UNIT 14

DEVELOPMENT OF FROG

Structure

14.1 INTRODUCTION

In Block 3 you were introduced to the basic concepts of the study of animal development. You learnt that though vertebrate development may vary among the different groups of animals, the early processes and stages of animal development are the same in all groups. You would recall that there are three main stages in early vertebrate development in which (1) the main body axes-dorsal/ventral and anterior/ posterior are to be set up; (2) the three germ layers-ectoderm, mesoderm and endoderm are formed and (3) patterning of the germ layers (early nervous system and mesoderm) takes place. Maternal genes provide factors in the form of RNAs and proteins in the cytoplasm eggs of most vertebrates (except mammals) as cues for the initial stages of development. Later the zygotic genes take over. These developmental stages shared by all vertebrates result in the formation of an embryo being born as either a larval stage which is different from the adult form and transforms into the adult form later, or as a young organism having the typical features of the adult of that species. You had also learnt that an organism's development is

ultimately the result of differential gene expression. This determines what proteins are formed and when they are formed so that they change the behaviour of cells at different stages of embryo formation.

In Unit 10 we discussed some animals that have been chosen as models to study and understand the stages and mechanisms of development. The African clawed frog *Xenopus laevis* is one organisms that has historically been used as an amphibian model due to its ease of procurement, its ability to reproduce the year round and its large sized eggs which are easy to observe and to manipulate surgically. Another advantage of using *Xenopus* as a model is that it is completely aquatic and is able to develop normally in tap water. The mechanisms involved in the changing of its larval form into an adult organism have been studied in depth and provides insights into the phenomenon of metamorphosis. Therefore, *Xenopus* and other species of frogs and some other amphibians have been used by early embryologists and present day developmental biologists to understand many mechanisms underlying development of vertebrates.

In this Unit we will study the various stages of development of *Xenopus laevis* starting from fertilisation, blastula formation, cell movements in gastrulation and formation of early nervous system in neurulation. We know that amphibians do not emerge from the embryonic stage as fully formed adults instead, they have a free swimming larval stage- the tadpole which undergoes metamorphosis to develop into a miniature adult. We will learn how the frog embryo develops from the fertilized egg to a stage in which it has distinct body axes. Another crucial process in early vertebrate development is the development of the three germ layers - ectoderm, mesoderm, and endoderm. In this unit we shall see how this is achieved in frog. After the initial patterning, the germ layers move into their appropriate positions in the body plan through the process of gastrulation. A key developmental event that accompanies gastrulation is the induction of the nervous system from the dorsal ectoderm by a complex cascade of signals from adjacent tissues, and we shall consider that here .You will see how the concepts you had learned in the previous units in Block 3 are exemplified in the various stages of frog development.

The 2012 Nobel Prize in Physiology and Medicine was awarded to John Gurdon for his pioneering work in nuclear transplantation experiments in frog eggs. His experimental techniques and studies became the forerunners of the modern stem cell research and cloning.

The process by which the larva transforms into an adult is called metamorphosis and this often involves radical biochemical and physiological changes in its form, usually controlled by hormones. We will learn about these changes in this unit. In some amphibians the larval stage also becomes the sexual stage and the larva does not undergo metamorphosis. This special stage of development is known as neotany, about which we will learn in the last section of this unit.

Objectives

After studying this Unit you should be able to:

- describe the various stages and processes in the development of frog;
- explain how body axes are formed in frog;
- describe the morphogenetic movements during frog gastrulation;

- ❖ correlate the fate map of frog with its outcome in development;
- ❖ describe the process of neurulation;
- ❖ explain the underlying process of metamorphosis and its hormonal control, and;
- ❖ define and discuss neotany in amphibians.

14.2 CLEAVAGE AND BLASTULATION

Before we discuss cleavage and formation of blastula in frog let us look into the structure of its egg and how the maternal factors that are already present in the cytoplasm of the egg control the laying down of the body axes before and after fertilisation. The anterior–posterior axis of the embryo though initiated after fertilization, is laid down only after gastrulation. We will first explore the relative roles of pre-existing maternal factors in the egg and the embryo's own developmental program in the formation of the body axes.

14.2.1 Setting the Embryonic Axes

The mature unfertilized egg of frog (in this unit we will use most of the times, unless specified otherwise the term frog to mean *Xenopus*) already has a distinct polarity (Fig.14.1) with a pigmented region referred to as **animal hemisphere** and a pale yolky region known as **vegetal hemisphere**(Refer again to unit 13).

The axis running from the animal pole to vegetal pole is referred to as **animal-vegetal axis**. The frog egg has many localised mRNAs and proteins that are laid down in the cytoplasm of egg while it is being formed. These 'maternal factors' control the development of the zygote till about 12 cleavages and then its own genes begin to be expressed. Of these a number of mRNAs are the signaling molecules that determine the animal-vegetal axis. ***Thus, the animal-vegetal axis is already laid down in the egg even before it is fertilized. The animal –vegetal axis of the egg is not exactly the anterior posterior axis of the tadpole but is related to it as the head will eventually develop from the animal region.*** Before fertilization you will recall that the egg is enclosed in a **vitelline membrane** surrounded by a jelly like coat (Fig.14.1). Within the first 90 minutes of sperm entry which can happen anywhere on the animal hemisphere, the rotation of the egg cortex with respect to inner cytoplasm is initiated. The cortex is a gel-like layer of actin filaments about 5 micrometres thick just under the membrane. The vegetal cortex opposite the sperm entry point moves towards the animal pole by about 30 degrees, relative to the rest of the cytoplasm. This cytoplasmic rearrangement is called the **cortical rotation** (Fig.14.2). Cortical rotation relocates some maternal factors that are originally located in the vegetal half of the egg to an area which will become the dorsal side. This leads to the formation of a 'signaling centre' opposite the site of sperm entry known as the blastula organizer or **Nieuwkoop Centre** (named after a Dutch embryologist Pieter Nieuwkoop who discovered the organising properties of the vegetal region). Signals from the Nieuwkoop center are required for the future development of all dorsal and

A signaling center is a localized region of the embryo that exerts a special influence on the surrounding cells and can thus determine how they will develop.

anterior structures. One of the main roles of the Nieuwkoop center is to specify another key signaling center, the **Spemann organizer,** which arises just above the Nieuwkoop center at the late blastula-early gastrula stage. Recall from Unit 10 that signals originating in the Spemann organizer are involved in further patterning along both the antero-posterior and dorso-ventral axes of the embryo, and in inducing the central nervous system. As a result of cortical rotation, there is reduction in the pigmentation of animal hemisphere opposite to sperm entry point (SEP) (Fig.14.2).

Fig.14.1: *Xenopus* egg with the pigmented animal hemisphere and light coloured vegetal hemisphere where the yolk is concentrated.

In most frogs (except *Xenopus*), thus, a crescent shaped surface area known as **gray crescent develops** on the surface of amphibian egg opposite to the point of sperm entry. The sperm entry point marks the **prospective ventral side**, while the region opposite to it becomes the **prospective dorsal side** of the embryo. ***Thus, the point of sperm entry lays down the initial dorsal-ventral axis of the larvae though it will become fixed only during gastