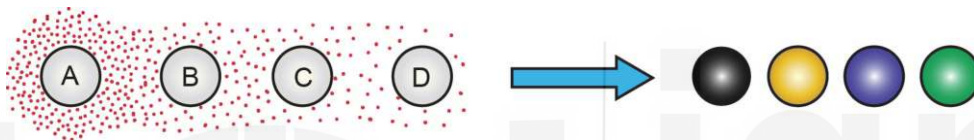


Fig. 11.9: Theoretical morphogen gradient induces different cell types. A type cells release the morphogen shown in red and depending on the concentration gradient and duration of exposure different cell fates are seen for cell type B, C, and D

In this way a morphogen gradient describes a mechanism by which cells of one part of the embryo can determine the location, differentiation and fate of many surrounding cells and provide a basis for understanding many patterning processes. The role of these signals range from establishment of initial polarity of embryos to specification of cell identity in particular tissues, notably in limb appendages and nervous system of both vertebrates and *Drosophila*. The best studied example of morphogen action is in *Drosophila* embryo which you have studied in the previous unit (Refer to subsection 10.6.1). The graded distribution of Bicoid a transcription factor from the anterior end to posterior end acts as a morphogen that establishes antero-posterior polarity in early embryo. Two different genes are expressed one named orthodenticle by high levels of Bicoid and the second named hunchback gene by low levels of Bicoid.

11.3.2 Embryonic Induction

The embryonic process during which the close range influence of one cell or group of cells on adjacent cell/cells resulting in the change of cell behaviour like change in shape, mitotic rate, cell fate is called **embryonic induction**. There are at least two components to every inductive interaction-the **inducer**, which is the tissue that produces a signal or signals that change the cell behaviour of the receiving tissue or **responder**. Not all tissues are capable of responding to the signals produced by the inducer, only the tissues that have the ability to respond can be induced. This ability to respond is called **competence** which is an acquired condition.

Let us understand this phenomenon by taking the example of the development of the vertebrate eye. When the development of eye is initiated, two bulges are seen in the brain that approach the surface ectoderm in the head region. The head ectoderm is competent to respond to the paracrine factors released by the brain bulges that are the **optic vesicles**. The head ectoderm cells are induced to form the **lens** tissue of the eye that is, the genes for expressing the lens protein are activated. The prospective lens cells in turn secrete another paracrine factor that instructs the optic vesicle to form the retina. *Note that the two cell types that co-construct the eye induce each other.* The important part is that the head ectoderm is the only region in the embryo that is competent to respond to the instructions from the optic vesicle signals. Experiments done with *Xenopus laevis* embryo show that if the optic vesicle is placed in another part of the head ectoderm it still can induce lens tissue but if the optic vesicle is implanted anywhere else say underneath the trunk ectoderm it will fail to induce the lens tissue, showing that only the head ectoderm is competent to respond to the inducing signal (see fig.11.10).

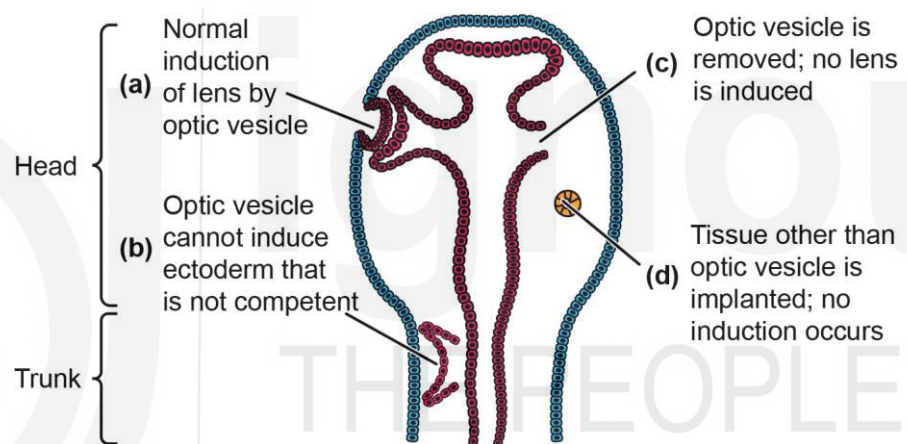


Fig. 11.10: Ectoderm's ability to respond to the optic vesicle inducer in *Xenopus* is due to competence of ectodermal cells in the head region. The optic vesicle is able to induce lens formation in the anterior portion of the head ectoderm a); b) but not in the presumptive trunk region; c) If the optic vesicle is removed no lens is formed by the overlying head ectoderm; d) most of the other tissues implanted under the head ectoderm are not able to substitute for the optic vesicle.

Further study in amphibians has shown that often one induction will give a tissue the ability to become competent to respond to another induction in a sequential manner. Recall that we had said that the competence is an acquired condition and in amphibians the head ectoderm becomes competent to be induced by the optic vesicle only through sequential signals from the underlying foregut endoderm and heart forming mesoderm in the early gastrula. The next signal is given by the anterior neural plate which instructs the synthesis of a transcription factor called PAX6 which is required for ectodermal competence to respond to the optic vesicle. *Thus we see that though the optic vesicle appears to be the inducer of lens formation the ectodermal cells had been made competent by at least two other tissue.* Their effect on the ectoderm is synergistic and cumulative. As lens induction begins at the gastrula stage the competence of the non-neuroal ectoderm becomes restricted to the head ectoderm in the regions fated to form the lens.

Another important feature of embryonic induction is that an inducer tissue is often induced by the receiver tissue as well. For example once the lens is formed it induces the optic vesicle to become the optic cup and the walls of the optic cup become distinguished to form two layers one, the neural retina and second, the pigmented retina. Such induction is called **reciprocal induction**.

At the same time the lens also induces the ectoderm above it to form the cornea. The cornea forming ectoderm also acquires its competence to respond to the signals from the lens tissue. Under the influence of the lens the corneal ectoderm becomes columnar and secretes multiple layers of collagen. The mesenchyme cells from the neural crest use this collagen matrix to enter the area and secrete proteins that further differentiate the cornea. A third signal which is hormonal, dehydrates the tissue and makes it transparent. Thus we can see that there is a sequence of induction events as shown in the development of eye in Fig.11.11.

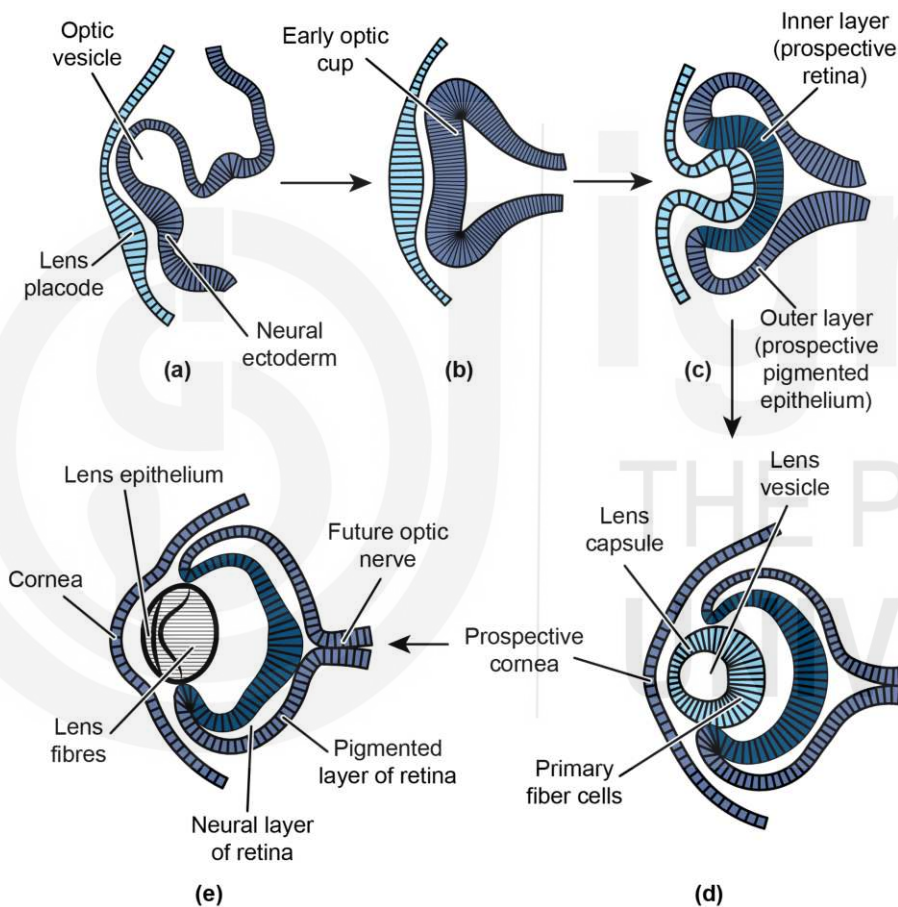


Fig. 11.11: Induction of lens in mouse. a) the optic vesicle extends toward the surface ectoderm from the forebrain. The lens placode (the prospective lens) appears as a local thickening of the surface ectoderm near the optic vesicle b) the lens placode enlarges and the optic vesicle has formed an optic cup; c) the central portion of the lens-forming ectoderm invaginates, while the two layers of the retina become distinguished; d) the lens vesicle has formed; e) the lens consists of anterior cuboidal epithelial cells and elongating posterior fiber cells. The cornea develops in front of the lens. The whole process takes about 4 days starting from day 9 in the embryo.

Induction interactions are called **instructive** if the signal from the inducing cell causes a *new gene expression in the responding cell*. In this case without the inducer cell the responding cell is not capable of differentiation into a particular

cell type. Example of instructive interaction is when the optic vesicle of *Xenopus* is placed in another part of the body's ectoderm and it fails to induce the lens. **Permissive** interactions during induction are seen when the responding tissue is already specified but needs the environment to express its tissue characteristics. A good example of permissive interaction is the extracellular matrix that is often required by tissues to develop. The matrix itself does not alter the tissue type but allows the already specified tissue to express the traits of the tissue they are supposed to be. Thus, permissive interactions lend to regulate the degree of expression of the remaining developmental potential of the already specified cells.

SAQ 4

Match the following statements with the given terms:

- i) Permissive Interaction
- ii) Morphogens
- iii) Competence
- iv) Instructive interaction

Statements

- 1) Compound or molecule that is diffusible and influences the movement and organisation of cells as per a gradient.
- 2) High levels of transcription factor produces cell type B and low levels of transcription factor A produces tissue type C
- 3) A signal from the inducing cell that is required to initiate a new gene transcription
- 4) The responding cell is already specified but its degree of expression is regulated by the inducer signal.
- 5) In the presence of tissue A tissue B develops in a certain way but in the absence of tissue A it does not develop in that way.
- 6) Signals from Tissue A induce the formation of a cell type B in one region of the embryo but when tissue A is transplanted to another region of the embryo it fails to induce the cell type B.

11.4 CELL DEATH

Death is the ultimate event in the life of all organisms. Death and degradation of cells takes place at particular times. Death of cells is also induced in cases of acute injury and infection, DNA damage, toxicity and trauma or when cells turn cancerous. Cell death may occur through 'cellular suicide' with the help of the intracellular organelles called lysosomes (autophagy); or when induced by acute injury or infection (necrosis); or through programmed cell death (apoptosis) Apoptosis is genetically controlled through a pre-designated

pathway. Autophagy and Necrosis cause cell death through alternate pathways.

11.4.1 Apoptosis

Apoptosis (*both p are pronounced*) was a term coined in 1972 for the process of active cell death resulting in the orderly breakdown of cellular structures. This is also known as **programmed cell death** since apoptosis is triggered by the expression of specific genes and blockage of mRNA or protein synthesis. It was best understood in the nematode *C elegans* where out of a total of 1090 somatic cells 131 undergo programmed death during development. This number of dying cells is invariant in the species. Since then the significance of programmed cell death was established in developmental biology of other species for genetically determined elimination of cells. Apoptosis occurs normally during development and aging as a homeostatic mechanism to maintain cell populations in tissues and as a defence mechanism in immune reactions and diseased conditions. Apoptosis is often initiated by removal of growth factors from cells and sometimes as an active response to a signal during the developmental process. For example, it is the process responsible for elimination of tissues during finger and toe formation in limbs of vertebrates, and loss of the tail in tadpoles during metamorphosis.

Some of the major events of apoptosis include chromatin condensation and nuclear fragmentation. As apoptosis proceeds, individual cells or clusters of cells become increasingly round and undergo **pyknosis** (the reduction of cell volume), the membrane bulges out in small portions known as **blebs** and organelles undergo modifications. However the cell does not lose its membrane integrity and the cell's contents are not released into the intercellular spaces and no inflammatory responses occur. The dying cells are usually phagocytosed typically by macrophages to prevent secondary necrosis. Apoptosis is an energy-dependent process. Three major biochemical events occur during apoptosis:

- i) **DNA and protein breakdown:** Early apoptosis is characterized by the breakdown of DNA into 50-300kb fragments that are then cut into oligonucleotides by endonucleases. This results in DNA fragments of a range of lengths.
- ii) **Activation of caspases:** Late apoptosis is characterized by the activation of cysteine proteases known as caspases that break down the protein and cytoskeletal components of the cell and activates the enzyme DNase, which continues the degradation of the cell's DNA. Caspases breakdown about 100 cellular proteins which bring about changes in the cell which are characteristic of programmed cell death or apoptosis, for example out of a family of a dozen caspases, one is inhibitor of DNA and causes DNA fragmentation; another called nuclear lamin brings about nuclear fragmentation. A cascade of caspases breaks the cell into small membrane-covered vesicles by folding the cell inwards. The cell membrane remains intact during the process despite vesicle or blebs budding on the cell surface. As apoptosis progresses, these cells will lose the cell-to-cell adhesions and will separate from the neighboring cells (Fig 11.12).

- iii) **Membrane modifications:** In which an intracellular transmembrane protein, is exposed on the surface by which phagocytes recognise the dying cell.

The process of apoptosis is divided into two pathways: the **intrinsic pathway** if the apoptosis is initiated in the mitochondria and the **extrinsic pathway** which is started by the binding of signalling molecules or ligands to transmembrane receptors that involve death receptors domains on the intracellular side and these lead to a cascade of reactions that start the apoptotic process.

11.4.2 Necrosis

Necrosis another cell death process is different from apoptosis. It can happen simultaneously or independently of programmed cell death. Necrosis is accidental death induced by an acute injury or a deadly disease or DNA damage as opposed to programmed cell death. Necrosis, therefore, is not a naturally occurring phenomenon but is induced by serious injury. In Greek necrosis means death.

Necrosis is considered to be a toxic process which is uncontrolled and passive that is, it does not require an energy source to take place. It affects a large field of cells and is mediated by two main mechanisms- injury to the cell membrane and interference with the supply of the energy to the cells. Some of the major morphological changes that occur with necrosis include cell swelling; formation of cytoplasmic vacuoles; disruption of ribosomes and organelle membranes; swollen and ruptured mitochondria and lysosomes; and eventually disruption of the cell membrane. This loss of cell membrane structure results in the release of the cytoplasmic contents into the surrounding tissue (Fig.11.12).

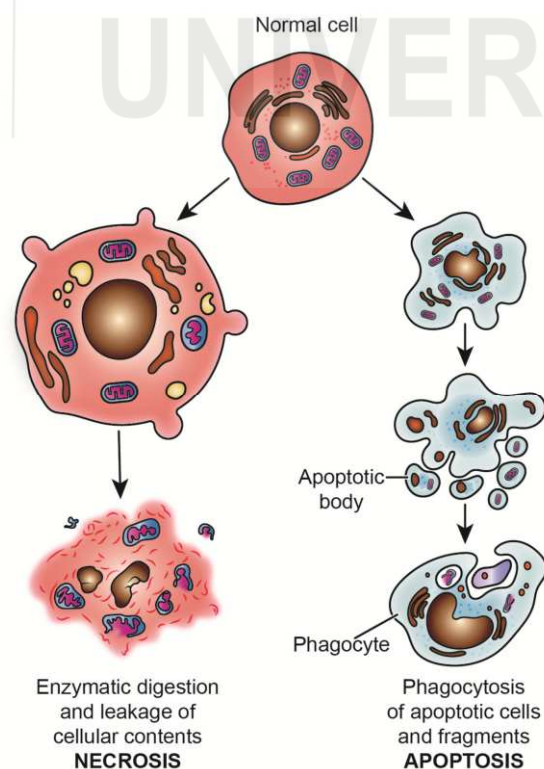


Fig. 11.12: Cell death by necrosis and apoptosis.

The unregulated release of products of cell death to the outside, initiates inflammatory response in the surrounding tissue. Leucocytes and nearby phagocytes eliminate the dead cells through phagocytosis. Damaging substance from leucocytes can cause damage to surrounding tissues and if untreated, necrosis results in building up decomposing dead tissue and cell debris at or near site of necrotic cell death (e.g. gangrene). Often necrotic tissue needs surgical removal.

11.4.3 Autophagy

Eukaryotic cells have their own method of removal of nonfunctional or damaged organelles or other cell components or even cells. Lysosomes are the organelles in the animal cell that carry out this function and hence they are also called “suicidal bags”. **Autophagy** is another form of cell death process by which lysosomes help to maintain homeostasis through recycling of the cells' own organic material (Fig. 11.13).

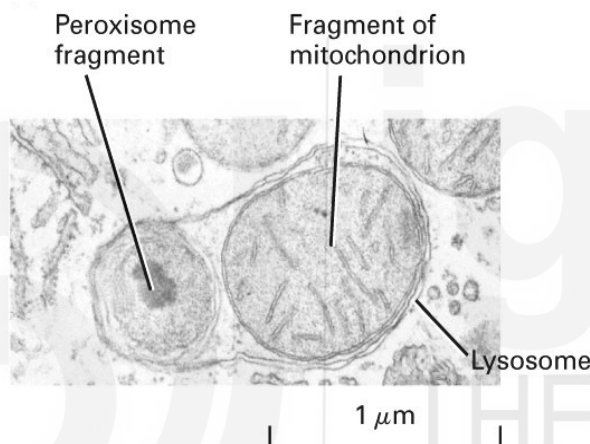


Fig.11.13: Electron micrograph of lysosome of a cell breaking down a damaged mitochondrion for its disposal.

The process of autophagy is evolutionary conserved, meaning whereby that the process of autophagy is the same in single celled organisms like yeast to humans. Similar to apoptosis, autophagy has an important role in development processes, human disease and cellular response to nutrient deprivation. In some circumstances, a correlation has been documented between reduced autophagy and cancer suggesting that autophagy is a safeguard mechanism against unrestricted cell growth. This self-degrading process is used for three key reasons and they are: (i) To recycle proteins and organelles that do not pass quality control or are in excess in an effort to maintain homeostasis, (ii) To sustain the cell in times of need by degrading less-essential cellular components as an alternative fuel source, and (iii) To defend itself against pathogens.

There are three main forms of autophagy known as macroautophagy (usually referred to as autophagy), microautophagy that involves the transfer of the cytosolic cargo directly into the lysosomes through membrane invagination and chaperone mediated autophagy in which proteins known as chaperones accompany the cargo inside the lysosome (Fig. 11.14).

The role of autophagy in cell death can be:

- i) Autophagy – associated cell death where induction of autophagy accompanies apoptosis or some other cell death pathway.
- ii) Autophagy - mediated cell death where autophagy pathways activate apoptosis pathways.
- iii) Autophagy – dependent cell death which occurs independent of both apoptosis and necrosis.

Autophagy is influenced by several genes, which if lost can result in some cancers.

The characteristic morphological features of autophagy are: enclosing cytoplasmic organelles in double or multi-membrane vesicles and delivering them to the cell's own lysosomes for degradation. In one sense the cell eats itself! The process of autophagy is dependent on constant protein synthesis and ATP. In general there are 4 stages in the process – (i) Induction which may be due to intracellular stimuli or signals to stimulate the protein **kinase**, a regulatory protein. (ii) formation of autosomes in which the cellular material to be disposed of is broken down and is called 'cargo' which is then packaged into an autophagosome (iii) fusion with lysosomes or vacuoles, (iv) autophagy body breakdown and recycling.

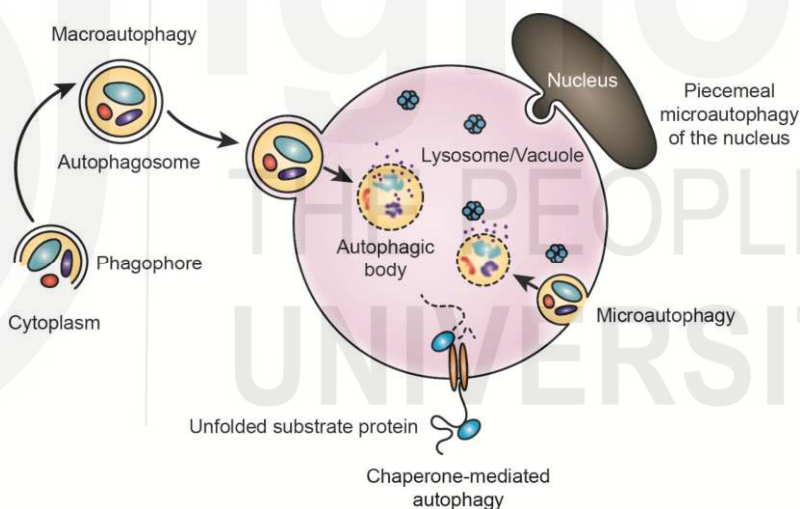


Fig 11.14: Various forms of autophagy and how they take place.

Macroautophagy in which the organelles and cytoplasmic parts are enclosed in a autosome that merges with the lysosome.

Microautophagy in which the cytoplasmic cargo is drawn in to the lysosome by involution of the membrane and **chaperone mediated autophagy** in which the cargo is delivered by the assistance of a chaperone protein into the lysosome.

SAQ5

In the table given below, fill in at least four distinguishing characteristics of apoptosis, necrosis and autophagy.

	Apoptosis	Necrosis	Autophagy

11.5 SUMMARY

In this unit you have learned that:

- All embryonic cells are either epithelial cells which are tightly connected to one another in sheets or tubes or mesenchymal cells which are unconnected or loosely connected. The process by which these cell types in the embryo get organised to give form and structure to the embryo is known as morphogenesis.
- Morphogenetic processes that give structure to the embryo involve key cell properties like cell movement, cell adhesion and cell to cell communications. Cell movements and cell shape changes take place by the rearrangement of the internal cytoskeleton made up of microfilaments and microtubules. Cells typically move by a crawling motion which is best seen and studied in fibroblast cells both in vivo and in vitro conditions. Moving cells in the embryo use the intercellular matrix as a substratum forming pathways for migration.
- Animal embryonic cells stick to one another and to the extracellular matrix due to cell surface proteins known as adherins. These belong to cadherin family or integrins molecules. Cadherins are transmembrane proteins and interact with other cell cadherins of the same kind for the cell to cell adhesions. Their intracellular domains consist of a protein known as catenin which also binds to the cytoskeleton filaments in the cell. Integrins bind the cell to the extracellular matrix. Adherin molecules are responsible for cell sorting and maintaining tissue boundaries.
- Cell to cell communication occurs by use of chemical signalling. The signalling proteins called ligands bind to receptor or proteins on target cells to set up a signal transduction pathway that often controls the gene expression of the target cell. Cell signalling can be categorised according to distance travelled in the embryo as paracrine, autocrine, juxtacrine and endocrine.
- An example of cell to cell interaction is seen in the epithelial-mesenchymal transition that can involve individual cells or sheets of epithelia in the embryo. EMT is also seen in the adult in the process of wound healing, regeneration and is crucial in cancer metastasis.
- Pattern formation is the result of cell to cell interaction. It is the process by which the cells find their positional information in the embryo. The two general modes of pattern formation are by morphogen gradients and through sequential embryonic induction. As the cells are exposed to different concentrations of the morphogens in a gradient they express different genes at different times and locations. Induction involves the influencing of a set of cells by another set of cells or a cell so that the behaviour of the influenced cells/cell changes. However only those target cells can be influenced by induction that are competent to respond to the influencer, signals.
- The development of the vertebrate eye is a good example of embryonic induction event. Often one induction event is necessary for the target cell to induce another target tissue leading to a cascade of induction events as seen in the development of lens of eye.

- Programmed cell death or apoptosis is also important in development. Apoptosis is a means of cell death without the spilling of cell contents into the surrounding. It involves molecular pathways where proteases like caspases cause the nucleus to condense and cell to shrink, displaying signals on the surface to attract phagocytes. Other cell death processes in organisms are necrosis and autophagy which have different pathways from apoptosis.

11.6 TERMINAL QUESTIONS

1. In what ways can inducing signals be transmitted in the developing embryo? Explain using examples.
2. What are cadherins? What is their role in cell to cell interactions?
3. Mark the following statements as true or false.
 - i) During development cells move by extending their cell processes in all directions without any polarity
 - ii) The cytoskeleton of the cell is actively involved in cell movement and cell shape change.
 - iii) Cadherin on one cell binds strongly to similar cadherins molecules on other cells
 - iv) Integrins are cell adhesion molecules that attach cells to other cells at the apical region
 - v) Permissive interaction involves gene expression in the induced cell
 - vi) Morphogens are paracrine signalling molecules
 - vii) In apoptosis the cell organelles are delivered to lysosomes and the cell contents are spilled out
 - viii) Cell death by necrosis indicates that cell has undergone a toxic reaction
 - ix) Optic cup is induced to form the retinal layers by reciprocal induction from the lens
 - x) Pattern formation is the result of morphogenesis
4. Define morphogenesis and list the cell behaviours responsible for it.
5. Why is cell death a necessary event in embryonic development?
6. What is a signal transduction pathway? Write out its step in the order they would occur in a target cell.

11.7 ANSWERS

Self-Assessment Questions

1.
 - i) cytoskeleton; adhesion
 - ii) extracellular matrix.
 - iii) chemical signals

2.
 - i) Differential adhesiveness of cadherins interactions. Similar cadherins attach to each other on cell surfaces while dissimilar cadherins do not attach or have very weak attachments.
 - ii) Cadherins depend on calcium and integrins are not calcium dependent. Cadherins bind to other cells and to the cytoskeleton while integrins bind the cells to the extracellular matrix and to the cytoskeleton.
3.
 - i) A small molecule that is released by one cell and binds to a complementary receptor on the membrane of the target cell or in the cytosol.
 - ii) Juxtacrine signalling happens when there is direct contact between the signalling molecule on a cell and receptor molecule of another cell while paracrine signal happens when a signal molecule is released from a cell and received by another cell which may or may not be a neighbouring cell.
 - iii) EMT is responsible for the formation of neural crest cells that are migratory and mesenchymal in behaviour from the dorsal most ectodermal cells of neural tube. These cells are epithelial in their behaviour.

In adults the process of EMT is needed for wound healing, regeneration of tissue and is the cause of metastasis of cancer cells, where the solid tumour cells detach from the tumour and migrate to other parts to form more solid tumours.

4. Statement i)-morphogen; statement ii)-morphogen gradient; statements iii) and v)- instructional induction; statement iv) -permissive interaction; statement vi)-competence
5. Distinguishing characteristics

	Apoptosis	Necrosis	Autophagy
1.	Programmed cell death Requires energy	Independent cell death not programmed, does not need energy	Signal comes from genes
2	Membrane of cell not disrupted contents of cell do not spill out, blebs formed	Injury to cell membrane, unregulated cell death, contents spill out	Lysosome mediated recycling of cell contents, to maintain homeostasis
3	No inflammation in surrounding tissue removed by phagocytes	inflammatory damage to surrounding cells, forms scars often has to be removed surgically	Autosomes formed that merge with lysosomes that breakdown the cellular components
4	Important in development of digits by elimination of cell populations,	Reaction to toxic event in the body	For homeostasis and recycling of cellular components

Terminal Questions

1. Refer to Section 11.3
2. Refer to Subsection 11.2.2
3. i) F; ii) T; iii) T; iv) F; v) F; vi) T; (vii) F; viii) T; ix) T; x) F
4. Refer to Section 11.2
5. To maintain cell numbers in tissue and organs, for homeostasis, to eliminate extra cells and tissues during morphogenesis and organogenesis e.g., formation of digits in vertebrates for recycling of cell content in times of need.
6. A signal transduction pathway is the series of events that take place in a target cell when a signal or ligand is received by a competent receptor on the cell. It is generally culminated in gene expression or in biochemical reactions inside the cell or regulate cytoskeleton elements.

Steps in the transduction pathway:

- 1) When a ligand binds to a transmembrane receptor it changes the shape of the receptor
- 2) The receptor activates a protein in the membrane on the intracellular side
- 3) Binding to phosphate groups and other enzymatic reactions are enabled in several steps
- 4) Ultimately the cell response is effected. If the signal is passed on to the nucleus, it will activate or repress a gene, or other biochemical reactions are elicited in the cytoplasm or cytoskeletal components are regulated.

UNIT 12

BEGINNING OF A NEW ORGANISM

Structure

12.1	Introduction	Formation and Maturation of Ova
	Objectives	Oogenesis in Amphibians
12.2	Modes of Reproduction in Eukaryotic Animals	Oogenesis in Mammals
	Asexual Reproduction	Egg Envelopes of Vertebrates
	Sexual Reproduction	12.5 Fertilization
	Sexual Reproduction in Multicellular Animals	Events Prior to Sperm-Egg Fusion
12.3	Spermatogenesis	Basic Events of Fertilization
	Phases of Spermatogenesis	Regulation of Sperm Entry in the Egg
	Spermiogenesis	Fertilization in Mammals
	Spermiation: Activation of Sperm	12.6 Summary
	Structure of Sperm among Vertebrates	12.7 Terminal Questions
12.4	Oogenesis	12.8 Answers

12.1 INTRODUCTION

In the previous two units you have studied about the universal principles that govern animal development and the various interactions that occur among cells during the process of development of an organism. You would have realized that when studying developmental biology you will have to take inputs simultaneously from morphology, genetics and molecular biology. In this unit, you will learn that continued generation of new individuals of a species is known as reproduction and that in most species especially in vertebrates, reproduction involves the transfer of genetic material from two individuals of a species to produce offsprings, thus ensuring the continuity of the species. You also will study that animals reproduce by asexual or sexual reproduction or by a combination of these two reproductive modes. Most vertebrates however,

reproduce by producing specialized cells or gametes that are generated by a process known as gametogenesis, which results in the formation of the sperm in males and ovum(plural:ova) in females. This will be followed by a study of the structure of sperm and ova. In addition, you will learn the various events associated with the fusion of sperm and egg, and subsequently the fusion of their pronuclei—the processes collectively known as fertilization. Fertilization results in the formation of a zygote which will give rise to a new organism. The process of development of the zygote into a fully formed new organism will be dealt in later units.

Objectives

After studying this unit you should be able to:

- ❖ discuss the different types of reproduction in animals;
- ❖ explain the process and purpose of gametogenesis;
- ❖ outline the mechanism of fertilization and explain how fertilization restores diploidy in most of the multicellular organisms;
- ❖ discuss the significance of activation of egg in the event of fusion of egg and sperm;
- ❖ list the events that prepare the fertilized egg for the next phase of development namely the cleavage.

12.2 MODES OF REPRODUCTION IN EUKARYOTIC ANIMALS

Reproduction is one of the main properties of life, Evolution is closely linked to reproduction, because the continuous replacement of aging parents with new offsprings provide the animal populations the means to adapt to a changing environment.

Two modes of reproduction occur in organisms:1) asexual and 2)sexual.

Animals of some taxa can reproduce both asexually and sexually. Unicellular, eukaryotic animals the protists earlier known as protozoan reproduce asexually for the perpetuation of the progeny. Several invertebrate animal phyla, such as cnidarians, bryozoans, annelids, echinoderms, and hemichordates reproduce asexually. Asexual reproduction is however, rare among vertebrates though it has recently been reported in some lower vertebrates such as in hammer- headed sharks.About at least 50 vertebrate species reproduce by parthenogenesis; some do not involve meiosis or involve only the early stages of meiosis before chromosome reduction occurs. Thus, the progenies due to parthenogenesis are essentially clones and could be categorized as asexual reproduction.

12.2.1 Asexual Reproduction

In asexual reproduction offsprings arise from a single parent or organism, and inherit the genes of only that parent. Thus, in organisms that reproduce only

asexually, special reproductive organs or cells are absent. Furthermore, in asexual reproduction gametes are not produced thus, fertilization of gametes does not occur. In addition, the number of chromosomes (unless mutations occur) almost never change during the entire process of asexual reproduction. Box 12.1 shows the types of asexual reproduction that occur in protists and some invertebrates. You can read Unit 1 of the course on Animal Diversity again to recall the modes of reproduction

Box 12.1

Asexual reproduction occurs in all protists and may occur by means of any of the following processes: i) fission (binary or multiple fission (schizogony), ii) budding iii) gemmulation, and iv) fragmentation.

- i) **Fission-** In fission the single celled eukaryote organism divides mitotically, into two or more parts and each separated part regenerates the missing structures to reform a complete separate organism which is identical to the original parent cell. The fission may be: (1) **binary fission**, or (2) **multiple fission**, in which a single entity produces multiple organisms.

In **Binary fission** the single organism produces two organisms or offsprings. In binary fission the organism may divide lengthwise, as in flagellate protozoa, or may divide transversely, as in ciliate protozoa. In **multiple fission**, or **schizogony**, the nucleus divides repeatedly before the division of the cytoplasm. This process thus, produces many daughter cells simultaneously. Spore formation, called sporogony, is a form of multiple fission. It is common among some parasitic protists, for example, in malarial parasites.

- ii) **Budding-** It is an unequal division of an organism. A new individual arises as an outgrowth (bud) from its parent, develops organs like those of the parent, and then detaches itself. Budding occurs in several animal phyla and is especially prominent in cnidarians, like *Hydra*.
- iii) **Gemmulation-** In gemmulation, new individuals are formed on the parent in the form of gemmules. A gemmule is an aggregation of cells, surrounded by a resistant capsule, which can survive under unfavorable conditions, like winter, even when the parent organism cannot survive. In many freshwater sponges, gemmules develop just before winter. This enables the gemmules to survive the cold temperature of winter in the dried or frozen body of the parent. In spring, the enclosed cells become active, emerge from the capsule, and grow into new sponge organisms.
- iv) **Fragmentation-** In fragmentation a multicellular animal breaks into two or more parts or fragments. Each of these fragments is capable of becoming a complete individual. A number of invertebrates reproduce asexually by simply breaking into two parts and then regenerating the missing parts of the fragments, for example, most anemones and many hydroids reproduce by fragmentation. Many echinoderms can regenerate lost parts, but this is not the same as reproduction by fragmentation.

12.2.2 Sexual Reproduction

Sexual or **bisexual** reproduction, as a rule involves two parents, each of which is diploid (having full genome quantity). Each parent contributes special **germ cells called gametes** or **sex cells** that are haploid as they contain half the amount of genome complement. In this type of reproduction each gamete is incapable of independently developing into a new individual. Once the two gametes sperm and ovum unite during the process of fertilization; they form a diploid **zygote**, which develops into a new individual. Thus, the union of the haploid gametes restores diploidy in the offsprings and results in offsprings having new genotypes that would be different from that of either parent. The individual formed from the zygote would have the characteristics of its species and may have some features similar to its parents. However, it would also have features that distinguish it from its parents as sexual reproduction would cause a recombination of genetic material received from both parents. The new genetic combinations of individuals that are produced add into the population of the species, and are responsible for the evolution of that species.

The processes of gamete formation, reproduction and fertilization occur sequentially and in many cases the preparation for one phase is initiated with the onset of the preceding phase.

The crucial event that enables sexual reproduction and distinguishes it from asexual reproduction is the distinctive type of cell division called meiosis or meiotic division that takes place during gamete formation in sexually reproducing animals. This type of cell division is a double division and differs from ordinary cell division (mitosis or mitotic division). In meiosis, the chromosomes during gamete formation duplicate once, but the cell divides twice, producing four cells, each with half the original number of chromosomes (the haploid number).

12.2.3 Sexual Reproduction in Multicellular Animals

Nearly, all vertebrates and many invertebrates have separate sexes, and such a condition is called dioecious (Gr. *di*, two, + *oikos*, house). In sexually reproducing multicellular eukaryotes, the gametes produced by the males (sperms) and females (egg or ovum), differ in size, mobility and in the quantity they are produced in. The ovum produced by the female, is large because it is the primary source of cytoplasm for the zygote and often contains stored yolk to sustain early development. In addition, the ova are non-motile, and are produced in relatively small numbers. The spermatozoa (sperms), produced by the male, on the other hand, are small, motile, and are generated in enormous numbers. Each sperm is a stripped-down package of highly condensed genetic material designed for the single purpose of reaching and fertilizing an egg.

The male-female distinction is evident in most animals. Organs that produce germ cells are called **gonads**. The male gonad called **testis (plural: testes)** produces sperms which are the male gametes and the female gonad called **ovary** produces female gametes called eggs or ova(**sing: ovum**). The **gonads** represent the **primary sex organs**, which are the only sex organs

found in certain groups of animals. Most animals, however, have various **accessory sex organs** (such as penis, vagina, oviducts, and uterus) that transfer or receive germ cells. In the primary sex organs, germ cells undergo many complicated changes during their development, the details of which are described in this unit.

In a few animals, an individual parent produces both the sperms and the eggs as it has both male and female reproductive organs respectively. These animals are called monoecious (Gr. monos, single, + oikos, house) or hermaphrodites (from a combination of the names of the Greek god Hermes and goddess Aphrodite) and the condition is called **hermaphroditism**

Several invertebrate as well as a few vertebrate (some fishes) animals are hermaphrodites. Hermaphrodites can fertilize themselves, but most avoid self-fertilization and exchange germ cells with another member of the same species. Some fishes are **sequential hermaphrodites**, in which a genetically programmed sex change occurs within an individual organism. In many species of reef fishes, such as wrasses, an animal begins life as either a female or a male (depending on the species) but later becomes the opposite sex.

Parthenogenesis

Parthenogenesis ("virgin origin") is the development of an embryo from an unfertilized egg or one in which the male and female nuclei fail to unite following fertilization. Classification of parthenogenesis as either asexual or sexual reproduction is difficult, since there are many different patterns which however, will not be dealt with in this unit. Parthenogenesis is surprisingly widespread in animals. Asexual or sexual parthenogenesis occurs in various groups of invertebrates and in a few groups of vertebrates. Certain populations of whiptail lizards of the American southwest are clones reproduced by parthenogenesis.

Gametogenesis

In multicellular eukaryotic organisms, the reproductive process begins with the generation of gametes by the process of gametogenesis. The gametes are formed from **germ cells** in the embryo. The germ cells are referred to collectively as the **germ line**, consisting of cells that will or can become the future gametes, and all other cells of the body are referred to as the somatic tissues or soma. During embryonic development germ cells undergo multiplication and will also often undergo a migration from the site of their formation to the gonad, which may be some distance away. The gonads arise from mesoderm and are initially composed entirely of somatic tissues. After the germ cells arrive they become fully integrated into its structure, and in postembryonic life undergo gamete formation. The sperms in males are produced by the process of **spermatogenesis** while the ova in the females are produced by the process of **oogenesis**. **These two processes are collectively known as gametogenesis.**

In the following subsection you will learn in detail about spermatogenesis while oogenesis will be dealt with in the forthcoming subsection.

Studies of ovarian development in mammals suggest that parthenogenesis does occur, but that the resulting cells (**parthenotes**) fail to develop into offspring. Parthenotes of mouse and rabbit form a small group of pluripotent stem cells—cells that are capable of developing into any cell type. Similar findings in the human may ultimately provide stem cells for therapeutic use to replace defective or dying cells in such diseases as heart failure, diabetes, Parkinson's disease, and spinal cord injury.

SAQ 1

What is the distinction between the following pairs?

- i) Asexual and sexual reproduction.
- ii) Hermaphroditism and parthenogenesis.

12.3 SPERMATOGENESIS

The process of spermatogenesis produces mature male gametes, commonly called sperms but more specifically known as spermatozoa. In humans, the entire process of spermatogenesis is variously estimated as taking 74 days. The paired testes of human males produce 200 to 300 million spermatozoa daily. However, only about half or 100 million of these become viable sperm.

We had said earlier that the primordial germ cells (PGC) migrate from their site of origin to the site where gonads in case of males, the testes (Fig.12.1a), are formed. They lie in the germinal epithelium, near the outer edge of the testes. This germinal epithelium also contains the Sertoli's cells (Fig.12.1b) which are supporting cells and play a role in sex differentiation.

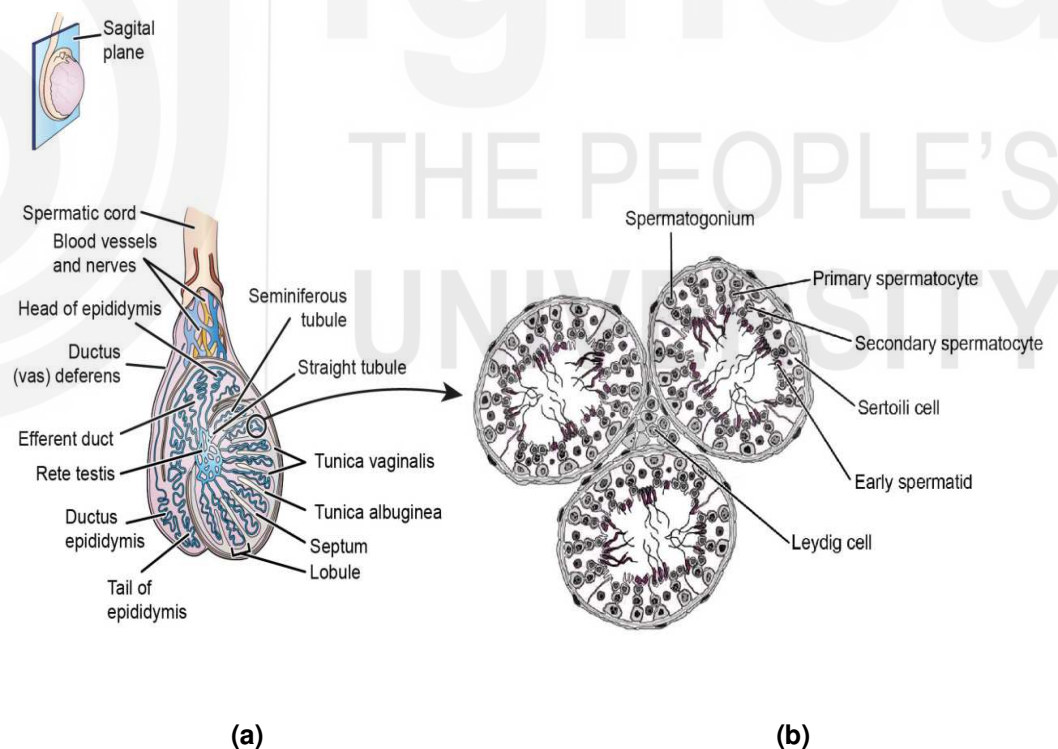


Fig.12.1: a) Saggital section of human testis showing seminiferous tubules; b) cross section of seminiferous tubules, showing Sertoli's cells, developing stages of sperms and Leydig cells between adjacent seminiferous tubules.

Spermatogenesis is a continuous process of various physical, chemical and morphological changes by which the spermatozoon is developed from the

undifferentiated mother cells known as the **spermatogonia**. Spermatogenesis takes place within the seminiferous tubules of the testis, through cell division and differentiation. Spermatogenesis in humans is initiated in the paired male testis at the beginning of puberty and usually continues uninterrupted until death; although a slight decrease in the quantity of production of sperms is seen with an increase in age. With the start of spermatogenesis at puberty, the germinal lining of the coiled seminiferous tubules exhibit two differing cell populations: (i) some are the Sertoli's cells while (ii) the majority are the germ cells in various stages of division and differentiation (refer to Fig 12.1 b again).

The Sertoli's cells in addition to playing a role in sex differentiation are also responsible for the barrier between "blood and testicles" (testicle refers to the testis present in men and other male mammals when enclosed in the scrotal sac) in the seminiferous tubules of human testes. This is of practical importance because haploid cells in the inner part of the tubule exhibit surface antigenic properties, different from all other body cells. Thus, they must thus be kept secluded from the immune system of the organism.

12.3.1 Phases of Spermatogenesis

The process of spermatogenesis (Fig. 12.2) can be broadly divided into three phases- (1) multiplication phase, (2) growth phase and (3) maturation phase.

1. Multiplication phase

Multiplication phase or the mitotic phase is the stage of multiplication or proliferation of the immature primordial germ cells within the seminiferous tubules of the testes.

The primordial germ cells which are located in the outer wall of the of the seminiferous tubules divide mitotically to give rise to the stem cells which further divide mitotically to produce spermatogonial cells, also called spermatogonia. Two types of spermatogonia are produced during the multiplication phase: i) "A type spermatogonia" and ii) "B type spermatogonia". A type spermatogonia, lack heterochromatin in their nucleus and continue to divide mitotically throughout the life span of the male human being, in order to provide additional spermatogonia so that there is a constant source of germ cells. Type B spermatogonia contain heterochromatin in the nucleus and are known as **primary sperm cells**. They are the prototypes of male sex cells that are destined to develop into mature sperm cells.

2. Growth Phase

Growth phase is characterized by the development of structural and functional features of the distinct sex cells. The primary sperm cells develop during this phase by increasing the amount of cytoplasm and developing the organelles within the cytoplasm. These **large, B Type spermatogonial cells** are now known as **primary spermatocytes** and their cytoplasmic and the nuclear contents become ready for subsequent changes. The primary spermatocytes now move towards the lumen of the seminiferous tubule and duplicate their DNA. The Growth phase is then followed by the maturation stage.

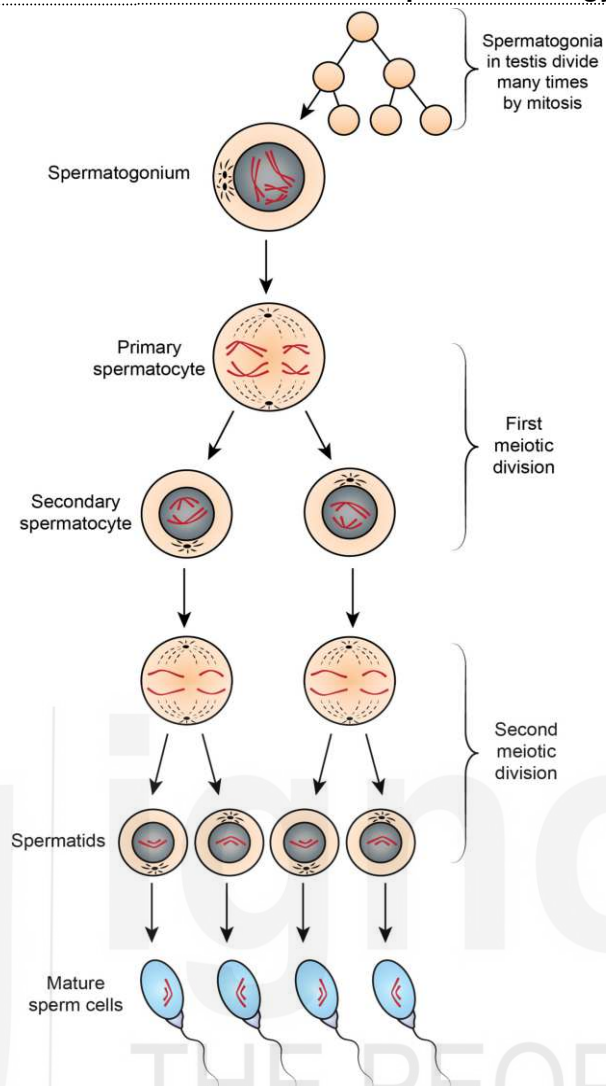


Fig.12.2: Stages in spermatogenesis

3. Maturation Phase

The maturation phase is the stage in which the diploid primary spermatocytes get transformed into haploid spermatids (Fig.12.2) by two successive meiotic divisions. The primary spermatocyte divides meiotically to produce two **secondary spermatocytes** each with a haploid nucleus. Each of the secondary spermatocytes undergoes second meiotic division, thus giving rise to four haploid **spermatids**. Each spermatid undergoes an intracellular differentiation to produce a spermatozoa. Maturation then takes place under the influence of testosterone. Humans have a diploid number of 46 chromosomes, while their haploid gametes have half the number of chromosomes ($n = 23$). The haploid spermatids undergo extensive changes in order to differentiate into sperms.

During maturation the unnecessary cytoplasm and organelles are removed. The excess cytoplasm, known as residual bodies, are phagocytosed by the surrounding Sertoli's cells in the testes. The mature haploid spermatids formed at this stage however, lack motility and are still not capable of functioning as male gametes. They are transformed into the motile, active spermatozoa or sperms by the process of spermiogenesis about which we will study in more detail in the next subsection.

12.3.2 Spermiogenesis

At the end of the meiosis the spermatids appear as simple spherical cells with a centrally located nucleus. Their differentiation into sperm requires an extensive morphological transformation. The various cellular organelles like mitochondria, Golgi body and centrosome contribute to such a transformation. The transformation of a spermatid into a spermatozoon is essential so that it (1) attains motility and (2) its motility and movement is improved by reduction of unnecessary or superfluous materials present in it.

The number of programmed steps needed to transform an ordinary looking round spermatid into a dynamically shaped spermatozoon varies from species to species. The steps range from six to eight steps in man; to 19 steps in rats and rabbits.

The fully formed sperm (Figs.12.3 and 12.4.) consists of the head and flagellum. The flagellum can be divisible into the following regions which consist of neck piece/ the middle or mid piece, principle piece which is usually the longest part of the flagellum and the tail end. The axoneme is the central cytoskeletal structure of the flagellum. It has the typical structure of a cilium and is composed of an array of microtubules, typically consisting of nine pairs surrounding two single central ones. You will be reading about the structure of the axoneme later in this unit. In mammalian sperms, as well as sperms of vertebrates, two accessory structures the outer dense fibers (ODFs) and the fibrous sheath are present between the axoneme and the plasma membrane, which enclose and extend along the length of the axial filament and terminate before the end piece of the flagellum.

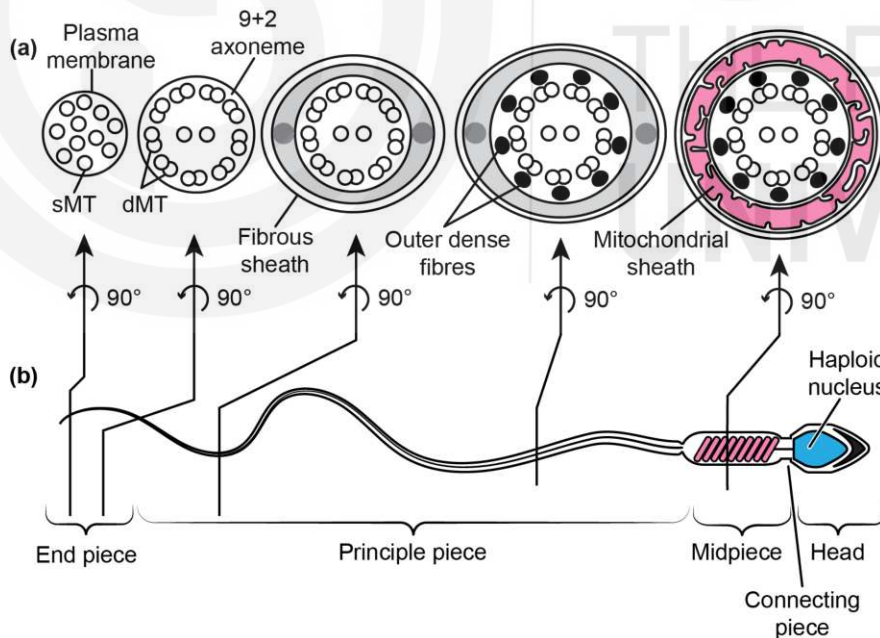


Fig.12.3: Mature spermatozoon. The genetic information is packaged in a tight bundle in the haploid nucleus in the head region. Surrounding it is an acrosomal vesicle that helps the sperm penetrate the ovum. The spermatozoa's motility is derived from its flagellum, which is powered by a group of mitochondria in the midpiece.

Formation of Head

The head region of a sperm consists of (Figs.12.3 and 12.4) the nucleus and the acrosomal vesicle or acrosome. The first step in the transformation of the

spermatid involves the formation of an acrosomal structure from the Golgi apparatus of the cell. The acrosome lies proximal to the nucleus and forms a cap over the sperm nucleus. The acrosome in the sperm head is located between the plasma membrane and the nuclear membrane of the sperm (Fig.12.4). It corresponds functionally to a lysosome and thus contains lysosomal enzymes (hyaluronidase among others) that play an important role in penetrating through the pellucida zona of the oocyte. The remainder part of the Golgi apparatus, which does not participate in the formation of the acrosome and also some of the cytoplasm of the sperm cell become reduced after acrosome formation.

Once, the acrosomal cap is formed in the sperm, the nucleus rotates and the cap faces the basal membrane of the seminiferous tubule. This rotation is essential because the flagellum is to arise from the centriole located on the other side of the nucleus.

Subsequent to the formation of acrosome, the nucleus flattens and condenses and there is a loss of cytoplasm. The nucleus of the spermatid retains, only its actual genetic material, the DNA and the proteins (histones) associated with it. The nucleus also loses its nucleolus, all its RNA, and most of the proteins. The chromosomes become condensed and tightly packed. The shape also changes from spherical to elongate (Figs.12.3 and 12.4). In different groups of vertebrate animals, the spermatid nucleus assumes different shapes. The variations in the shape of nucleus in animal groups are considered as an adaptation for propulsion in a liquid medium.

Formation of the Flagellum

The flagellum forms a propulsion system for the sperm. The flagellum or tail of the spermatozoon or sperm has four distinct segments (see Figs.12.3 and 12.4) that are: 1) the neck or connecting piece, which connects to the proximally (anteriorly) located head with its overlying acrosome; 2) middle piece, or mid piece of the flagella; 3) the principal piece of the flagella and 4) end piece of the flagella.

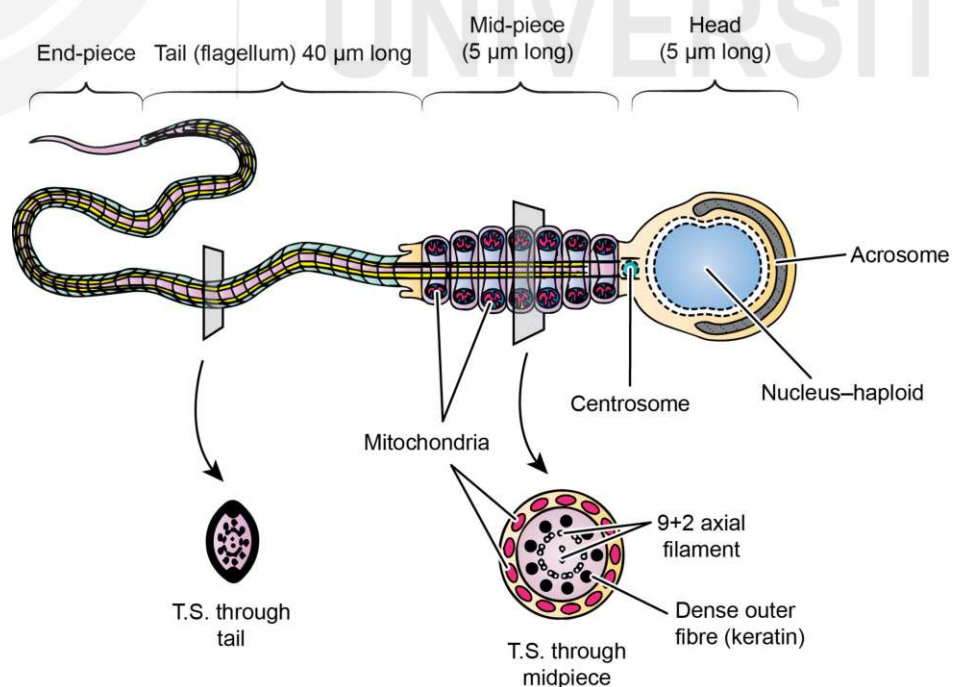


Fig12.4: Structure of a human sperm showing cross section of the flagellum at its different segments.

The means by which sperm are propelled vary according to how the species has adapted to environmental conditions. In most species, an individual sperm is able to travel by whipping its flagellum. The major system for its movement is the **axial filament or axoneme** which is the central, internal cytoskeletal structure of the flagellum and is formed from one of the two centrioles of the centrosome present at the base of the sperm nucleus in the neck region of the sperm. Microtubules emerge from the centriole and form the axoneme of the flagellum. The axoneme has been well conserved throughout evolution. The core of the axoneme consists of two central microtubules surrounded by a row of nine doublet microtubules (Fig.12.5). These microtubules are made exclusively of the protein **tubulin**. However, there are other proteins critical for flagellar function. The force for sperm propulsion is provided by dynein, a protein attached to the microtubules. Dynein is an ATPase—an enzyme that hydrolyzes ATP, and converts the released chemical energy into mechanical energy that is used to propel the sperm. This energy allows the active sliding of the outer doublet microtubules. For the development of axoneme, the developing spermatozoa in the seminiferous tubules orient themselves so that their tails point towards the center of the lumen, away from the germinal epithelium.

There is very little variation in axonemes between vertebrate species and the '9+2' microtubule arrangement with dynein arms has been conserved throughout the eukaryotic group of animals. Thus, it suggests that this arrangement is extremely suitable for transmitting energy required for movement. In the sperm of some fishes and amphibians, there is an undulating membrane, which stretches along most of the tail length and takes an active part in the locomotory activity of the sperm

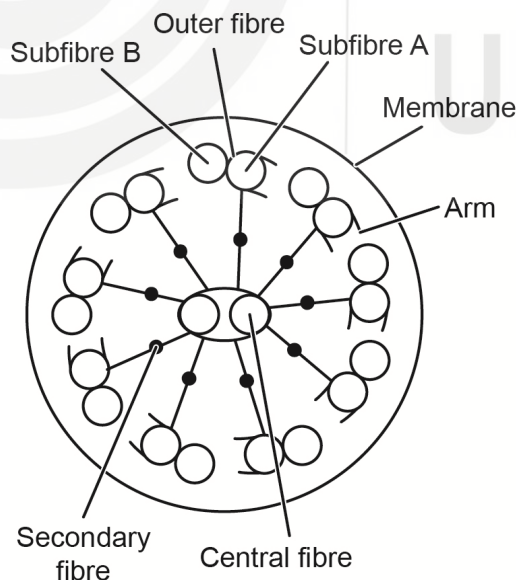


Fig.12.5: 9+2 arrangement of microtubules in the axoneme that has been conserved in vertebrates.

The middle piece or mid-piece is the central part of the flagellum of the sperm cell, just behind the neck region of the flagellum. Unlike the sperm head that contains genetic material, the middle piece contains tightly packed

mitochondria that provide the sperm with the energy required for swimming to locate and fertilize the ova. In the spermatids of mammals including, humans, most of the mitochondria become concentrated around the axial filament. These mitochondria then lose their individuality; fuse together and the fused mitochondria become spirally arranged (Figs.12.3 and 12.4).

After the middle piece, the remainder of the flagellum is long and is called the principle flagellum, till just before the tail or end part of the flagellum. In mammals, the axoneme or axial filament, which forms the central part of the flagellum extends from the neck to just before the end of the tail and becomes covered by, two accessory structures the outer dense fibers (ODFs) and the thick fibrous protein sheath. The outer dense fibers (ODFs) are enclosed by the thick fibrous sheath. In the mid-piece of the flagellum the two accessory structures namely the outer dense fibers (ODFs) and the thick fibrous protein sheath are covered by the mitochondrial sheath. The two accessory structures are absent in the end part of the flagellum called tailpiece, which as a result is naked (Figs.12.3 and 12.4).

The production of sperm cells require a certain milieu. The testes contained within the scrotum have a testicular temperature which is 2-3 °C lower than body temperature which is essential for sperm production. In addition, a slightly elevated pressure within the testes is also necessary.

Figure 12.6 shows the transformation of the spermatid into a sperm.

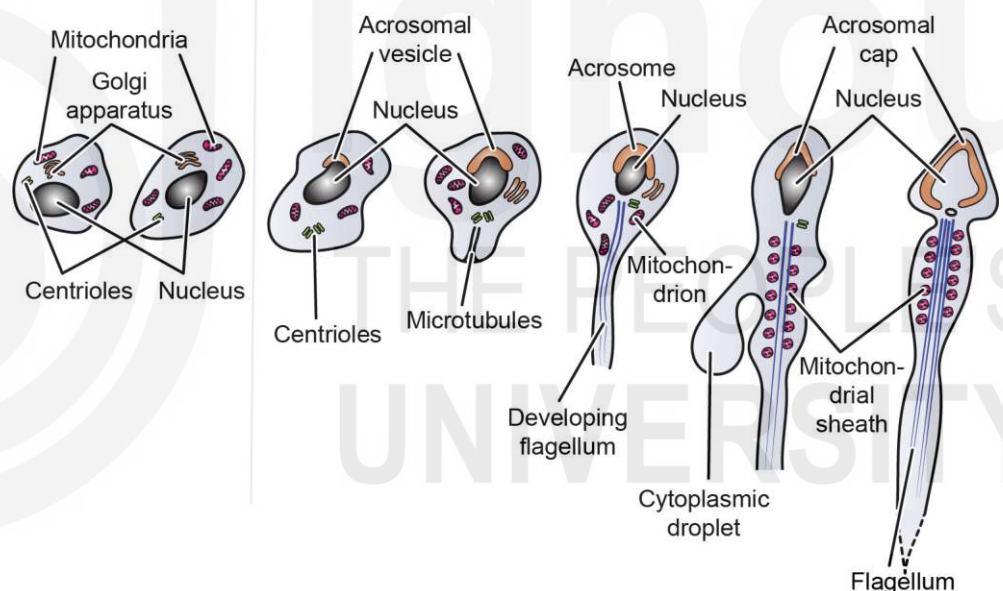


Fig.12.6: Process of Spermeiogenesis in which the spermatid transforms into a sperm.

12.3.3 Spermiation: Activation of Sperm

The mature though inactive, non-motile spermatozoa or sperms contained in the testicular fluid which is secreted by the Sertoli's cells, move inwards towards the fluid-filled lumen (cavity) of the seminiferous tubules and are released in it. After which the spermatids are transported from the lumen of the seminiferous tubule into the epididymis (highly convoluted duct behind the testis) and then via the vas deferens to the outside of the body through the penis, by means of peristaltic contractions. While travelling in the epididymis, the non-motile spermatozoa transform into the motile spermatozoa or sperms, by the process of spermiation and become capable of fertilizing the ovum.

The motile sperms are microscopic, remain alive, and have the capability to fertilize an ovum from 24 to 48 hours, right after they the female genital tract.

In humans the whole process of spermatogenesis, from spermatogonia to spermatozoa takes approximately 74 days (Fig.12.7) while in mouse it takes 34.5 days to complete the whole process of spermatogenesis.

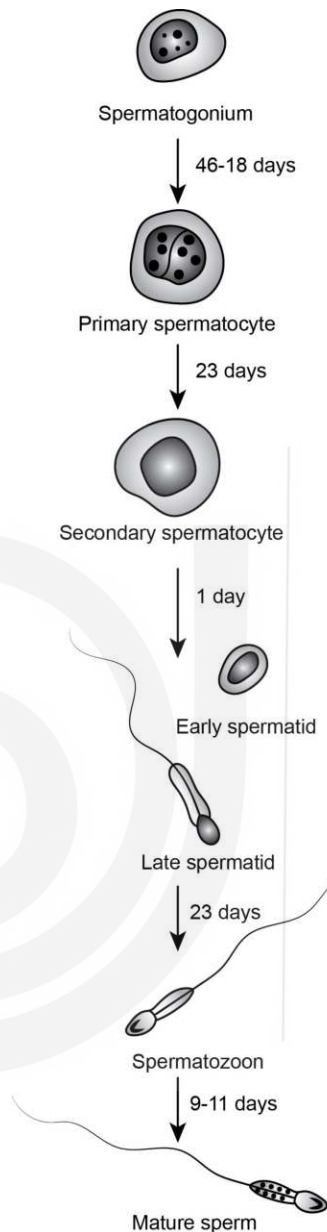


Fig. 12.7: Development of human spermatozoa: Each of the stage of spermatogenesis in humans, has its own specific duration. The spermatogonia take 16–18 days to form the primary spermatocytes; the primary spermatocytes take 23 days to make the secondary spermatocytes; the secondary spermatocytes take only one day to become spermatids, and the spermatids spend another 23 days maturing into spermatozoa. This does not add up to 74 days because after release of the spermatozoa into the lumen of the seminiferous tubules, the spermatozoa still require maturation. The released spermatozoa are not yet motile, and they are transferred to the epididymis by movement of the luminal fluid, and not by motility of the sperm. In the epididymis, the spermatozoon undergoes maturation processes that progressively enable it to bind to the zona pellucida of the ovum and to fertilize the ovum.

SAQ 2

Fill in the blanks:

- i) refers to the formation of sperm cells in male gonads.
- ii) In the tubules different stages of sperm production can be observed.
- iii) The three stages in sperm formation are (a) phase, (b) phase and (c) phase.
- iv) At the end of maturation division during spermatogenesis diploid are transformed into haploid
- v) The process of formation of sperm from spermatids is known as

12.3.4 Structure of Sperms among Vertebrates

The structure of sperms throughout the Animal Kingdom is highly conserved. The sperm is covered by a plasma membrane, which contains species-specific proteins called bindin. The bindin proteins recognize and bind to the receptors present on the egg plasma membrane of the same species. While studying the sperm morphology (Fig 12.8) the following features are considered: 1) The size of the sperm; 2) The shape and size of the nucleus of the sperm and the structure of the acrosome; and 3) The flagellum or tail.

The size of the sperm or basically the length of sperm may vary in different animal groups and different species. It can range from being as tiny as 0.02 mm as in crocodile to being as large as 2 mm as in *Balanoglossus*. In general, the width and length of the sperm head varies due to variations in the size and organization of the nucleus and acrosome. The shape of the head of sperms is species specific in animals including vertebrates (Fig.12.8). The shape of the sperm head is spherical in teleost fishes, rod or lance shaped in amphibians, spirally twisted like a corkscrew in birds, and in mammals it is spoon shaped in man, flattened in buffalo or hooked in mouse and rats. These shapes are believed to be an adaptation for propulsion in fluid media.

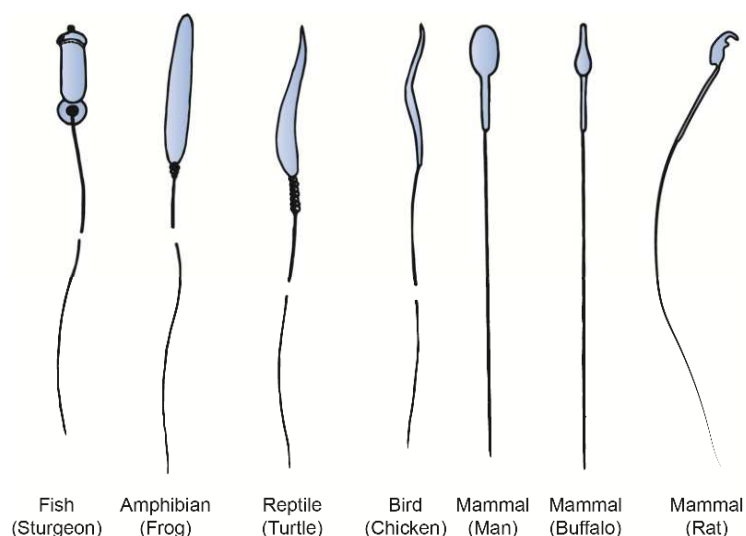


Fig.12.8: Sperms of some vertebrates (the break in the flagellum of sperm indicates that a portion is omitted), showing the head which varies in size and shape. The flagellum also varies in length.

SAQ 3

- i) List the main components of a mature sperm?
- ii) What is the purpose of motility of spermatozoa?
- iii) What is the source of energy for the motility of spermatozoa?

12.4 OOGENESIS

Oogenesis begins in the ovary of the female embryo. The embryonic ovary contains primordial germ cells, which as you have read in section 12.2 migrate to the ovary in the same manner as the primordial germ cells of the males. The primordial germ cells give rise to the **diploid oogonial cells, or oogonia** which are also known as the mother cells. These give rise to the haploid ovum. The mechanism of oogenesis varies much more among animal species as compared to spermatogenesis. In some species, hundreds and thousands of eggs are produced routinely as seen in the sea urchin and frog. Whereas in mammals only a few eggs are produced. Oogenesis like spermatogenesis involves meiosis to produce the haploid ovum. Oogenesis is a complex process as the ovum (egg) acquires its developmental potentialities from the molecular information, encoded in its DNA and the accumulation of materials present in it, which is required for building the structures of the early embryo as well as for providing energy till the embryo becomes self-sufficient. You have learnt in the subsection on spermatogenesis that the differentiation of sperms occur after meiotic events. In oogenesis the process of the first meiotic division is very prolonged and it is during this process that growth and differentiation of oocyte takes place. In many animals most events related to oocyte differentiation occur during prophase I of the first meiotic division. Even though the mechanisms for oogenesis vary in animal species the basic mechanism of formation and maturation of ovum is more or less similar in all organisms (Fig. 12.9). It essentially includes the same steps as in spermatogenesis but the steps and the products of the process are modified.

12.4.1 Formation and Maturation of Ova

Oogenesis (Fig. 12.9) mainly involves 3 phases: I) proliferative or pre-meiotic phase; II) meiotic phase and III) cell growth phase.

I) Proliferative or pre-meiotic phase

The primordial germ cells that enter into the ovary divide mitotically and produce oogonial cells, which as a result, increase in number. This is the proliferative or pre-meiotic phase as there is increase in the number of oogonial cell, within the ovary, usually during embryonic life. These oogonial cells grow in size and become the **primary oocytes**. The proliferative phase occurs in all vertebrates but the timing is different for different groups of animals. For example, in higher animals like reptiles, birds, and mammals, the proliferative phase is completed long before sexual maturity whereas, in amphibians, each year, a new crop of egg is generated by mitotic proliferation from a population of **gametogenic stem cells**.

II) Meiotic phase

The primary oocytes after attaining their full size undergo, a meiotic division. For this process, the chromosomes present in the primary oocytes replicate. Shortly after prophase I of the first meiotic division the replicated chromosomes condense and remain in an arrested stage of division for a prolonged period. This period of arrested division may last for months and even years. In many animals most events related to oocyte differentiation occur during prophase I of the first meiotic division. In human females, each primary oocyte is contained within a small follicle, which is a cavity lined with protective cells. At birth, the paired ovaries together contain about 1-2 million primary oocytes, of which about 500 mature between puberty and menopause.

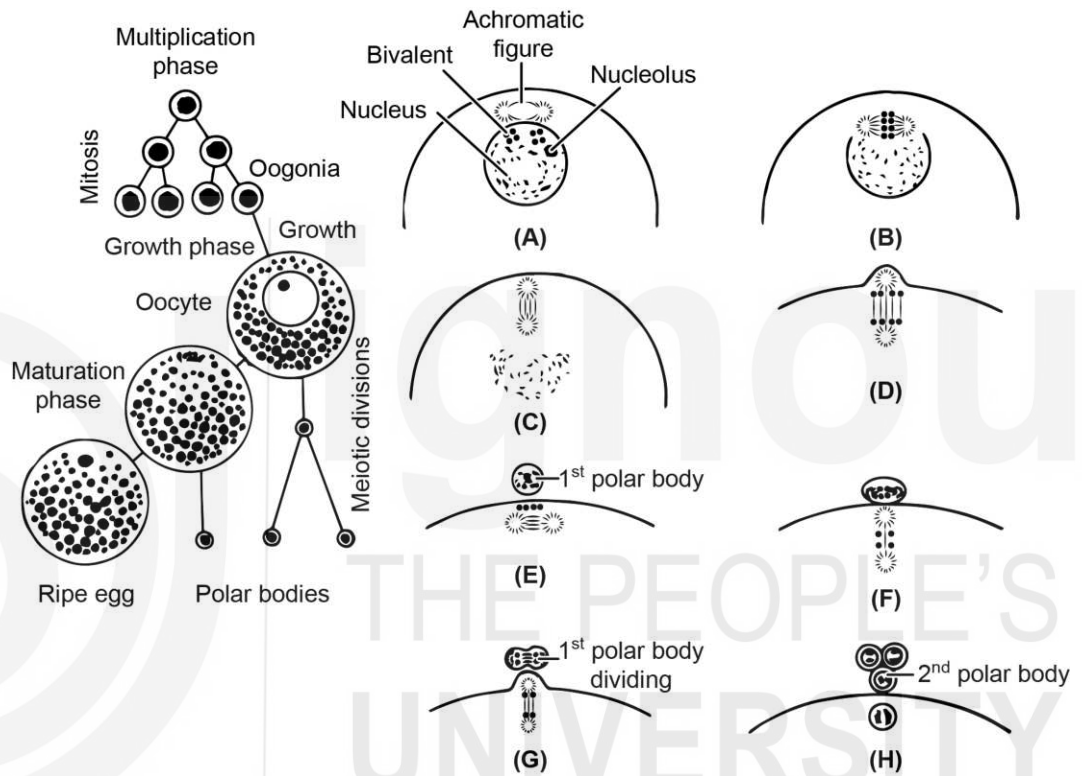


Fig.12.9: Diagrammatic representation of (1) stages in oogenesis and (2) of the first (A to E) and second (F to H) meiotic divisions in the oocyte.

In most vertebrates and many invertebrates, the egg does not actually complete the meiotic division before fertilization. The second meiotic division may be completed only outside the ovary after fertilization. The general rule is that development is arrested during prophase I of the first meiotic division (in the primary oocyte phase). Meiosis resumes and is completed either at the time of ovulation (birds and most mammals) or shortly after fertilization (many invertebrates, teleost fishes, amphibians, and reptiles). In humans, the ova begin the first meiotic division at about the thirteenth week of foetal development. Then their development arrests in prophase I until puberty, at which time one of these primary oocytes typically develops into a secondary oocyte in each month for ovulation. When the first meiotic division or maturation (reduction) division is completed, the cytoplasm is divided unequally. One of the two daughter cells, called the **secondary oocyte**, is large and receives most of the cytoplasm; the other is very small and is called the first polar body. Each of these daughter cells, however, receives half of the chromosomes and so are haploid cells.

The terminology used in describing the female gamete can be confusing. In general, an egg, or ovum, is a female gamete capable of binding with the sperm and being fertilized. An oocyte is a developing egg that cannot yet bind to the sperm or be fertilized. The confusion arises because the eggs of different species are in different stages of meiosis. The human egg, for example, is in second meiotic metaphase when it binds with the sperm, whereas the sea urchin egg has completed all of its meiotic divisions when it binds with the

The secondary oocyte undergoes the second meiotic division to produce a large **ovum** called **ootid** and another small polar body. In humans, meiosis II is completed only when a spermatozoon penetrates the secondary oocyte. If the first polar body also divides in this division, which sometimes happens then there are three polar bodies and one ootid (Figure 12.9). The ootid develops into a functional, haploid ovum. Polar bodies are nonfunctional, and they eventually degenerate.

The formation of polar bodies during ova production is necessary as the excess chromosomes that result from each nuclear division during meiosis, is disposed off through them. In addition, the unequal cytoplasmic division results in the formation of a large cell with the cytoplasm that contains a full set of cytoplasmic components needed for early development. Thus, a mature ovum or egg has N (haploid) number of chromosomes, the same as a sperm. However, each primary oocyte gives rise to only one functional ova instead of four as in spermatogenesis.

III) Growth Phase of Oocyte

During the **growth phase** period of the oocyte the gene products and nutrients are stored and accumulated in the oocyte. The growth of oocyte is so enormous that it becomes almost the largest of the body cells. For example, it ranges in size from 10 μ m to 200 μ m in mammals; it is 2000 μ m in frog *Lithobates pipiens* (earlier known as *Rana pipiens*) and is 40,000 μ m in birds. The time required for this growth varies with species. A greater amount of RNA polymerase enzymes, histone proteins, mitochondria, ribosomes etc. are also formed as they are required for cell division. During this growth phase, both qualitative and quantitative changes occur in the nucleus as well as cytoplasm.

In many animals, the most obvious feature of egg maturation is deposition of yolk. We shall discuss oogenesis in two groups of animals, (1) the amphibians as an example of organisms, which produce moderately yolky eggs and (2) mammals, as an example of organisms whose eggs do not contain any yolk.

10.4.2 Oogenesis in Amphibians

Eggs of amphibians like those of other animals are derived from stem cells, which produce oogonial cells. The time taken for oogenesis varies in different species. For instance, in the frog, *Lithobates pipiens* (*Rana pipiens*), oogenesis takes 3 years after metamorphosis to be completed, out of which the first 2 years consist of the growth phase, when the size of the primary oocyte increases. In the third year, the primary oocyte rapidly accumulates the yolk in its cytoplasm. While in *Xenopus* however, the period for oogenesis is only several months and hence, it becomes a better model for studying amphibian oogenesis

Development of amphibian egg is divided into 3 broad stages:

- I. **Previtellogenesis** (before the deposition of yolk) stage.
- II. **Vitellogenesis** (major period of yolk deposition) stage and
- III. **Post-vitellogenesis** or final maturation stage, in which the oocyte is released from its meiotic block by the action of progesterone.

I. Previtellogenesis stage

Previtellogenesis is the period up to the early diplotene phase of meiosis. During this period, the following changes are observed in the egg:

1. Changes in the nucleus:

- i) The nucleus increases several hundred times in volume to becomes highly enlarged. The increase in volume is due to the production of a large amount of nuclear sap. Such a nucleus is called as **germinal vesicle** in the egg.
- ii) The chromosomes are four-stranded bivalents, characteristic of meiotic prophase, but they remain active during oogenesis and display numerous protruding loops of chromatin. Because of this appearance they are called **lampbrush chromosomes** (Fig.12.10). These loops in the lampbrush chromosomes represent folds of multiplication or gene amplification (number of copies of a gene is increased without a proportional increase in other **genes**) of the DNA. This increases the synthesis of transcriptional mRNA, t-RNA and small r-RNA which are needed to support protein synthesis during early development of the embryo.
- iii) A large number of nucleoli are formed (for example upto 1000 to 1500 nucleoli are formed in *Xenopus*) which are distributed beneath the nuclear membrane.

2. Changes in cytoplasm:

- i) The **cytoplasm** increases in quantity and quality.
- ii) During the process of oogenesis the animal-vegetal polarity of the oocyte arises. A special cytoplasmic region rich in mitochondria called the **mitochondrial cloud** is formed. Its location determines the future vegetal pole of the egg. The vegetal pole lies opposite to the mitochondrial cloud.
- iii) **Golgi complex** becomes well developed. It forms a spherical mass, which becomes located in the subcortical region of the cytoplasm. The Golgi complex in addition to performing its normal function also manufactures the **cortical granules** containing proteins and mucopolysaccharides. Proteolytic enzymes required for the synthesis of the fertilization membrane and for post fertilization phase to prevent polyspermy are also present in the cortical granules.
- iv) **The Endoplasmic reticulum of the Golgi complex** are transformed from granular form to annulated lamellae (ring-like) structures. The annulated lamellae structures consists of stacks of cisternae with some RNA. The function of this RNA is still unknown.

II. Vitellogenesis stage

Yolk which is the major nutritional reserve for the oocyte, is formed and accumulated during the vitellogenesis phase. Thus it is responsible for the

rapid increase in the size of the maturing egg. The formation and accumulation of yolk occurs in the oocyte, during the diplotene stage of the prophase of meiosis I. This stage is also characterized by active synthesis of RNA by lamp brush chromosome (Refer again to Fig. 12.10) present in the nucleus (see the marginal remark). The major precursor of yolk vitellogenin is synthesized outside the ovary in the liver and is transported by the blood. Vitellogenin is incorporated into the developing oocyte by the process of micropinocytosis (incorporation of macromolecules or other chemical substances into cells by membrane invagination and the pinching off, of relatively minute vesicles).

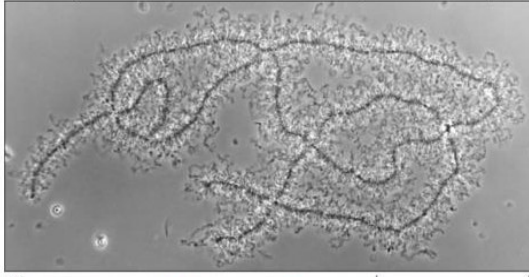


Fig.12.10: Micrograph of Lampbrush chromosome from amphibian oocyte.

<https://emb.carnegiescience.edu/science/faculty/joseph-gall>.

In **mature oocytes**, the yolk contains two proteins, which are derived from vitellogenins—a phosphorous containing protein, the phosvitin and a lipid containing protein called lipovitellin. These two yolk proteins are stored in the form of yolk platelets. The yolk in addition to proteins also contains phospholipids, neutral fats in the form of lipochondria (temporary storage vacuoles of lipids found in the Golgi apparatus) and carbohydrates in the form of glycogen granules.

As the oocyte grows (Fig. 12.11), various structures are synthesized in its cytoplasm. Thus, a pattern of organization gradually emerges in the oocyte. The upper half of the oocyte is called the animal hemisphere. The animal hemisphere contains the nucleus of the oocyte which is called the germinal vesicle, a large amount of yolk free cytoplasm and dense concentration of pigment granules. The opposite half of oocyte is yolk rich and constitutes the **vegetal hemisphere**. Therefore, amphibian eggs are highly asymmetric (**telolecithal**). Such differentiation of the egg into the animal–vegetal axis as you will recall is known as polarity of the egg. A transitional zone is also present between the 2 hemispheres which is known as the **marginal zone**

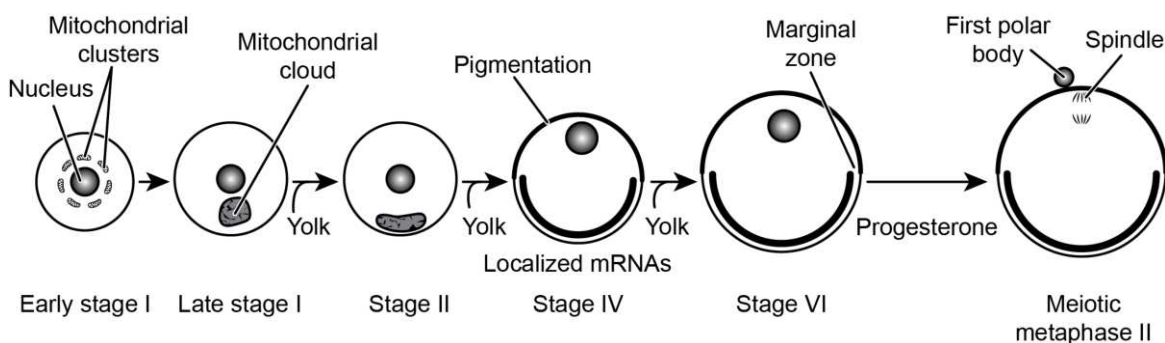


Fig.12.11: Oogenesis in *Xenopus*. There is considerable increase in the size as the oocyte matures.

III. Post –Vitellogenesis stage

Post-Vitellogenesis is also referred to as the, “Final Maturation Phase” and is regulated by several factors such as the Mitotic Promoting Factor (MPF), cyclin-dependent kinase (cdk), c-mos protein etc. The following processes take place during the post–vitellogenesis stage:

- i) Hormone progesterone, induces the release of egg from its first meiotic block.
- ii) Breakdown of the germinal vesicle occurs
- iii) Completion of the first meiotic division takes place, and
- iv) The first polar body is formed.

Hormonal Induction during oogenesis

The interactions of hormones secreted by the hypothalamus, pituitary and follicle cells of the ovary regulate further events of oogenesis. Once the mating season arrives, the gonadotropin-releasing hormone from hypothalamus causes the pituitary gland to release the gonadotropic hormones into the blood. These gonadotropic hormones stimulate the follicle cells to secrete estrogen, which in turn activates the liver to synthesize and release vitellogenin, which is taken up actively by the oocyte. Progesterone, another hormone is secreted by follicle cells in response to gonadotropins of the pituitary gland. The action of the progesterone releases the primary oocyte from meiotic arrest and causes the breakdown of the germinal vesicle. The action of the progesterone thus, activates the oocyte for the completion of the first meiotic division and also causes the release of the egg from the ovary by the process of ovulation (Fig. 12.12).

Towards the end of vitellogenesis, the cytoplasm of the amphibian oocyte shows distinct regions. The golgi apparatus, mitochondria and pigment granules are present at the periphery of the cell and form the cortex of the oocyte. In the interior of the egg, yolk is largely accumulated in the vegetal hemisphere of the egg while the largely clear cytoplasm is present in the animal hemisphere and contains glycogen granules, ribosomes, lipochondria and endoplasmic reticulum.

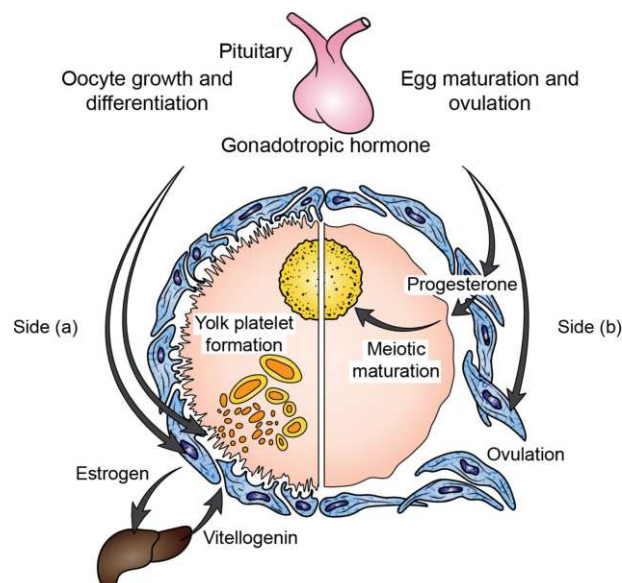


Fig.12.12: Diagram showing a) the regulation of amphibian oocyte growth and differentiation; and b) egg maturation and ovulation.

12.4.3 Oogenesis in Mammals

In mammals and the all vertebrates as you have studied earlier, the oogonial cells are derived from primordial germ cells. The multiplication phase, which comprises all the oogonial divisions and the transformation of the oogonia into oocytes, is completed either before or shortly after birth. All oocytes that are produced are held in meiotic arrest in prophase I.

In mammals, as you will recall the period of oogenesis basically covers the entire life of an individual from birth to ovulation. The meiotic arrest of the oocytes is released at the time of puberty after which groups of oocytes begin development during each ovulatory cycle. A large percentage of oocytes fail to undergo maturation and therefore degenerate.

The oocytes (Fig.12.13 a) in mammals are found in close association with non-germ cells that are present in the ovary. The non-germ cells or the accessory cells function to: (i) produce steroid hormones, (ii) transport some of the essential cytoplasmic components into the oocyte and are (iii) involved in the formation of cellular or non-cellular layers that surround the fully differentiated egg. The accessory cells that surround the egg are of two types: (1) follicle cells and (2) nurse cells. The follicle cells are somatic cells, and surround the oocyte as a single layer of the cells the forming follicular epithelial cells. The nurse cells are derived from the germ cell line and are connected with the oocyte by cytoplasmic bridges.

During the growth of the secondary oocyte, the single layered follicular epithelium proliferates and becomes multilayered. The follicular cells of this multilayer are then referred to as **granulosa cells**. The granulosa cells and the oocyte are separated by a space, filled with sulphated glycoproteins. This layer becomes the **zona pellucida** of the oocyte. When the proliferation of the granulosa cells is complete, they secrete a fluid that accumulates in the intercellular spaces. These fluid filled spaces coalesce to form a cavity called **antrum**. With the formation of antrum the oocyte is displaced to one side of the follicle and is ready, to be released by the process of ovulation (Fig.12.13 b). When the oocyte, is surrounded by the antrum, and is enclosed by the zona pellucida layer, which is covered by the follicular layers of cells (referred also as the granulosa cells), then this entire structure is called a Graafian follicle (Fig.12.13 b). In humans, the ova or oocytes are extremely minute, measuring about 0.2 mm. in diameter, and are enclosed within the Graafian follicle. As a rule, each Graafian follicle contains a single oocyte, but sometimes two or more are present.

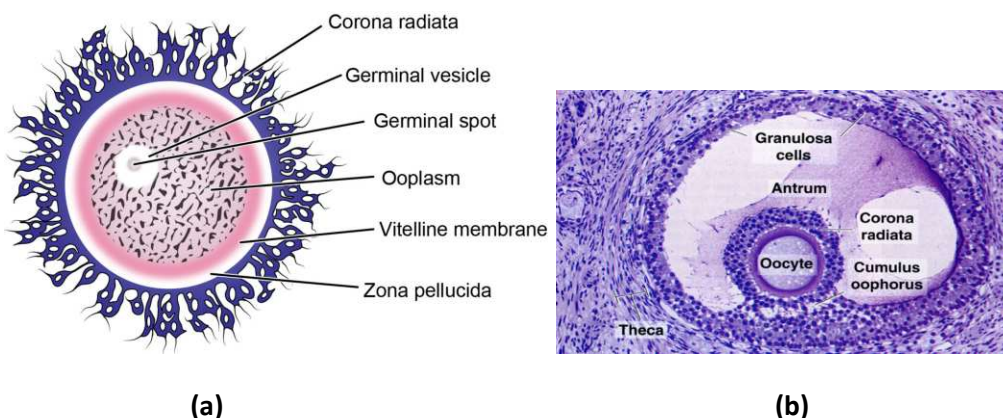


Fig.12.13: (a) Young oocyte of a mammal surrounded by follicle cells and; b) photograph from a histological slide of a mature Graafian follicle or tertiary follicle, in which the oocyte is present on one side of the secondary oocyte.

The rupture of a Graafian follicle (Fig. 12.14), at the surface of the ovary causes the release of the oocyte, which is conveyed by the uterine tube called oviduct to the cavity of the uterus. If the released ovum or oocyte is fertilized, then it forms a zygote, which is retained within the uterus. Within the uterus, the embryo develops into an offspring. However if fertilization does not occur then the ovum does not undergo any further development and is discharged from the uterus to the outside of the body.

The human ovum is alecithal with no yolk but with a large amount of cytoplasm. Cytoplasm is differentiated into an outer, smaller and transparent exoplasm or egg cortex and inner, larger and opaque endoplasm or ooplasm. Egg cortex contains some cytoskeletal structures like microtubules and microfilaments, pigment granules and cortical granules of mucopolysaccharides. Endoplasm contains cell-organelles, informosomes, t-RNAs, histones, enzymes etc. Nucleus of ovum is large, due to large volume of nucleoplasm and is called germinal vesicle. The nucleus is excentric in position so the human ova have a polarity. The side of ovum with nucleus and polar body is called animal pole, while the opposite side is called vegetal pole.

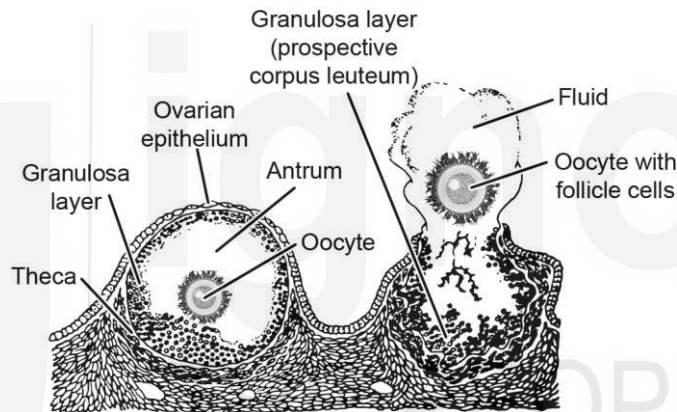


Fig.12.14: A Graafian follicle and ovulation of egg in mammals.

12.4.4 Egg Envelopes of Vertebrates

All ova like any other cell, are covered by a **plasma membrane (PM)** also referred to as **oolemma** or **cytoplasmic membrane** which consist of two layers. The collective thickness of the two layers is about 50 Å (unit membrane) and these two layers are separated by a gap of 60 Å. In addition to possessing the oolemma the eggs are also surrounded by special egg envelopes or extracellular coats which develop during the course of oogenesis. These egg membranes can be classified according to their site of origin as primary, secondary or tertiary types.

I. Primary Egg Envelopes

Primary egg envelopes develop in the ovary between oocyte and follicle cells in the space occupied by the interdigitating microvilli. Such envelopes have been named variously in different animals and given below:

- a) The **vitelline membrane** or primary envelope may be a non cellular, transparent layer of mucoprotein, supplemented by extensions of membrane glycoprotein from the plasma membrane and by proteinaceous structures that attach the vitelline envelope to the membrane. This membrane is essential for species- specific binding of the sperm and is found in insects, molluscs, amphibians and birds.

- b) In tunicates and fishes, the primary envelope is known as **chorion**. In many sharks and bony fishes the primary envelope has a striated appearance due to the degraded microvilli of the growing oocyte and is referred to as **zona radiata**. The perforation in the zona radiata becomes the micropyle through which the spermatozoa can enter the egg.
- c) In mammals, the primary egg envelope is called the **zona pellucida**. It is an unstriated and modified layer formed as a result of joint efforts of egg and follicle cells (Fig. 12.13). While escaping the Graafian follicle the mammalian oocyte carries on the surface of zona pellucida a layer of follicle cells known as **corona radiata**.

The primary envelopes usually stick closely to the egg surface. These later on participate in the formation of the fertilization membrane.

II. Secondary Egg Envelope or coat

The secondary egg envelopes are secreted outside the primary egg envelope by the layer of follicle cells that surrounds the oocyte and are referred to as:

- Chorion in insects, ascidians and cyclostomes which is in the form of a chitinous shell in these animals.
- Corona radiata in mammals.
- Outer vitelline membrane in birds.

III. Tertiary Egg Envelope

The tertiary egg envelope is secreted by oviducts and other accessory parts of the genital organs while the egg passes through them from the ovary to the exterior, towards the cloaca.

- In ovoviviparous sharks the tertiary egg envelope surrounding the egg is in the form of a hard shell which is secreted by shell glands of the oviduct. The shell is drawn out as twisted horns which help to entangle the egg among sea weeds.
- In amphibians, the tertiary egg envelope layer is in the form of **a jelly or albumin layer** which surrounds the egg and protects it from adverse effects of sun rays, abrasion as well as from predators (because of its bad taste). The jelly absorbs water and swells up (Fig.12.15).

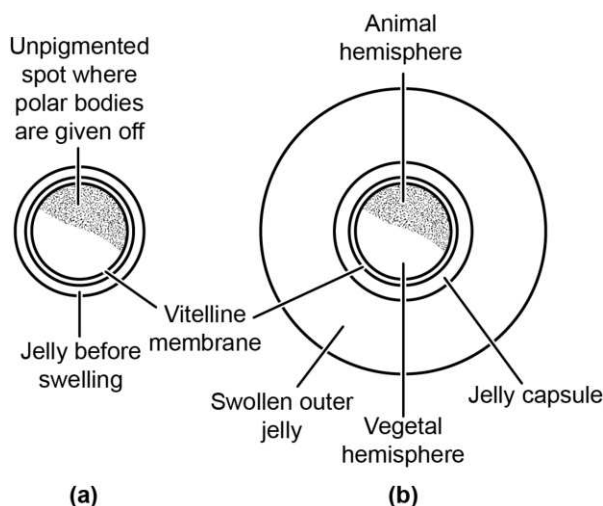


Fig.12.15: An amphibian egg a) as taken from oviduct and; b) sometime after in spawning in water with swollen jelly membrane.

In birds, reptiles and monotremes, the tertiary envelope includes the egg white or albumin as well as its chalazae which is the twisted albumin in form of spiral strands. Both of these surround the yolk of the ovum and are covered by inner and outer shell membranes which are in close contact with one another and are also known as the tertiary envelope. The two shell membranes are enclosed by the outermost calcareous shell which is porous (Fig 12.16). The calcareous egg shell is also a tertiary envelope. The egg shell of birds is elastic and compressible when the egg is laid but it hardens on exposure to the atmosphere.

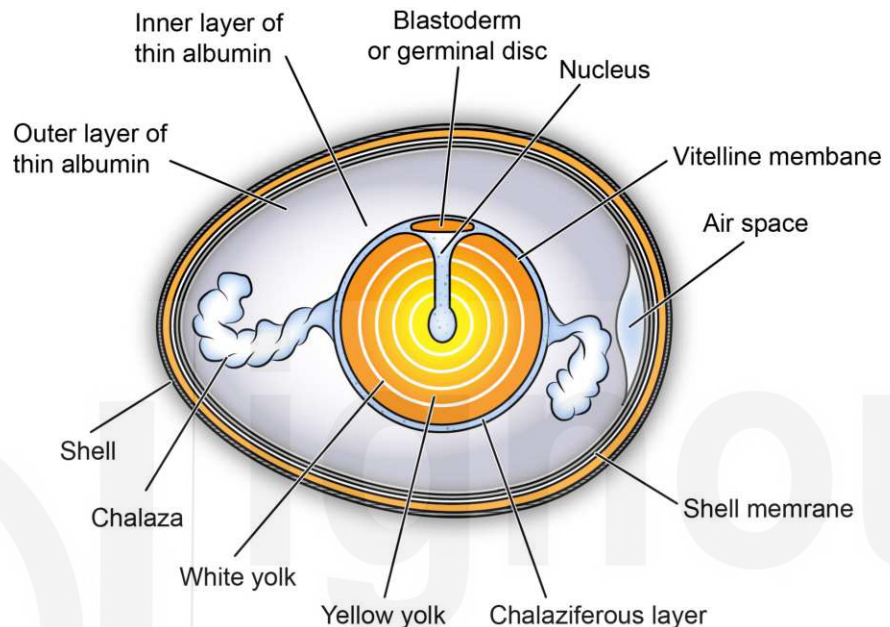


Fig.12.16: A median longitudinal section of hen's egg to show the egg envelopes.

SAQ 4

State whether the following statements are true (T) or false (F):

- Vitellogenesis is the stage at which much of the oocyte growth occurs. ()
- Mammalian eggs are characterized by a high yolk content. ()
- Each polar body derived as a result of meiosis is a potential ovum. ()
- In mammals the primary egg envelop is called zona pellucida. ()
- The first batch of eggs in *Lithobates pipiens* (*Rana pipiens*) matures a year after metamorphosis and then a batch matures once in every three years. ()
- Amphibian eggs are highly symmetrical in the distribution of yolk in them. ()
- In amphibians the yolk is formed and accumulated in the egg during the diplotene stage. ()
- Follicle cells and nurse cells are two types of accessory cells in the mammalian oocyte. ()

- i) Follicles with fluid filled space antra are called Graafian follicles. ()
- j) The shell of hen's egg is elastic and compressible at the time of laying. ()

12.5 FERTILIZATION

In the earlier sections, you have learnt about the formation and differentiation of the male and female gametes. In this section, we shall describe the process by which the sperm and the egg unite to form a zygote. This process is known as fertilization. Fertilization results in (i) combination of genes derived from 2 parents (fusion of nucleii or amphimixis) resulting in restoration of the diploid chromosomal number in the nucleus, and (ii) generation of a new organism by activating the developmental process.

In this section we will describe the fertilization of sea urchins in which most studies have been done, and the fertilization in mammals which poses several practical problems of study. Prior to the actual fusion of the sperm and the egg nuclei, the germ cells prepare themselves for the fertilization process. In the first subsection, we shall describe such preparatory events. Then you will learn about the fusion process and finally there will be a discussion about the various events that occur subsequent to the zygote nucleus formation.

12.5.1 Event Prior to the Sperm-Egg Fusion

In a number of organisms that have been studied, the sperm remains immotile in the testis or in the semen. The sperm become active and motile when they are close to the eggs. Several factors have been attributed for the activation of the sperm, which include pH, oxygen tension, presence of certain ions etc. Detailed studies in sea urchin have shown that sperm motility is activated only after the sperm have been released in the seawater that changes the pH (Fig. 12.17). In mammals, the definite cause of activation is not known and it is presumed that sperm motility begins with the ejaculation of the semen into the female reproductive tract.

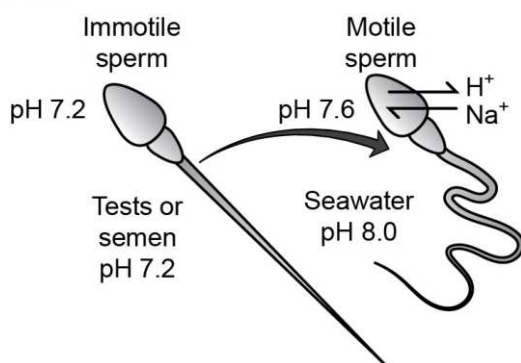


Fig. 12.17: The role of pH in the activation of sperm motility in sea urchins.

12.5.2 Basic Events of Fertilization

The events of fertilization vary from species to species however, it generally consists of 4 major events which are similar both in external and internal fertilization and are as follows:

- Contact and recognition between sperm and egg which ensures that both the gametes are of the same species.
- Regulation of sperm entry into the egg. This allows generally for only one sperm to enter the egg and inhibits the others.
- Fusion of genetic material of sperm and egg.
- Activation of egg metabolism to start development.

Contact and Recognition Between Egg and Sperm

A major problem in sexual reproduction is to ensure that the spermatozoa and ovum come close to each other. This is achieved in different vertebrate animals by different means. It can be accomplished by the secretion of certain chemicals and mobility of the sperm.

Activation of the sperm does not ensure that the sperm will meet the egg. In instances where fertilization occurs outside the organism, as in external fertilization, chemotactic mechanisms have been evolved to attract the sperm towards the egg. The chemotactic molecules differ even between closely related species. Species-specific sperm attraction has been documented in several species. In fish eggs the substances found near the micropyle attract the sperm. The substances found near the micropyle make the sperm move rapidly to the vicinity of the egg.

This direction for swimming is provided by small chemotactic peptides known as sperm-activation peptides. One such peptide is **resact** (sperm respiratory activating peptide) that has been isolated from the egg jelly of the Atlantic purple sea urchins (*Arbacia punctulata*). It has been shown that injection of a small quantity of resact causes randomly distributed sperms to change their swimming behaviour and accumulate near the site of injection within seconds. Binding of the sperm membrane to the resact causes the influx of calcium ions from the sea water into the tail and activates cGMP. Increase in cGMP and calcium ions activates the mitochondrial ATP generating apparatus and dynein ATPase which stimulate flagellar movement in the sperms that swim towards the egg. Thus, resact works both as a sperm activating and sperm attracting peptide.

Acrosome reaction

Contact and **interaction** between the sperm and egg jelly of the sea urchin results in the acrosome reaction (Fig. 12.18). The acrosomal vesicle fuses with the sperm cell membrane and releases, by exocytosis its contents consisting of proteolytic enzymes like chymotrypsin, **acrosomin** and proteosomes. The released contents of the acrosomal vesicle digest the jelly coat present on the egg surface and make a path through it, to the vitelline envelope of the egg. The second part of the acrosome reaction involves the extension of the acrosomal process. The inner membrane of acrosome protrudes out as the acrosomal process. The influx of Ca^{2+} which is essential for this process, is mediated through protein **RhoB** present in the acrosomal and mid-piece regions of the sea urchin sperm. The sperm's contact with the egg's jelly coat provides the first set of species-specific recognition events. Another critical

species-specific process is the binding event that must occur after the sperm penetrates the jelly and the acrosomal process makes contact with the surface of the egg. This recognition of the extracellular coat is mediated through protein **bindin**, which is again species specific.

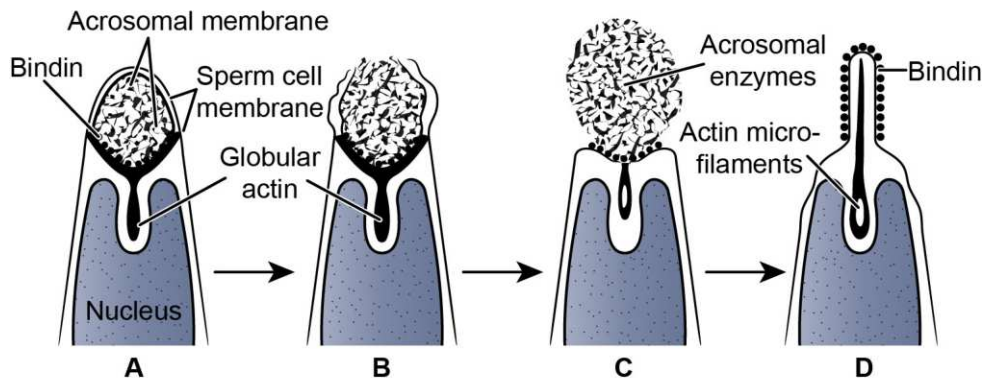


Fig.12.18: Events in the acrosome reaction in sea urchin sperm. A) A sperm head before the beginning of acrosome reaction. The acrosome is intact and is surrounded by the plasma membrane of the sperm; (B&C) At the beginning of the acrosome reaction, there is partial fusion of plasma membrane with the acrosome membrane. Also the acrosomal contents are released and this includes the protein bindin; D) Later stage of acrosome reaction in which acrosome has disappeared and the acrosomal process has extended further, exposing the bindin at the base. The assemblage of actin molecule to produce the microfilaments also occurs at this stage

Fusion of egg and sperm cell membranes

In sea urchin all regions of the egg cell membrane are capable of fusing with sperm while in other species only certain regions can do so. After the acrosome reaction, the fusion of the egg and sperm membrane causes the polymerization of the actin in egg to form a **fertilization cone** (Fig.12.19). Actin from both the sperm and egg forms a cytoplasmic bridge between them and the sperm nucleus and tail passes through the bridge into the egg. In sea urchin bindin plays a second role as a fusiogenic protein. In sea urchins, the accumulation of Ca^{2+} ions in the sperm appears to control the fusion of acrosomal and sperm membranes. Drugs which inhibit the Ca^{2+} ion movement into the cell are seen to prevent the occurrence of acrosome-plasma membrane fusion.

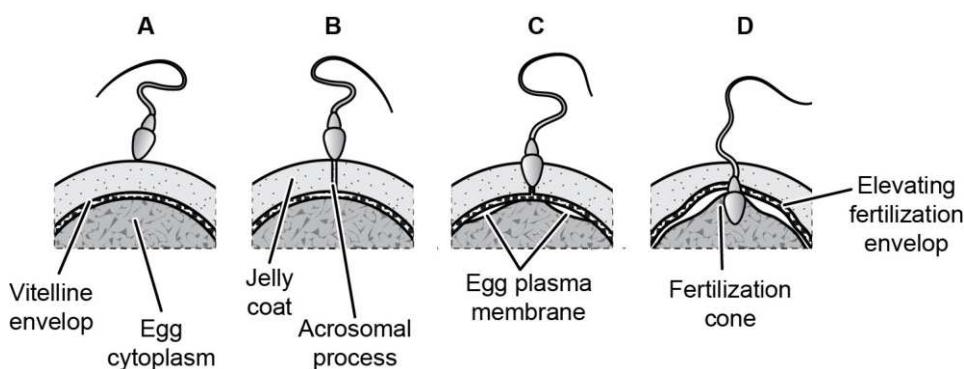


Fig.12.19: Events that occur during the sperm-egg fusion in sea urchin.

12.5.3 Regulation of Sperm Entry in the Egg

When the sperm enters the egg it is important to ensure that only one sperm is allowed to fuse with the egg so that the diploid ($n+n=2n$) number is maintained. To prevent polyspermy (union of more than one sperm with an ova), different mechanisms have evolved in animals. Two such responses of eggs to prevent entry of more than one sperm are seen in sea urchins: (i) the early response and (ii) the late response. Thus, prevention of polyspermy occurs in two phases: (1). the fast block phase and (2). the slow block phase.

Fast Block Phase

In sea urchins, the fertilization potential causes a temporary change in the voltage across the plasma membrane of the egg from negative to positive potential—from -70 to $+20\text{mV}$. Such potential changes prevent the fusion of more than one sperm with the egg. Recent studies have shown that the potential change is brought about by insertion of a positively charged fusion protein by the first sperm when it fuses with the egg. This fusion protein promotes sperm-egg fusion and the fertilization potential. Once the egg membrane becomes positively charged, insertion of another positive fusion protein by another sperm is not possible, as like charges repel.

Slow Block Phase

The slow block to polyspermy is achieved by cortical reaction, which is a slower mechanical block. The slow block is necessitated by the fact that the fertilization potential is only a temporary phenomenon and the potential of the egg membrane soon returns to negative value. Therefore, at time of fertilization the permanent prevention of entry of additional sperms is necessary.

The cortical reaction begins at the point of entry of the sperm into the egg (Fig. 12.20). The entry of the sperm triggers a wave of exocytosis of cortical granules which spread around the surface of the egg in one minute and fuse with the egg membrane and release their contents into the space between the vitelline envelope and the cell membrane. The perivitelline space is filled with hydrated proteins and mucopolysaccharides which along with the vitelline envelope form the **fertilization membrane**. The fertilization membrane acts in three ways: (i) the membrane increase the distance between the extra sperms attached to its outer surface and the egg plasma membrane through absorption of water; (ii) The peroxides and the peroxidases, released by the cortical granules harden the fertilization membrane and make it resistant to sperm proteases and (iii) the trypsin-like proteases of cortical granules the “cortical granule serine protease” clip off the Bindin receptors and destroy the glycoprotein receptors of the vitelline membrane thereby, dislodging the extra sperms.

Several experiments have demonstrated that Ca^{2+} ions are directly responsible for the cortical granule reaction. This influx of Ca^{2+} is not from outside but from the egg cell.

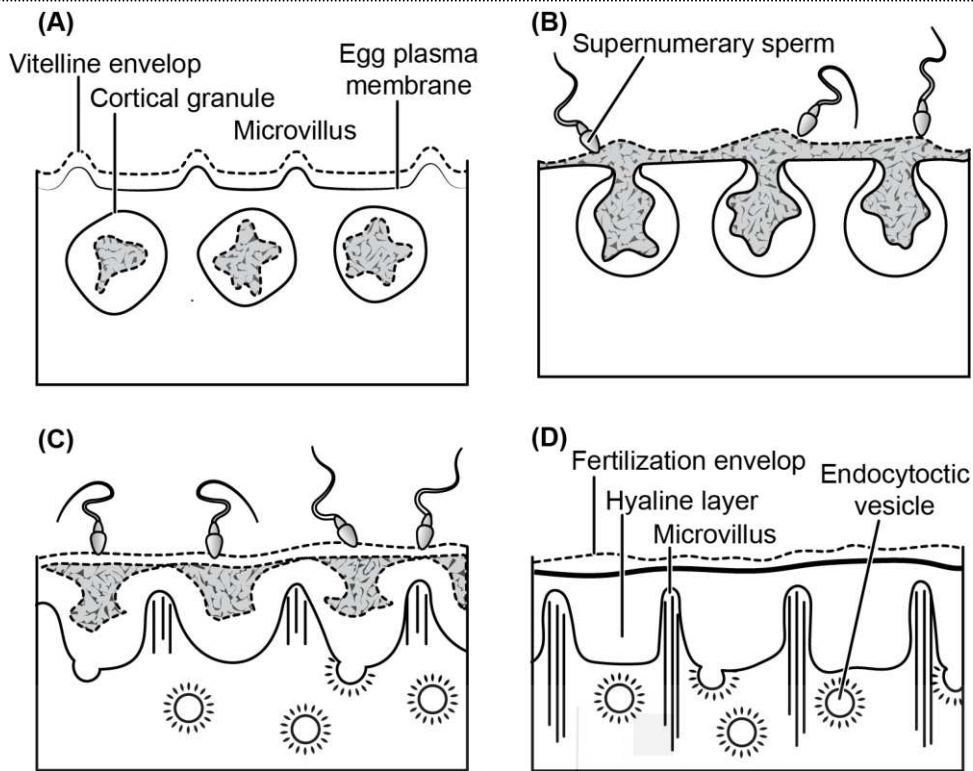


Fig.12.20: Diagram showing cortical reaction and fertilization membrane formation in sea urchin: A) Unfertilized egg with cortical granules, vitelline membrane and plasma membrane with microvilli; B) Just fertilized egg showing exocytosis of cortical granules and beginning of formation of fertilization membrane; C) Elevation of fertilization membrane and completion of cortical granule exocytosis; and D) Fertilized zygote showing elevated fertilization membrane, fully elongated microvilli and hyaline layer.

III. Fusion of Genetic Material

In sea urchins, the completion of second meiotic division in the ovum occurs after the entry of the sperm into the egg. The haploid egg nucleus after the entry of the sperm is known as the **female pronucleus**.

The male pronucleus is also formed in the egg by the breakdown of the sperm nuclear envelope, decondensation of chromatin and the formation of the pronuclear envelope. After the sperm enters the egg, the male pronucleus, separates from the mitochondria and tail and rotates 180° so that the sperm centriole now lies between the sperm and the egg (Fig12.21).

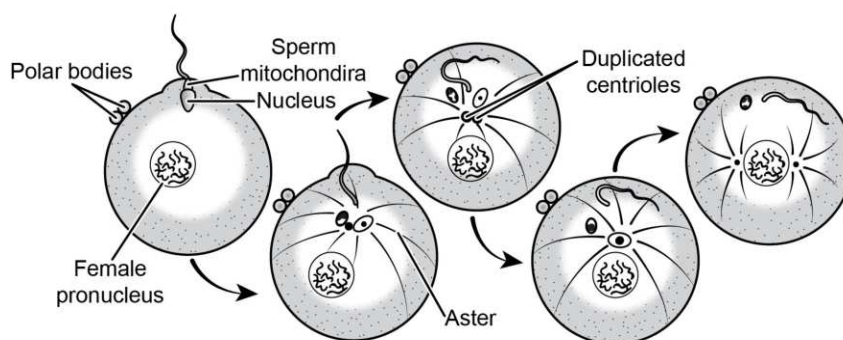


Fig.12.21: Diagrams showing the movement of pronuclei during fertilization in sea urchin.

The centriole brought in by the sperm now acts as a microtubule organizing centre to form the aster by the integration of the microtubules of both egg and the sperm. The two pronuclei migrate towards each other along these microtubules and then fuse to form the diploid zygote nucleus or synkaryon. Usually the nuclear fusion occurs between 10 to 20 minutes after attachment of the sperm to the egg. The mitochondria of the sperm ultimately disintegrate in the cytoplasm.

12.5.4 Fertilization in Mammals

Scientists have found it very problematic to study fertilization in mammals as it is difficult to mimic the conditions that occur inside the oviduct and furthermore, the ejaculated sperm population is highly heterogeneous, containing spermatozoa at different stages of maturation. In addition to this, the female oviduct or reproductive tract is not a passive passage for sperms but is a highly dynamic structure which regulates the transport and maturity of both ovum and sperms. Therefore the sperms undergo two unique processes before they make actual contact with matrix of the egg which contains the **cumulus oophorus**.

The first process is **translocation** which refers to the transport of sperms from vagina to the site of fertilization which is the ampullary region of the oviduct. The transport of sperms is brought about by uterine muscle contractions and to some extent by sperm flagellar motility.

The other process is **capacitation**, in which it is essential for the newly ejaculated sperms to undergo maturation in the female reproductive tract so that they become competent to fertilize the ovum. In mouse, the capacitation period is one hour and in humans 5 to 6 hours. Capacitation mechanism in mammals is generally poorly understood. Usually capacitated sperms consume more oxygen and this is believed to assist the mammalian sperm in penetrating through the surface coats, which protect the eggs and are also responsible for specific sperm-egg interaction.

The processes of both translocation and capacitation are influenced by different regionally specific factors such as Ca^{2+} channel, hyaluronidase enzyme, thermal gradient and chemotactic compounds such as the hormone progesterone.

Acrosome reaction

In mammals, the acrosome reaction commences when the sperms approach the zona pellucida of the egg. This reaction is characterized by the fusion of the outer membrane of the acrosome of the mammalian sperm with the plasma membrane of the egg. After the fusion, the acrosomal membrane vesiculates and releases the acrosomal contents which include several enzymes including **hyaluronidase**. Subsequently, the outer portion of the acrosomal membrane disappears and only the inner portion adjacent to the nucleus remains intact (Fig.12.22). Hyaluronidase acts on the cell surface polysaccharide hyaluronic acid and the sperm plasma membrane protein, PH20 acts as an enzyme and digests the hyaluronic acid. In addition a corona penetrating enzyme, **proteosomes** is also released. The function of these

enzymes is to loosen the corona radiata cells and help the sperm to reach zona pellucida. It is believed that the sperm using a protease called **acrosin** present on the sperm head penetrates its way through the zona pellucida. Zona pellucida is believed to possess species specific sperm receptors that prevent interspecific fertilization in mammals. For example, in mouse there are three glycoproteins in the zona pellucida termed ZP_1 , ZP_2 , and ZP_3 , of which ZP_3 is the sperm receptor.

The acrosome reaction in mammals is initiated by an influx of Ca^{2+} into the sperm. In the glycoprotein ZP_3 , the sugar moiety acts as a sperm receptor and the protein portion is believed to initiate the acrosome reaction.

Fusion of Sperm and the Egg

In mammals the fusion begins at the equatorial region of the sperm head. The plasma membranes of the two cells become continuous to form a cytoplasmic bridge through which the sperm nucleus enters the egg cytoplasm. Usually the entire sperm including the nucleus, centrioles, mitochondria, plasma membrane and even the flagellar axoneme enters the egg cytoplasm. Once the sperm enters the egg, the fertilization cone forms. The fertilization cone is an extension of the egg cytoplasm which is around the head of the entering sperm. Microfilaments in the fertilization cone virtually draw the sperm into the egg. Inhibitors of microfilament formation such as cytochalasin B inhibit the formation of fertilization cone and sperm entry into the egg. **Zonadhesin** a protein from the acrosome is believed to fix the sperm to the point of attachment and so provides a pivot point for the sperm to enter the zona pellucida.

Prevention of Polyspermy

In mammals fast block to polyspermy has not yet been detected, however a slow block occurs by enzymes from the cortical granules, which modify the zona pellucida proteins (sperm receptors), such that they can no longer bind to the sperm.

The cortical granules contain hydrolytic enzymes which are released into the perivitelline space during cortical reaction. This hardens the zona pellucida which becomes refractive to penetration by extra sperms. The changes in the zona pellucida are called **zona reaction**.

Fusion of egg and sperm pro-nuclei

The mammalian sperm enters almost tangentially to the surface of the egg and the glutathione in the egg cytoplasm allows the uncoiling of sperm chromatin. In mammals, the sperm enters the oocyte in which the nucleus is "arrested" in metaphase of its second meiotic division. The calcium oscillations brought about by the sperm entry inactivates MAP kinase and allows DNA synthesis. The centriole of male pronucleus produces asters, joining the two pronuclei of the sperm and egg and enabling them to migrate towards each other. They become apposed to each other but do not fuse. They remain adjacent to each other until the meiotic divisions, then their nuclear envelopes break down and the paternal and maternal chromosomes mix at a common metaphase plate. Thus a true diploid nucleus in mammals is first seen not in the zygote but at the two-cell stage.

Egg activation

Egg activation requires transient rise in Ca^{+} ions. This rise in calcium seems universal in the fertilization process in the animal kingdom. This rise in calcium is responsible for exocytosis of cortical granules, completion of 2nd meiotic division, resumption of DNA synthesis and general metabolic activation. The 2nd meiotic division results in expulsion of another polar body

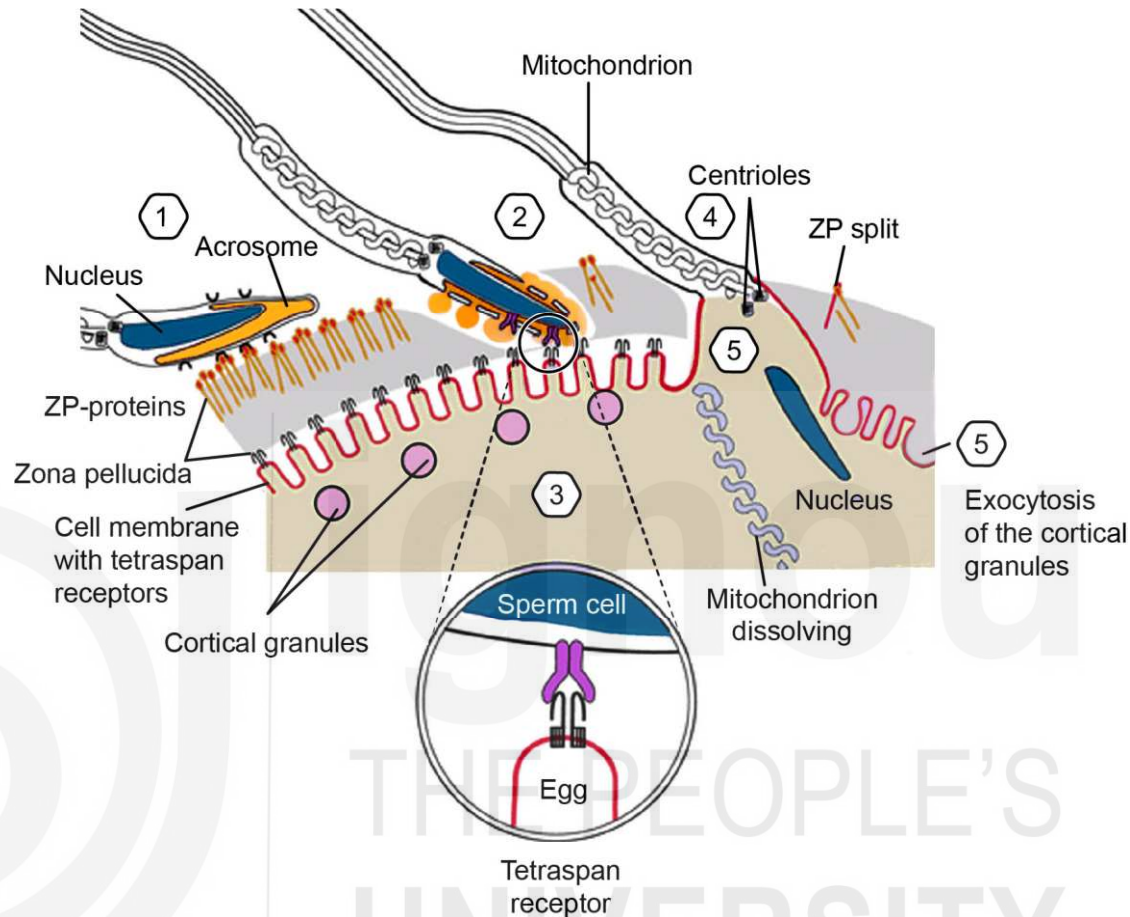


Fig.12.22: Fertilization in a mammal: (1) The sperm head docks with its equatorial zone onto the ZPprotein complex of egg envelope (zona pellucida). (2) Upon contact the acrosomal vesicle is fenestrated and releases a collection of hydrolytic enzymes which by their action form a slit into which the rotating spermatozoon moves, propelled by the flagellum. (3) The opening of the acrosomal vesicle exposes a still unidentified ligand (purple) that is bound by a complex of tetraspan protein complex in the egg cell membrane. (4) The mutual binding triggers the vesiculation of the membranes of the spermatozoon and the inner components of the sperm are released. The egg cell engulfs the nucleus, mitochondrion and centrioles of the sperm by endocytosis. (5) Mitochondria of the sperm are destroyed and degraded in the egg cell while the centrioles remain. (6) By means of exocytosis the cortical granules release enzymes (*blue*) that degrade the sperm-binding ZP-complex. A cortical reaction similar to that seen in the sea urchin egg and enzymatic linking of zona pellucida proteins provide a hardened fertilization envelope that hinders further sperm from entering the egg cell.

SAQ 5

- I) Choose the correct answer from the alternatives provided.
- a) Fertilization is responsible for the activation/arrest of development.
 - b) Activation of the sperm ensures/does not ensure that sperm will meet the egg.
 - c) In organisms with external/internal fertilization, chemotactic mechanisms have been evolved to attract the sperm towards the egg.
 - d) A period of maturation in the female reproductive tract required for the transformation of sperm is known as activation/capacitation.
 - e) Sperm using an enzyme called acrosin/hyaluronidase penetrate their way through zona pellucida.
 - f) The acrosome reaction in mammals is initiated by an influx of calcium ions/glycoproteins.
- II) Fill in the blanks with suitable words.
- a) is the extension of egg cytoplasm around the entering sperm head.
 - b) Inhibitor of microfilament formation such as prevent the formation of fertilization cone.
 - c) The early response for the entry of sperm into the egg is prevention of
 - d) The for polyspermy is mediated by the electrical depolarization of egg plasma membrane.
 - e) The slow block to polyspermy is achieved by reaction.
 - f) is the fusion nucleus of female and male pronuclei.

12.6 SUMMARY

After studying this unit you have learnt:

- There are several modes of sexual reproduction in animals. Reproductive development involves the formation of male and female sex cells or gametes by a process of gametogenesis. The basic mechanisms of gametogenesis are common to all sexually reproducing animals involving meiotic division which gives rise to haploid gametes.
- Sperm or male gametes are produced and differentiated in large numbers in the seminiferous tubules of the testes. A sperm consists of the head and its flagella. The flagella consist of a neck, middle piece, principle piece and tail end piece. Sperm head carries the sperm nucleus and acrosome. The neck piece has two centrioles. The middle piece has the energy supplying power plant in the form of mitochondria. Sperm tail

consists of typical flagellum or cilium. The basic purpose of sperm organization is to carry the paternal information to the egg and hence the motility is acquired.

- Fertilization brings about the activation of quiescent and stationary egg and restores the diploid state in the zygote for the next generation. The spermatozoa reach the egg by chance or in some cases because of chemical attraction. The release of acrosomal contents clears the passage through the egg envelopes and sperm nucleus eventually passes through the cytoplasmic bridge to the egg cytoplasm, and is then called the male pronucleus. The male and female pronuclei fuse, thus, completing the process of fertilization.
- Polyspermy is blocked by the depolarization of egg plasma membrane and formation of fertilization membrane resulting from the cortical reactions in most of the cases.
- Activation of egg involves the removal of inhibitory factors from the cytoplasm because of increase in the Ca^{2+} and rise in the pH. The cumulative effects of such changes bring about the sudden burst in protein synthesis, increase in oxygen consumption and ultimately initiation of cleavage of fertilized egg.

12.7 TERMINAL QUESTIONS

1. Briefly describe the spermiogenesis process.
2. What is the role of pH in sperm motility of sea urchin?
3. What are the major differences in the development and differentiation of an egg and a sperm?
4. Describe the mechanisms evolved by eggs to prevent polyspermy.

10.9 ANSWERS

Self-Assessment Questions

1. i)

S. No.	Asexual reproduction	Sexual reproduction
1.	Offsprings arise from a single parent or organism, and inherit the genes of only that parent.	Sexual reproduction, as a rule involves two parents, each of which is diploid (having full genome quantity).
2.	The gametes are not produced in asexual reproduction	Each parent contributes special germ cells called gametes or sex cells that are haploid as they contain half the amount of genome complement.
3.	Fertilization of gametes does not occur in asexual reproduction.	In sexual reproduction the haploid male gamete and haploid female gametes unite by the process of fertilization to form a diploid zygote.

4.	The number of chromosomes (unless mutations occur) almost never change during the entire process of asexual reproduction.	The number of chromosome is diploid in the parents and haploid in the gametes.
5.	The offsprings produced are clones of the parent,	The offsprings produced have different genotypes from the parents and other members of their species.

- ii) **Hermaphroditism** means the a single animal has both the male and female reproductive organs. Such animals are called monoecious. Hermaphrodites can fertilize themselves but most avoid self-fertilization and exchange germ cells with another member of the same species. While **parthenogenesis** means the development of an embryo from an unfertilized egg or from an egg in which the male and female nuclei fail to unite following fertilization.

2.
 - i) Spermatogenesis
 - ii) Seminiferous
 - iii) Multiplication, growth and maturation
 - iv) Primary spermatocytes, spermatids
 - v) Spermiogenesis
3.
 - i) Head (with acrosome, nucleus) middle piece (with mitochondria and Centrioles) and tail.
 - ii) To approach the stationary egg.
 - iii) mitochondria in middle piece.
4.

a) True	b) False	c) False
d) True	e) False	f) False
g) True	h) True	i) True
5.
 - I)
 - a) activation
 - b) does not ensure
 - c) external
 - d) capacitation
 - e) acrosin
 - f) calcium ions
 - II)
 - a) fertilization cone
 - b) cytochalasin

- c) polyspermy
- d) fast block
- e) cortical
- f) synkaryon

Terminal Questions

1. Refer to Subsection 12.3
2. Activation of stationary and quiescent egg, amphimixis, restoration of diploid state, generate genetic variations revitalization of cytoplasm.
3.

	Spermatozoa	Ovum
i)	After the completion of maturation division the spermatids undergo the phase of differentiation (spermiogenesis so that haploid	Even before the completion of maturation division, the female gamete is functional, and in some cases, no differentiation is needed after maturation division.
ii)	Only short duration of growth.	Growth period is much prolonged.
iii)	All the four spermatids become functional male gametes.	Only one ovum becomes functional female gamete and rest three are discarded as polar bodies
4. Refer to Subsection 12.5.3.

UNIT 13

CLEAVAGE AND GASTRULATION

Structure

13.1	Introduction	Products of Cleavage: Morula and Blastula
	Objectives	
13.2	Cleavage	Mechanism of Cleavage
	Types of eggs on basis of Yolk present	13.3 Gastrulation
	Influence of Yolk on Cleavage	Setting up Body Axes
	Planes of Cleavage	Morphogenetic Movements
	Patterns of Cleavage	13.4 Summary
		13.5 Terminal Questions
		13.6 Answers

13.1 INTRODUCTION

In the previous Unit of this Block you have studied how beginning of a new organism occurs with the onset of gametogenesis and fertilisation in which spermatozoa reach the ovum by either chance or chemical attraction. Eventually, one spermatozoon fuses with the ovum to restore the diploid genomic condition of the species and activates all the potentials in the fertilised egg cell or zygote to develop into a new individual of the next generation. But the zygote is one cell and the adult body in Metazoa is constituted of many cells - from a few hundred to many billions of cells. This implies that the unicellular zygote must enter a phase of rapid divisions in quick succession to convert itself into a multicellular body. Such a series of divisions of the zygote is known as **cleavage**. In this multicellular structure formed as a result of cleavage, the various cells or cell groups later become rearranged as layers and sub layers during a process called **gastrulation**.

Cleavage and gastrulation are significant phases in ontogenetic development because cleavage transforms the unicellular zygote into a multicellular body and gastrulation lays the foundation of primary organ rudiments so as to initiate the formation of organs according to the body plan of the particular metazoan group of animals to which the particular individual belongs. In this

unit, we will discuss the various types of cleavage patterns in animals and how these depend on the absence or amount and distribution of yolk in the egg as well as inherited maternal factors.

We then go on to study the process of gastrulation and the cell movements which give new positions to the blastomeres as the multilayered plan of the embryo is established. It is advisable that you revise Unit 11 again before studying this unit, as the underlying molecular mechanism of both cleavage and gastrulation have been discussed in it.

Objectives

After studying this unit you should be able to:

- ❖ explain the various planes of cleavage furrows;
- ❖ list different cleavage patterns;
- ❖ explain the purpose of gastrulation, and;
- ❖ describe the influence and the role of yolk in determining the pattern and course of cleavage and gastrulation in vertebrates.

13.2 CLEAVAGE

Cleavage is a series of cell divisions of the fertilised egg through which it is converted into a multicellular structure, called **blastula**. Practically no growth takes place during cleavage only the large volume of the zygote's cytoplasm is divided into numerous smaller cells called blastomeres. Except for mammals, in most other animals the initial rate of division and the placement of blastomeres in relation to each other is controlled by maternal proteins and mRNAs stored in the oocytes. Only in later stage, the division of cells is under the direct control of the genome of the zygotes. During cleavage the cell division is mitotic and has some striking peculiarities which makes it different from mitotic division in somatic cells. The main characteristic features of cleavage are as follows:

- a) All the divisions of the zygote are mitotic and occur in quick succession.
- b) Synchronisation of cell divisions of blastomeres: The early blastomeres divide simultaneously (synchronously) producing two blastomeres from zygote followed by 4,8,16,32,64 blastomeres and so on, in most cases. However, such synchronisation is lost, during later cleavage divisions.
- c) There is no interphase between two successive cleavage divisions in blastomeres, or the Interphase period in the cleavage divisions is very short and does not involve growth so that the resulting blastomeres becomes smaller in size as their number increases i.e. there is no growth in the amount of cytoplasm in the derived blastomeres with the result that the size of daughter blastomeres continues to decrease during successive cleavages.

- d) The size of the nucleus remains practically unchanged. Therefore, the nucleus: cytoplasm ratio, which is very small in the fertilized egg cell or zygote, continues to increase in the blastomeres, derived from successive cleavage divisions.
- e) The rate of cell divisions is very rapid and a very large number of cells are produced during cleavage. For example in fast cleaving embryos, such as embryos of frog *Xenopus laevis*, the cell division results in 37000 cells in 43 hours and in *Drosophila melanogaster* about 50,000 cells or blastomeres are produced in just 12 hours. This is possible due to absence of G₁ and G₂ phases in the cell cycle in their blastomeres. which just have a mitotic phase M and a synthesis Phase S, where only the nuclear material is replicated (see Fig.13.1 a) and so blastomeres go directly from M to S without the intervening G₁ or G₂ stages as present in the somatic cell cycles(see Fig.13.1 b). In both *Xenopus laevis* and *Drosophila melanogaster* the cell cycle of blastomeres after the midblastula transition have a G₁ and G₂ phase. The cell division rate slows down later on before gastrulation starts.

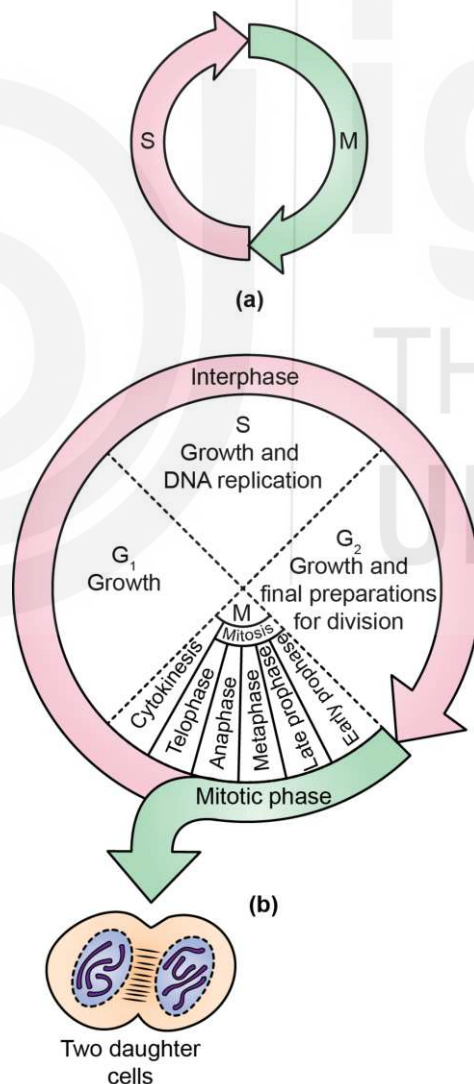


Fig.13.1: a) modified cell cycle of cleavage where G₁ and G₂ are omitted. b) phases in the normal somatic cell cycle. During G₁ RNA and other components needed for growth are synthesized and during S phase the cell synthesizes DNA, in G₂ phase it makes proteins and prepares to divide and division occurs during the M phase.

13.2.1 Types of Eggs on basis of Yolk Present

The yolk present in the egg is important as a nutritive material for the developing embryo. Furthermore, the amount and distribution of yolk determines the type and structure of the egg, and it also influences the rate and pattern of its cleavage. In other words, cleavage depends, to a large extent, upon the amount, distribution and orientation of yolk in the egg. In general yolk inhibits cleavage. When one pole of the egg is rich in yolk it is known as **vegetal pole** and the opposite pole is known as **animal pole**. Usually the animal pole has a sparse distribution of yolk and the cell's nucleus is present and positioned in the animal pole.

Types of Eggs

A) The eggs in various animal groups based on the amount of yolk, are of the following types (Fig.13.2):

- i) **Alecithal or yolkless eggs** as in the eutherian mammals. (Fig.13.2 A). In the absence of yolk the nutrition is provided by the placenta.
- ii) **Microlecithal or oligolecithal eggs** have little yolk which is in the form of granules, e.g., echinoderms, *Amphioxus*, molluscs (except cephalopods), annelids, flatworms (Fig.13.2 B).
- iii) **Mesolecithal eggs** have moderate amount of yolk, e.g. tunicates and amphibians (Fig.13.2 C). Since the eggs of these animals do not have much yolk they usually have larval stages that are voracious feeders.
- iv) **Megalecithal or macrolecithal or heavily yolked eggs**, e.g. cephalopod molluscs, bony fishes, reptiles, birds and egg laying mammals. The yolk occupies almost the whole of the interior of the egg with a small disc-shaped clear area of cytoplasm near the animal pole where the germinal vesicle (or nucleus) lies. Most of such eggs are large sized and the entire nutrition for the embryo is provided by the yolk (Fig.13.2 E).

B) The eggs can be categorized as follows on the basis of how yolk is distributed in them:

- i) **Isolecithal egg** in which the yolk is more or less evenly distributed yolk e.g., (echinoderms, *Amphioxus*, molluscs (except cephalopods), annelids. (Fig.13.2 B)
- ii) **Telolecithal egg** in which the yolk granules or yolk mass occupying the vegetal hemisphere is highly telolecithal as the yolk fills up almost the entire interior of the egg leaving only a small disc of clear cytoplasm containing the germinal vesicle (nucleus) near the animal pole of the egg. (Fig.13.2 C, D). Such types of eggs are seen in fish, reptiles and birds.
- iii) **Centrolecithal egg** – In insects the yolk granules are concentrated in the interior of the egg whereas the cytoplasm is

distributed as a thin peripheral layer around the yolk. Cell division takes place only in this rim of cytoplasm. (Fig.13.2 E).

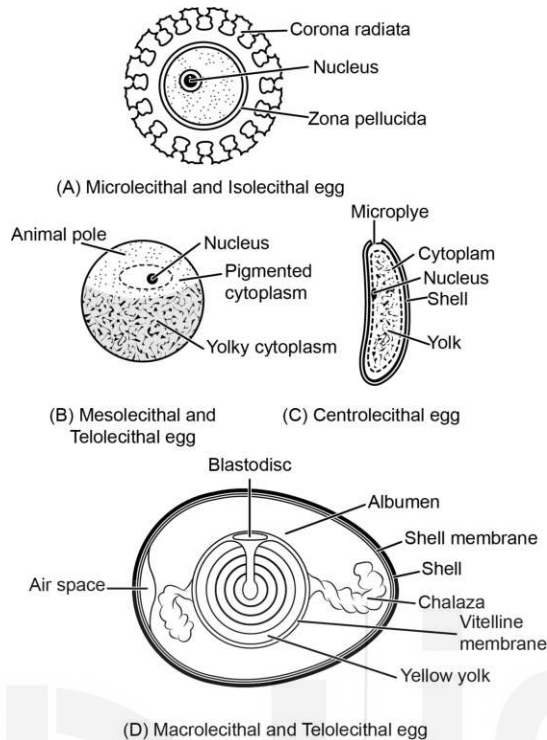


Fig. 13.2: Different types of eggs. A) microlecithal and isolecithal egg; B) mesolecithal and telolecithal egg; C) centrolecithal egg; D) macroleithal egg of hen.

13.2.2 Influence of Yolk on Cleavage

Though the biological significance of yolk is to provide nourishment to the developing embryo however, it is not part of the active cytoplasm. Yolk is the dead and inert component of the egg and does not participate in cellular activities, but, it influences cleavage in the following ways:

- With the gradual increase in the amount of yolk stored in the zygote, the total amount of the active cytoplasm tends to decrease.
- Cell division is the activity of only the nucleus and cytoplasm. With an increase in the yolk amount, the formation of spindles, cell membranes and cleavage furrows in the zygote takes place in the active cytoplasm which is restricted to a relatively smaller area of the zygote and its daughter blastomeres.
- The speed of cleavage is inversely proportional to the amount of yolk present. In fertilized telolecithal eggs, the blastomeres nearer to the animal pole divide at a faster rate than the blastomeres located towards the vegetal pole because of the passive behavior of the inert yolk in the yolk parts of the zygote and its daughter blastomeres obstructs the formation of cleavage furrows.

Therefore, the nature of various metabolic activities of the egg and the blastomeres derived from it depend upon the amount and placement of the yolk mass in the zygote.

SAQ 1

Fill in the blanks with appropriate words:

- i) During cleavage zygote and blastomeres divide by
- ii) There is no or between two consecutive divisions of blastomeres during early cleavage.
- iii) The egg of frog on the basis of yolk present in it is described as.....
- iv) Large size of hen's egg is due to large amount of.....

13.2.3 Planes of Cleavage

The ova of most of the animal groups (except some specific cases like insects) are spherical or nearly spherical having their own actual centre which is comparable to the earth shapes. Similar to north and south poles on earth, the egg has animal and vegetal poles (Fig.13.3). The yolk platelets have more density than the active cytoplasm which also contains the nucleus. The yolk platelets are concentrated more towards vegetal hemisphere. Therefore, when the egg lies in any fluid medium (the fundamental feature of most of the eggs even in the apparently terrestrial eggs like those of birds etc.), the vegetal pole tends to face the centre of gravity and animal pole is away from it.

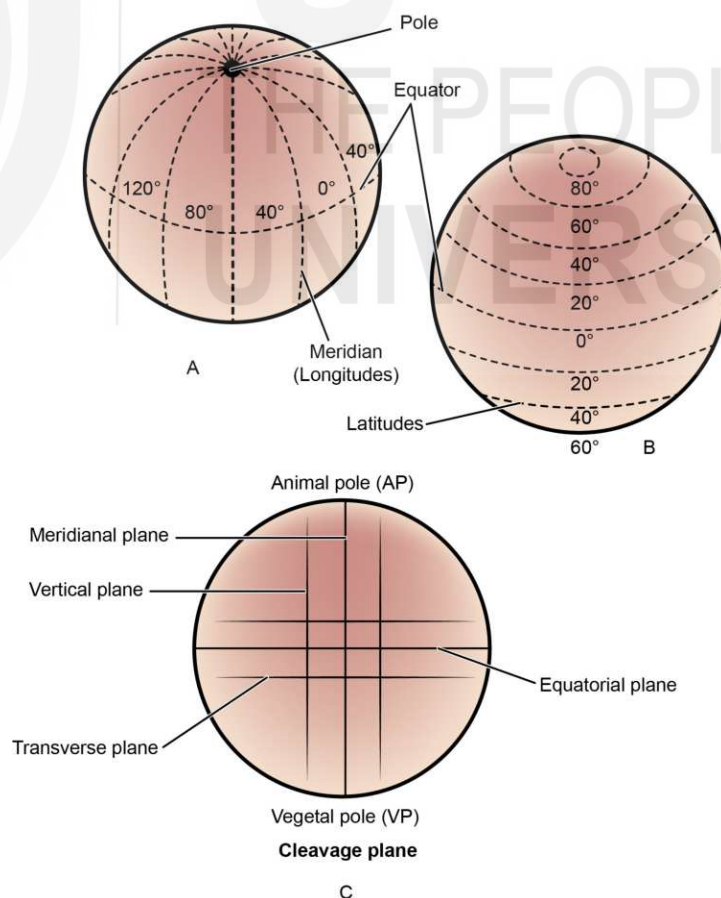


Fig. 13.3: A) Meridians (longitudes); B) Latitudes (imaginary lines on the earth surface which are comparable to the cleavage planes of a spherical egg); and C) Cleavage planes of egg.

With this picture in mind, we can now define the planes of cleavage of zygote or blastomeres, keeping in mind the imaginary lines (latitudes and longitudes) drawn on the earth surface (Fig.13.3).

The basic planes along which the egg and its daughter blastomeres are divided during early cleavage are as follows:

- i) **Meridional Plane** – the cleavage furrow passes from the animal pole to the vegetal pole through the centre of the spherical egg or the blastomeres, so as to divide the egg into two equal halves, e.g., first cleavage furrow in the chick and first as well as second cleavage furrow in the frog's egg (Fig.13.4 A, B).
- ii) **Vertical Plane** – the cleavage furrow may lie on either side of the central meridional plane passing through animal-vegetal axis. The cleaved cells may be unequal in size. e.g., (2) **Vertical plane**: When cleavage furrow passes from the animal pole to the vegetal pole, but it does not pass through the median axis of the egg. Example: 3rd cleavage plane of chick (Fig.13.4 C and 13.8 C), Bowfin (*Amia calva*) and Gar fish (*Lepidosteus*). (Fig.13.4 C).
- iii) **Equatorial Plane** – the cleavage furrow bisects the egg at right angle to the median axis exactly half way between the animal and vegetal poles. The cleavage furrow appears along the equator of the spherical egg e.g., the third cleavage plane of sea urchin (Fig.13.4 D).
- iv) **Latitudinal or transverse or horizontal plane** – it is like equatorial but the cleavage furrow passes through the egg cytoplasm on either side of equator along the latitudes of the egg sphere, e.g., third cleavage plane of amphibian eggs (Fig.13.4 E).

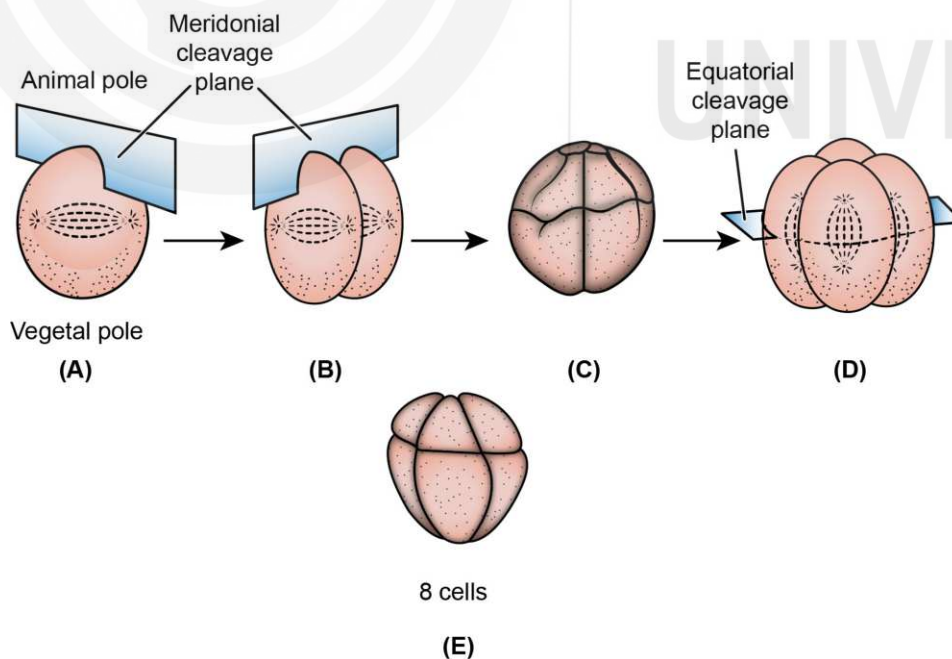


Fig.13.4: Planes of cleavage. A) and B) Meridional cleavages; C) Vertical cleavage; D) Equatorial cleavage; and E) Latitudinal cleavage plane the upper tier of blastomeres (micromeres) are smaller than the lower tier of cells (macromeres) as there is more yolk in the vegetal pole.

13.2.4 Patterns of Cleavage

The pattern of embryonic cleavage in animals is determined by two major parameters: the amount and distribution of yolk protein within the cytoplasm, and factors in the egg cytoplasm that influence the angle of the mitotic spindle and the timing of its formation.

In most of the animal groups with spherical or almost spherical egg in which yolk is absent (alecithal) or occurs in little or moderate amounts (micro-or mesolecithal eggs), the first and second divisions result in four blastomeres of almost equal size (Fig.13.4, A, B, C). The third cleavage due to greater concentration of yolk platelets in the vegetal hemisphere divides the 4 blastomeres in the latitudinal plane giving rise to 8 cells that are arranged in two tiers of 4 blastomeres each. The first tier consists of 4 small blastomeres (micromeres) and lies in the animal hemisphere while the second tier consists of 4 large blastomeres (macromeres) and lies in the vegetal hemisphere (Refer again to Fig.13.4 E). The arrangement of blastomeres in these two tiers is very distinct and on this basis, the cleavage may be of 4 types (Fig.13.5):

- a) **Radial type:** If each of the blastomeres of upper tier lies exactly over the corresponding blastomeres of the lower tier the pattern of cleavage is radially symmetrical. Radial cleavage is a characteristic of deuterostomes, and results in indeterminate blastomeres (cells that can individually give rise to a complete embryo, and they don't have a determined embryological fate early on during the development of the embryo). In other words, you can take a single cell from a developing embryo, and given the right condition, that single cell can give rise to a whole embryo e.g. echinoderms, *Amphioxus*, amphibians (see again Fig.13.4 E also see Figs.13.7 D-F).
- b) **Spiral type:** The upper tier of blastomeres of 8-cell stage embryo may be shifted with respect to the lower tier. They are not located exactly on top of one another; instead, they are located at a slight angle. This position results from the oblique (tilted) position of mitotic spindle so that, from the start, the two daughter blastomeres do not lie one above the other. Spiral cleavage is a characteristic of protostomes (annelids, molluscs, some helminthes) and results in determinant blastomeres (cell that have a determined embryological fate early on during the development of the embryo). In other words, determinant blastomeres are programmed to become a specific type of cell, early on during the process of development. Spiral cleavage is influenced by maternal cytoplasmic determinants. The right handed or clock wise displacement of the micromeres is called dextral spiral cleavage. The left handed or anticlockwise displacement of micromeres is called sinistral spiral cleavage. A good example is the left handed and right handed coiling of the shell in snail which is the result of the spiral cleavage as the direction of cleavage and shell coiling is the same (Fig.13.6).

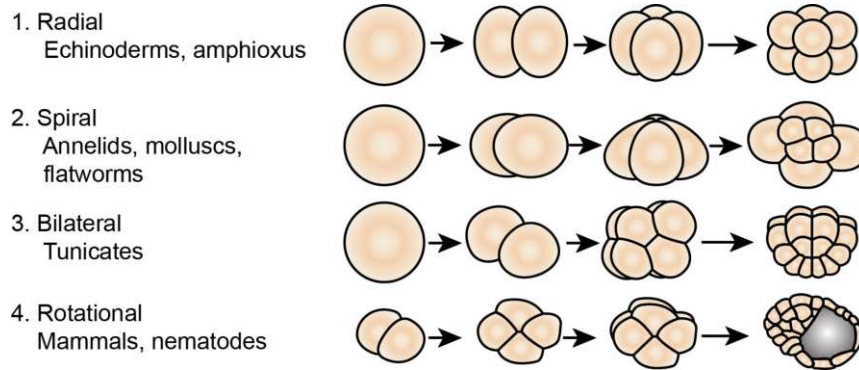


Fig.13.5: The four types of cleavage that occur in microlecithal (A to C) zygotes and D) the fourth type of cleavage that takes place in alecithal egg of nematodes and mammals(except for monotremes).

The turn of spiral in the snail as seen from above may be in a clockwise direction (dextral) or counter clockwise (sinistral) direction, (Fig.13.5 A,B). In many animals such as snails, it is a genetic character.

- c) **Bilateral cleavage:** In some animals (e.g., tunicates and nematodes, although in (different manner), the arrangement of 4 blastomeres after second cleavage is almost radially symmetrical as in the radial type of cleavage, but two of these are larger as compared to the other two blastomeres establishing a plane of bilateral symmetry in the developing embryo. During subsequent cleavages the bilateral arrangement of blastomeres may be still more obvious.
- d) **Rotational cleavage:** Zygote of mammals display rotational cleavage. Rotational cleavage involves a normal first division along the meridional axis, which gives rise to two daughter cells. The way in which this cleavage differs is that one of the daughter cells divides meridionally, whilst the other divides equatorially.

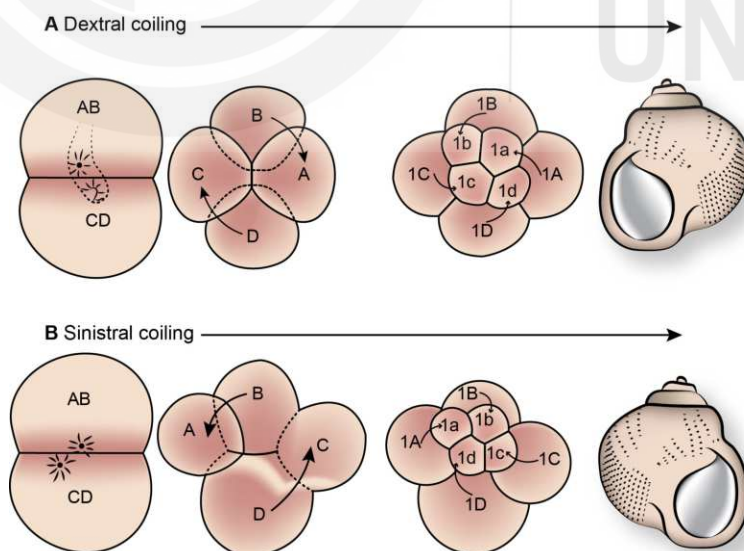


Fig. 13.6: Spiral cleavage in mollusc snails. Looking down upon the animal pole, the blastomeres are arranged either: A) clockwise (dextral) or B) anti-clock wise (sinistral). It happens to be a genetic character resulting in dextral or sinistral coiling of the shell of snails.

Furthermore, based on whether a particular cleavage furrow may divide the egg completely or partially, cleavage has been described as:

- A) **Holoblastic or complete cleavage:** Each cleavage furrow divides the entire egg completely in a particular plane. Holoblastic cleavage may be:
- i) **Equal holoblastic cleavage:** It occurs in alecithal (eutherian mammals) or microlecithal (*Amphioxus*, echinoderms) eggs where each cleavage furrow divides the egg so as to produce blastomeres of approximately equal size (Fig.13.7 A to G).

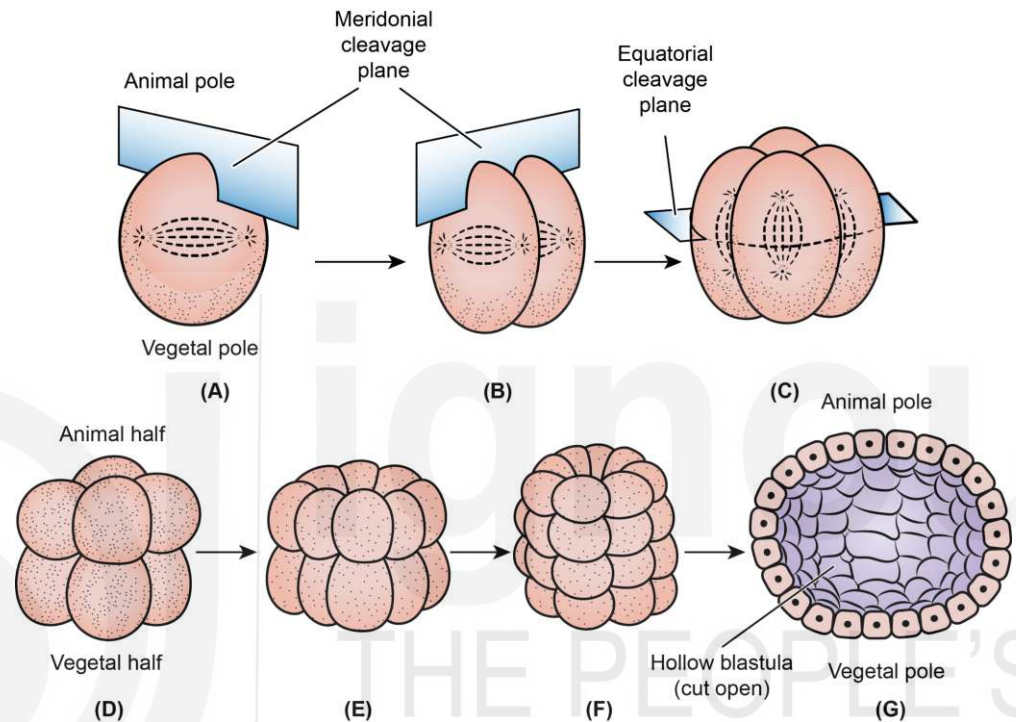


Fig. 13.7: Holoblastic and radial cleavage in the microlecithal egg of *Synapta digita* (Echinoderm) leading to the hollow blastula (G). A-B indicate the meridional planes of 1st and 2nd cleavage; C-equatorial plane (3rd cleavage); D, E, F showing the radial arrangement of blastomeres.

- ii) **Unequal holoblastic cleavage:** This takes place in mesolecithal and moderately telolecithal eggs (lower groups of bony fishes and amphibians), in which the yolk is largely concentrated in the vegetal hemisphere. In these eggs the first and second cleavage divisions take place along the meridional planes, producing 4 equal blastomeres. But, because of the yolk being concentrated in the vegetal region, the third cleavage furrow is latitudinal above the equator and closer to the animal pole. The furrow divides each of the 4 blastomeres completely but unequally into small and larger daughter blastomeres. The third cleavage is therefore, an unequal holoblastic cleavage which produces 4 small blastomeres (micromeres) in the animal region and 4 large blastomeres (macromeres) in the vegetal region (Figs.13.8 and refer again in Fig.13.4 E). Subsequently the micromeres containing relatively less yolk divide at a much faster rate than the large, yolkly macromeres.

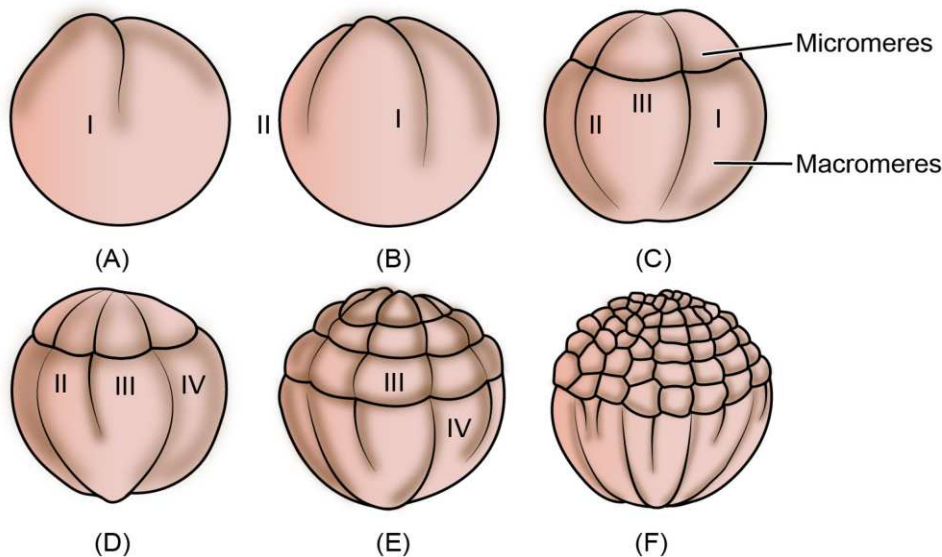


Fig. 13.8: Unequal Holoblastic Cleavage in frog's egg (A-F) cleavage furrows are designated, by Roman numerals indicating the order of appearance.

- B) Meroblastic or partial cleavage:** The fertilized egg does not divide completely because divisions are restricted to only a part of the egg while the rest of the egg remains entirely uncleaved. It is of two types:
- Discoidal meroblastic cleavage:** It takes place in the heavily, yolked macrolecithal and highly telolecithal fertilized eggs, as for example in cephalopod molluscs, reptiles, birds (Fig. 13.9) and monotremes (egg laying mammals). The cleavage is restricted to the cytoplasmic germinal disc situated at the animal pole. Even the germinal disc divides incompletely while the entire yolk mass remains undivided.

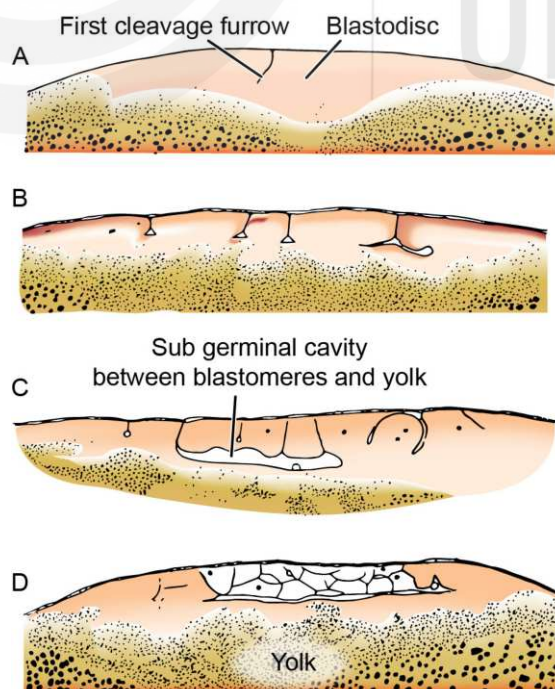


Fig. 13.9: Diagrams of sections of the fertilized chick egg. Discoidal meroblastic cleavage in which the blastodisc lies on top of yolk. Cleavage takes place only in the blastodisc.

- ii) **Superficial meroblastic cleavage:** This occurs in the centrolecithal eggs of insects. Cell divisions are restricted to the peripheral cytoplasmic layer while the centrally located yolk is left undivided (Fig.13.10).

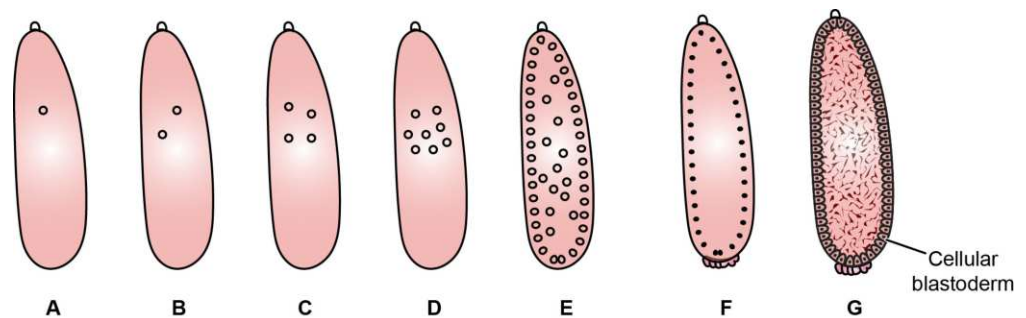


Fig.13.10: Diagrammatic representation of superficial cleavage in insect embryo. (A) Undivided zygote nucleus in the yolk. (B) – (E) After 1st, 2nd, 3rd and more divisions of the zygote nucleus. (F) Daughter nuclei have migrated from interior of the egg to peripheral cytoplasm which is still undivided. (G) Peripheral cytoplasm divided into separate cells to form cellular blastoderm around the undivided yolk.

We can now summarise the various types of cleavage in Table 13.1:

Table 13.1: Summary of Cleavage Types

Cleavage Patterns	Position of Yolk	Cleavage	Representative Animals
HOLOBLASTIC (Complete-cleavage)	Isolecithal (oligolecithal)	Radial /Spiral	Echinoderms, <i>Amphioxus</i> , most molluscs, annelids, flatworms, round worms.
	Yolk absent or sparse and evenly distributed	Bilateral/ Rotational	Tunicates eutherian mammals
	Mesolecithal, moderately telolecithal	Radial	Amphibians, lower bony fishes
MEROBLASTIC (Incomplete-cleavage)	Highly Telolecithal (Dense yolk)	Bilateral / Discoidal	Cephalopods molluscs reptiles, bony fishes, birds, egg laying mammals.
	Centrolecithal (Yolk concentrated in the centre of egg)	Superficial	Arthropods especially insects.

SAQ 2

- i) List out various planes of cleavage.
- ii) Fill in the blanks with appropriate word:
 - a) Meroblastic cleavage occurs in the and eggs.
 - b) The centrolecithal eggs of insects undergo cleavage because the cell division is restricted to the layer of the whereas the centrally located remains undivided.

13.2.5 Products of Cleavage: Morula and Blastula

In most cases, the blastomeres in early cleaving stages tend to assume a spherical shape like that of the egg before cleavage. Although their mutual pressure (resulting from limited available space within the egg envelopes) flattens the surface of blastomeres in contact with each other, the free surfaces of each blastomere remain spherical. As a result, after some cleavage divisions have taken place the embryo has a shape resembling mulberry. Because of this superficial resemblance this stage of embryonic development of many animals has been referred to as **morula** (Latin for mulberry) (Fig.13.11).

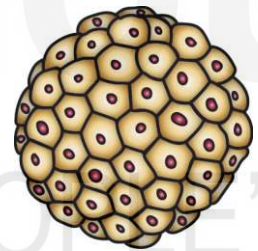


Fig.13.11: A morula which is a solid ball of cells.

As cleavage continues the subsequent arrangement of the blastomeres in the morula may vary in different groups of animals. In some the blastomeres are packed together without any space between them, or a small cavity appears but is soon obliterated. In both cases the result is the formation of a solid blastula called **stereoblastula** e.g. some flatworms, annelids, molluscs and coelenterates. In such blastulae some of the blastomeres lie externally and others in the interior.

But, in most animals the cavity appearing between the blastomeres persists and may enlarge. This cavity is called **blastocoel**. As the cleavage progresses, the adhesion of the blastomeres to one another increases, being bound by cadherins, and will usually have a system of tight junctions forming a seal between the external environment and the internal environment of the blastocoels. The blastomeres arrange themselves as an epithelial layer around the blastocoel. This stage of embryonic development is called **coeloblastula** (hollow blastula) or simply **blastula**. The layer of blastomeres is referred to as **blastoderm**.

The structure of blastula becomes modified in various animal groups. The modifications are related to the amount of yolk deposited in the egg, as you will see from the accounts of blastula structure in some deuterostome animals.

The blastula of most echinoderms consists of a fluid filled blastocoel surrounded by a single layer of cuboidal blastomeres, which constitute the simple epithelial blastoderm. In the blastulae of sea urchin and *Amphioxus*, the blastoderm surrounding the blastocoel is an epithelium consisting of a single

layer of columnar cells (blastomeres). However, the vegetal blastomeres are larger than animal blastomeres so that the epithelium is thicker at the vegetal pole and thinner at the animal pole. Thus the polarity of the egg persists in the blastula (Fig.13.12, A, B).

Animals with larger amount of yolk (e.g. amphibians) show considerable differences in size among the cells of the blastoderm, and the blastocoel is distinctly acentric nearer to the animal pole instead of being in the centre. The blastoderm is not a simple epithelium of a single of cells but is two or more cells thick (Fig.13.12 C)

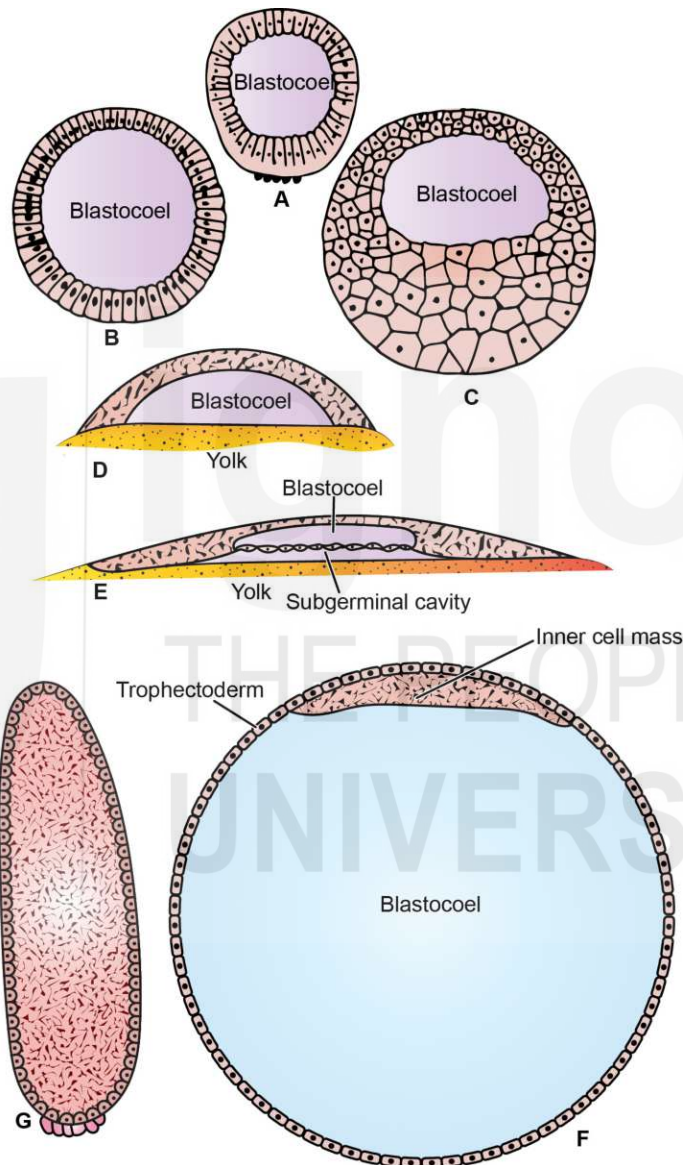


Fig. 13.12: Diagrammatic comparison of blastulae. (A) in echinoderms.

(B) *Amphioxus*; (C) Amphibian ; (D) bony fish ; (E) bird (F) blastocyst of mammals and (G) insect.

The cells of the inner side of blastoderm are loosely connected to one another but those at the external surface adhere with each other very firmly because of the presence of tight junctions between them (recall cell adhesion from Unit 11). The blastoderm at the animal pole and most of the animal hemisphere is made up of micromeres forming the dome-shaped roof of the blastocoel while the blastomeres (macromeres) of the vegetal hemisphere form the floor of the blastocoel). The amphibian blastula is also a coeloblastula but it is modified as

described above due to larger quantity of yolk mostly located in the vegetal hemisphere of the egg (Fig.13.12 C). The embryonic stage comparable to a blastula occurs in a still more modified form in the sharks, bony fishes, reptiles, birds and egg laying mammals, all with macrolecithal and highly telolecithal eggs. As you have learnt in the earlier subsection, in the egg laying amniotes reptiles, birds, monotremes the active cytoplasm is restricted to a small disc (cytoplasmic germinal disc) on top of the yolk near the animal pole. Cleavage is meroblastic occurring only in the germinal disc and gives rise to a disc-shaped blastoderm made of several layers of cells lying on top of the uncleaved yolk. Such a blastula is called discoblastula. Between the blastoderm and yolk there is a narrow space called sub-germinal space (or segmentation cavity), which is not comparable to blastocoel. In the birds a true blastocoel appears later between the upper layer of blastoderm (epiblast) and the lower layer (hypoblast) which is formed by cells migrating from the blastoderm (Fig.13.12 E).

In insects having centrolecithal eggs, the blastula stage does not have any cavity. It is characterized by one cell thick epithelial blastoderm enclosing the yolk filled interior. Such a blastula is called superficial blastula (Fig.13.12 F).

Cleavage of the yolkless eggs of eutherian mammals gives rise to a solid ball of cells (morula). Fluid is secreted into the space between the cells of morula which grows in size to become the **blastocyst**. This is the blastula stage of the embryos of eutherian mammals. Structurally it consists of a single layer of cells (trophectoderm) enclosing a large fluid filled blastocoel. At one end of the blastocoel pressed up against the inner surface of trophoctoderm there is a group of cells referred to as the **inner cell mass (ICM)**. The entire body of the embryo is formed from cells of ICM (Fig.13.12 G).

SAQ 3

- i) Explain why, that inspite of little or no yolk present in the fertilized eggs of echinoderms and eutherian mammals the cleavage patterns of their fertilized eggs follow entirely different courses.
- ii) A list of various animals is given below. Mention the type of cleavage and the resultant blastulae formed:

Animals	Types of Cleavage	Type of blastula
a) Rat (<i>Rattus rattus</i>)		
b) Labeo rohita (rohu fish)		
c) <i>Rana tigrina</i> (frog)		
d) <i>Calotes versicolor</i> (garden lizard)		
e) Pigeon		
f) Sea urchin		
g) <i>Nereis</i> (Annelida)		

13.2.6 Mechanism of Cleavage

Like mitotic divisions that takes place in any cell, cleavage is the result of two events: mitotic nuclear division (karyokinesis) followed by cytoplasmic division (cytokinesis). The two events involve numerous metabolic processes. Recall we had said in the beginning of the section that in most animals the egg cytoplasm contains regulative factors which fulfill all the requirements for these processes before the egg leaves the ovary. These factors are synthesized and stored in the cytoplasm during oogenesis as a result of the activity of maternal genes present in the oocyte. Fertilization by sperm activates the metabolic processes in the egg cytoplasm and initiates cleavage according to the programme already set by the maternal genes during oogenesis. There is much evidence to prove that cleavage is guided by the genetic information received by the egg cytoplasm from the mother during oogenesis. There is little or no transcriptional activity in the zygotic nucleus during early cleavage. Therefore, the effects of paternal genes that come into the egg with sperm nucleus are transcribed only later.

The regulative factors for such biphasic cleavage are said to lie in the egg cytoplasm itself.

The Cytoskeletal Mechanism

Two co-ordinated processes occur during cleavage (or cell division) as shown in Fig.13.13.

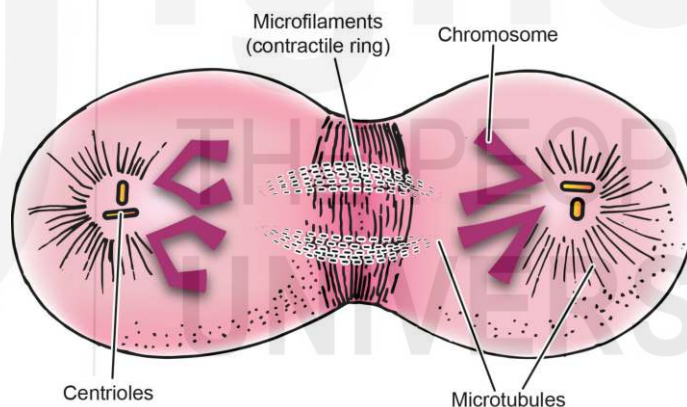


Fig. 13.13: Role of microtubules and microfilaments in cell divisions. In the telophase cell shown here, the chromosomes are being drawn to the centrioles by microtubules while cytoplasm gets pinched in through the contraction of the microfilaments.

1. **Karyokinesis:** The mitotic division of nucleus depends upon the formation of the mitotic spindle. The mitotic spindle is constituted by microtubules of which the tubulin protein is the structural unit. If the egg is treated with the drug colchicine the microtubules are disrupted and karyokinesis is arrested at metaphase.
2. **Cytokinesis:** Division of the cell depends upon the contractile microfilaments of which protein actin is the structural unit. A ring of microfilament appears in the cortex around the cell where the cleavage furrow is formed. Contraction of the microfilament ring in a purse-string manner deepens the furrow, ultimately cutting the cell into two (Fig.13.13). Treatment of the egg with **cytochalasin B** inhibits the organisation of contractile ring of microfilaments so that cleavage furrow is not formed and cytokinesis does not take place.

Karyokinesis and cytokinesis are co-ordinated processes with the latter following the former. However, the exact mechanism which brings about this coordination is not known so far. The available evidence suggests that mitotic spindle dictates the location of cleavage furrows. The furrow always forms perpendicular to the long axis of the spindle.

Although karyokinesis and cytokinesis are coordinated they are however, independent processes. Nuclear divisions can take place without being followed by cytoplasmic division. As you had learnt in Unit 10 in the case of syncytial egg of insects, cytoplasmic divisions take place only later when all these nuclei have migrated to the peripheral cytoplasm (Refer again to Fig.13.9). Similarly cleavage of cytoplasm can take place even if karyokinesis is blocked, e.g. if the zygotic nucleus of a fertilized egg is removed the enucleate egg cytoplasm undergoes cleavage divisions up to about the blastula stage.

The Formation of New Membranes

Division of the egg or a blastomere increase the total surface area of the two daughter cells that are required to be covered by the membrane at each cleavage. The existing membrane of the parent cell is insufficient. From the evidence available so far, it is indicated that this insufficiency of membranes for daughter blastomeres during cleavage is made up from two sources:

- i) A portion of the membranes covering the daughter cells is provided by stretching and extension of the original plasma membrane of the zygote or the blastomeres.
- ii) A portion of the cell membrane is newly synthesized by the daughter cells.

Thus, the furrow membranes are a mosaic of different parts.

SAQ 4

- i) Define:
 - a) Karyokinesis.
 - b) Cytokinesis.
 - ii) How is cleavage division affected by treatment of the egg with:
 - a) Colchicine.
 - b) Cytochalasin B.
-

13.3 GASTRULATION

The end of cleavage of the zygote results in the formation of a blastula, which in different species could be, a solid structure without a cavity or its cells may be arranged in the form of a one cell or several cells thick epithelium around a

cavity or around or on top of the yolk. In any case the blastula has no resemblances to the shape or organization of the body. Therefore, through the subsequent developmental stage, the simple blastula would transform itself into a more complex embryonic structure upon which the adult like body may be built up. Such a process of transformation is known as **gastrulation**. It is a very significant phase of embryological development, which marks the beginning of the form and organization of the adult body.

In the blastula all the cells are located on the surface forming the blastoderm. During gastrulation there occurs displacement of the parts of blastoderm which gives rise to three germinal layers viz. ectoderm, mesoderm and endoderm from which all the future organs will develop. The presumptive endodermal and mesodermal cells move from the surface of blastula into the interior of the embryo where the respective organs are formed during the course of further development. The cells of the presumptive ectoderm remain on the surface. With the exception of some parasitic flatworms a new cavity called the archenteron (future alimentary canal) is formed which is surrounded by endoderm and which thus, initiates the tube within tube structure of the triploblastic animals.

Thus, gastrulation is a dynamic process involving large scale movement of blastula cells resulting in their arrangement in a way which establishes the basic body plan according to which the embryo has to develop further. Since these movements lay the foundation for the form and organization of the body they are called **morphogenetic movements**. These involve the movements of the epithelial cells layers as a whole as well as the independent movements of the cells which break loose from epithelium and become mesenchymal. **In general gastrulation results in the following outcomes:**

- The formation of the three germ layers namely ectoderm, mesoderm and endoderm.
- The formation of the embryonic gut or archenteron
- The appearance of the major body axes. Though in some animals the information specifying the body axes is already present in egg in the form of cytoplasmic determinants and or the polarity of the yolk. However, the polarity becomes actually visible during gastrulation.
- Rearrangement of cells of the embryo by means of morphogenetic movements.
- The rhythm of cell divisions slows down. Growth, if any, is insignificant.
- There is intensification of the process of oxidation in the cells.
- The nuclei become more active in controlling the activities of the embryonic cells. The influence of paternal genes becomes evident during gastrulation.
- Proteins of many new types that were not present in the egg or blastula begin to be synthesized.

13.3.1 Setting up Body Axes

In Unit 10, you had been introduced to the formation of the body axes in the developing embryos. During gastrulation before the cell movements and migrations occur, the embryo must have the information to send the mobile(moving) cells to their positions so that the body tissues and organs are formed. For this the orientation for the anterior- posterior position; dorsal-ventral surfaces and left and right sides of the body have to be determined first. This happens even before the three germ layers are established. We have said earlier in this unit that research over the years has shown that in some animals the embryo is already patterned as a result of maternal factors that are laid down in the egg during its development in the ovary. We also know that this is the case in most insects (*Drosophila*). But does this happen in all vertebrates as well? Early experiments in embryology have shown that is the case in amphibians, and fish, (in *Xenopus* and Zebra fish, the favourite anamniotes models of developmental biologists), maternal factors are indeed present and these are mRNAs and proteins that are laid down while the egg is being developed in the ovary. The *Xenopus* egg already has a distinct polarity even before fertilization- the animal pole (top region where the egg nucleus lies) and vegetal poles (bottom yolk heavy region). This defines the animal-vegetal axis and also the cleavage pattern. The animal-vegetal axis of the egg is related to the anterior-posterior axis of the tadpole. However, which side of the animal region will form the head is not clear until the dorsal side of the embryo is specified. The dorsal-ventral axis is specified only after fertilization as the entry of the sperm sets in motion a series of events which will decide the dorsal-ventral axis. The dorsal side is opposite to the point of entry of the sperm. Sperm entry can happen anywhere on the egg and causes the outer layer of the cytoplasm (cortex) to loosen and shift by 30 degree away from the point of entry of the sperm. This causes the maternal factors, originally located in the vegetal region to move to sites on the equator. The second cleavage division causes this factor to be located only on two blastomeres that become the dorsal region. The establishment of the dorsal region allows the organizer region to develop. As we had read in Unit 11 the organizer is found to be active in the early period of development.

In chick embryo gravity defines the polarity of the egg and the blastoderm comes to rest on top of the yolk. The embryo's own genes are expressed from the beginning of cleavage and it is unlikely that maternal genes influence early patterning. The body axes are determined once the primitive streak starts to elongate. In mammals from which we take mouse as an example, there is no yolk and early development involves the formation of extra-embryonic membranes and placenta which connects to the mother for providing nourishment to the embryo. There is no clear polarity in the egg and the role of maternal genes in early development has not been established in mammals as the zygote's genes are active from the beginning. Under the direction of signals from embryonic and extra embryonic tissues the body axes are established later.

The details of the process of gastrulation are not easy to understand without the knowledge of positions of the cells of three germinal layers in the blastula. Recall from Unit 11 that a chart or diagram showing the prospective fate of

each part of blastula or embryo at any stage of development is called a "fate map". We have already studied the various ways of constructing the fate maps of blastulae and each method has its own advantages and disadvantages

13.3.2 Morphogenetic Movements

When you look at fate maps it becomes clear why the cells have to move during gastrulation. For example, if you look at the amphibian fate map you can see the presumptive ectoderm, endoderm and mesoderm all as adjacent areas in an epithelial sheet (Fig.13.14). The endoderm and mesoderm must move inside the embryo. Endoderm and mesoderm will separate from the ectoderm and move inside to form the internal gut. Gastrulation is thus a dynamic process involving a variety of coordinated movements of cells of different areas of the blastula. **The movement of cells in the embryo from one place to another to form particular structures is known as morphogenetic movements.**

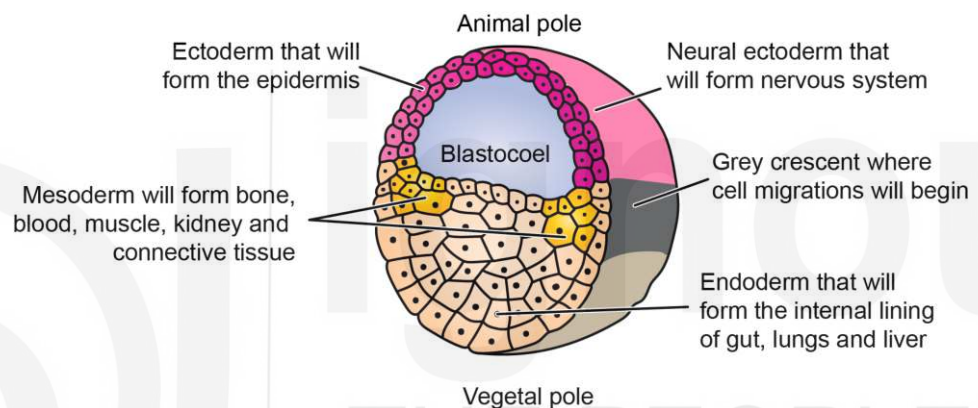


Fig.13.14: Fate map of Frog embryo to show the presumptive ectoderm, endoderm and mesoderm.

In the embryo the morphogenetic movements of cells from one place to another in order to establish a particular form or structural arrangement, occurs during embryonic development from the beginning of gastrulation onwards as well as in the adult body.

In Unit 11 you had learnt that the primary force for morphogenetic movements is provided by cell shape change, cell adhesion, ability of individual cells to migrate. For convenience, the types of cell movements are described separately but it should be understood that two or more of them may occur simultaneously. Broadly, there are two groups of morphogenetic movements in embryonic development i.e., epiboly and emboly.

i) **Epiboly**

Epiboly means to throw on or to extend upon (Fig.13.15). It is the movement of epidermal cell sheets spreading over as a unit to cover the deeper layers of the embryo. It occurs only in the presumptive ectodermal layers (epidermal and neural areas). The cells of this area have an inherent property of flattening, expanding and stretching. The cells of the presumptive ectodermal areas remain on the surface, eventually forming the outer layer covering the entire embryo and enveloping the inwardly migrating presumptive mesodermal and endodermal layers.

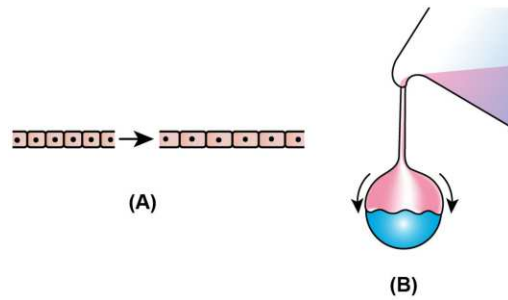


Fig.13.15: A) Epiboly: During epiboly, a sheet of cells spreads by thinning while its overall surface area increases in the other two directions. Epiboly can involve a monolayer (i.e. a sheet of cells which is one cell layer thick), in which case the individual cells must undergo a change in shape. In other cases, however, a sheet that has several cell layers can become thin by changes in position of its cells. In this case, epiboly occurs via intercalation. **B) Diagram showing the analogy with epiboly in which a viscous liquid is poured over a sphere. The liquid slowly spreads covering the surface of the sphere.**

ii) Emboly

Emboly means to throw in or to thrust in. Such movements bring about the migration of presumptive mesodermal and endodermal cells from the external surface of the embryo into its interior.

Emboly includes several different types of cell movements which are as follows:

a) Invagination

Invagination specifically includes the process of infolding or rolling in of the presumptive endodermal areas (Fig.13.16) and is the most widely observed embolic movement during gastrulation in most animals, e.g. echinoderms, *Amphioxus* and amphibians etc. Invagination may be passive, occurring as a result of the activity of other cells, or active, resulting from the inherent forces within the invaginating cells.

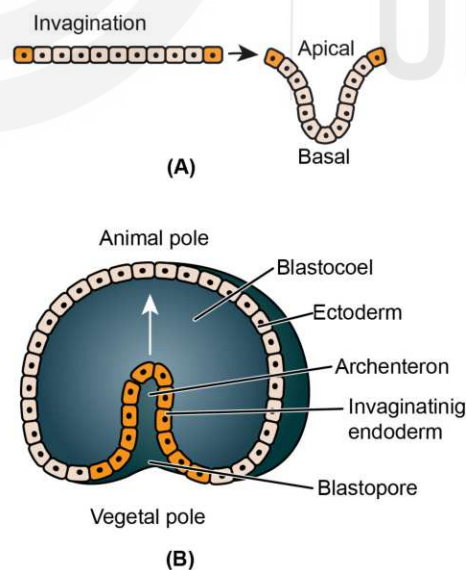


Fig. 13.16: (A) Diagrammatic representation of gastrulation by invagination. During invagination, an epithelial sheet bends inward to form an inpocketing. One way to think of this in three dimension is by imagining that you are poking a partially deflated balloon inwards with your finger. The resulting bulge or tube is an invagination; **(B) Gastrula.**

Various causes have been attributed to the process of invagination:

- Absorption of blastocoelic fluid by certain cells.
- Differences in the characteristics of blastocoelic fluid and external medium.
- Higher relative alkalinity of blastocoelic fluid which causes local surface tension changes in the membrane of certain cells.

It should be kept in mind that not any one factor causes invagination but a combination of different factors may be involved in various animals.

b) Involution

Involution denotes inward movement of an expanding outer layer so that it spreads over the internal surface of the remaining external cells (Fig.13.17). Involution of mesodermal blastomeres has been observed in *Amphioxus*, amphibians, birds, reptiles, monotremes and even in some eutherian mammals.

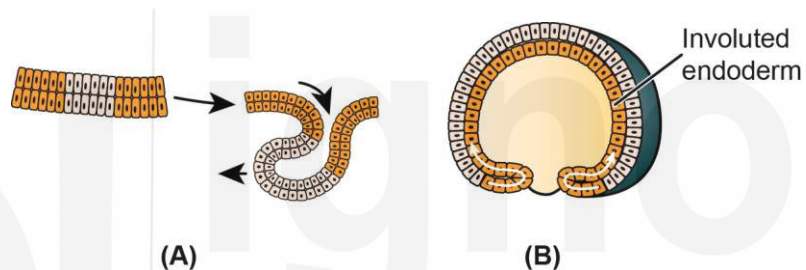


Fig. 13.17: During involution, a tissue sheet rolls inward to form an underlying layer via bulk movement of tissue. (A) One helpful image here is of a conveyor belt. As the material moves in from the edges of the sheet, material originally at the sites of inward rolling (shown in blue here) is free to move further up underneath the exterior tissue. (B) involution of endoderm in sea urchin.

c) Intercalation

Intercalation is another form of morphogenetic movement. During intercalation, two or more rows of cells move between one another, creating an array of cells that is longer (in one or more dimensions) but thinner. The overall change in shape of the tissue results from cell rearrangement. Intercalation can be a powerful means of expanding a tissue sheet. A specialized form of intercalation is convergent extension (Fig.13.18).

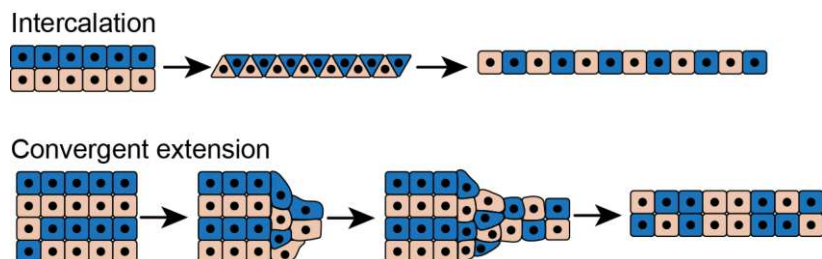


Fig.13.18: Intercalation involves two or more rows of cells that move between one another creating an array of cells.

d) **Delamination**

Delamination (Fig.13.19), denotes the separation of groups of cells from other cell groups to form separate cell layers. It includes splitting of a pre-existing sheet (layer) of cells into two more or less parallel sheets, usually with a space separating them.

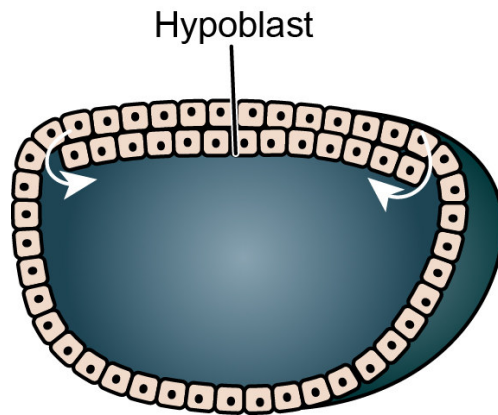


Fig.13.19: Delamination results in the formation of the hypoblast from epiblast in amniotes.

e) **Ingression**

In this process, migration of individual cells from the surface blastoderm or blastodisc into the embryo's interior takes place. Individual cells become mesenchymal (i.e., separate from one another) and migrate independently into the cavity or spaces developed within the embryo (Fig.13.20). Primary mesodermal cells of sea urchin embryo become internal by this process. Neural crest cells are an example of a mesenchymal cell type that emigrates out of an epithelium.

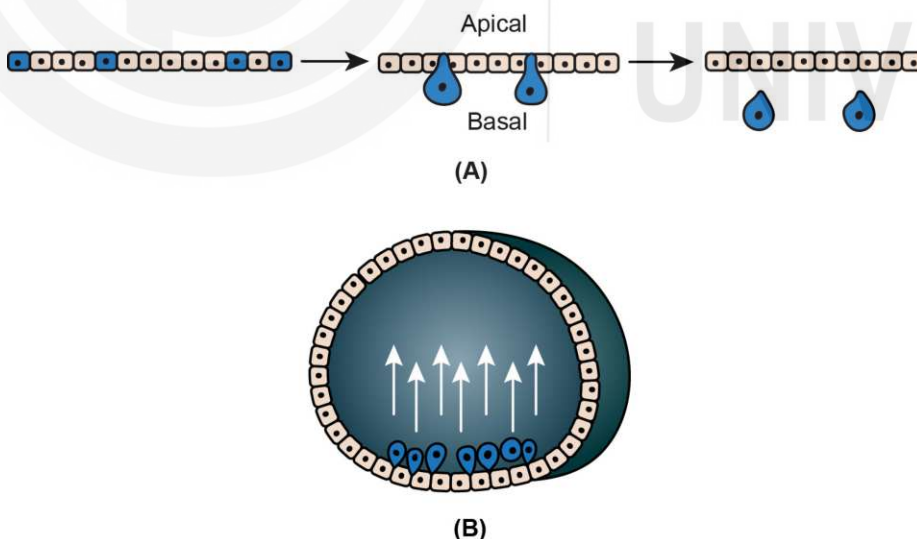


Fig.13.20: Ingression: (A) During ingression, cells leave an epithelial sheet by transforming from typical epithelial cells into freely migrating mesenchyme cells. To do so, they must presumably alter their cellular architecture and their adhesive relationship to the surrounding cells. (B) Diagrammatic representation of ingression of primary mesenchymal cells in sea urchin embryo.

In this section you have studied the general types of cell movements during gastrulation that are responsible for repositioning cells in the embryo. In the next block of this course you will see how these cell movements place cells in a different environment with the potential to receive new signals for further development. We will study the embryonic development in two representative vertebrates namely, the frog (anamniote) and the chick (amniote). You will see how all the general principles of development are applied in these two examples.

SAQ5

List the various morphogenetic movements through which the gastrulation may take place.

13.4 SUMMARY

After studying this unit you have learnt:

- Cleavage transforms the unicellular zygote into a multicellular structure. The divisions during cleavage are essentially mitotic but they lack G₁ and G₂ phases (or collectively interphase). The important characteristics of cleavage are synchronization, little or no displacement of cytoplasmic substances, no change in the embryonic shape and considerably high nuclear/cytoplasmic ratio.
- The amount of yolk determines the structure of the egg zygote which may be alecithal (eutherian mammals), microlecithal (Amphioxus), mesolecithal (amphibians) and macrolecithal (bony fishes, cephalopod molluscs, reptiles birds and egg laying mammals), depending upon the amount of the yolk; or isolecithal (sea urchin), telolecithal (amphibian, reptiles, aves, and egg laying mammals) and centrolecithal (insects) depending on the placement of the yolk materials within the egg zygote.
- The amount of yolk influences the size of egg, plane of cleavage furrows, rate of cleavage as well as the size of resultant blastomeres.
- The general shape of the egg is spherical or nearly spherical (except in insects). Hence, the planes of cleavage are meridional, vertical, equatorial or latitudinal.
- Based on the arrangement of the blastomeres, the cleavage may be described as radial, or spiral or bilateral or rotational. According to whether cleavage furrows divide the egg completely or partially, the cleavage is either holoblastic or meroblastic or superficial.
- Cleavage produces morula which is converted into the blastula or a blastula-like structure. The organization of blastula is dependent on the amount of yolk originally deposited in the egg.
- Rearrangement of various groups of blastomeres is brought about during gastrulation which is a dynamic process, i.e. the blastomeres and their derivatives actually move from one place to another and the three germ layers ectoderm, mesoderm and endoderm are established.

- Gastrulation is effected by many morphogenetic movements of its cells- epiboly e.g. cell proliferation, thinning out, spreading, expansion and extension of ectoderm and emboly e.g. invagination, involution, delamination, ingression of mesoderm and endoderm.

13.5 TERMINAL QUESTIONS

1. If the alecithal egg of a eutherian mammals is filled with an extremely large amount of yolk and enclosed inside a hard porous shell will the following statements qualify such an imaginary situation (put 'T' for true statement and 'F' for false one):
 - a) It will behave as the macrolecithal egg of reptiles, birds and egg laying mammals. []
 - b) It will behave as the macrolecithal eggs of bony fishes or cephalopod molluscs. []
 - c) Such a situation is impossible in case of eutherian mammals according to the law of irreversibility of evolution. []
2. What are the important factors governing the early ontogenetic development (embryogenesis) of an animal:
 - a.
 - b.
 - c.
3. Write 'T' for true statement and 'F' for false statement:
 - a) Embryo is not a living entity. It becomes alive at the moment of hatching or birth as the case may be. []
 - b) Different embryonic stages are the different phenotypic expressions of the same genome at various stages of embryonic development. []
 - c) Morphogenesis is the basic developmental process that is most dependent on cell movement. []
 - d) The amount of yolk in the egg may determine the events of hatching or birth. []
4. Define the following terms:
 - a) Blastoderm
 - b) Morphogenetic movements
 - c) Meroblastic cleavage
 - d) Fate maps
 - e) Coeloblastula
5. In most animals except birds and placental mammals factors for determining the body axes are already present in the egg. Explain the statement

13.6 ANSWERS

Self-Assessment Questions

1.
 - i) mitotic cell division/mitosis
 - ii) cytoplasmic growth or G_1 or G_2 phases
 - iii) mesolecithal
 - iv) yolk
2.
 - i) meridional, vertical, equatorial, latitudinal
 - ii)
 - a) macrolecithal, centrolecithal.
 - b) superficial, meroblastic, peripheral, cytoplasm, yolk.
3.
 - i) In echinoderms the cleavage pattern is determined by maternal cytoplasmic factors, while in mammals the zygotic genes determine the cleavage pattern.
 - ii)

Animals	Types of cleavage	Types of Blastula
Sea urchin	Radial holoblastic	Coeloblastula
Rat	Rotational, holoblastic	Blastocyst
<i>Labeo rohita</i> , <i>Calotes versicolor</i> and Pigeon	Discoidal, merolastic	Discolastula
<i>Rana tigrina</i>	Radial holoblastic (Unequal)	Coeloblastula
<i>Nereis</i>	Holoblastic	Solid blastula

4.
 - For i) refer to Subsection 13.2.6
 - ii)
 - a) Arrests karyokinesis
 - b) Inhibits cytokinesis.
5.
 - i) refer to subsection 13.3.2.

Terminal Questions

1.
 - a) T; b) F; C) T.
2.
 - a) The phylogenetic group to which the particular animal (species) belongs.
 - b) Amount of yolk deposited in the egg during the growth of oocyte.
 - c) Site of development (whether inside the maternal body or external environment).
3.
 - a) F, b) T, c) T d) T.
4. Refer to the relevant parts of the text.
5. Refer to section 13.3 for (a and d); Refer to subsection 13.2.4 for (c); Refer to subsection 13.3.2 for c.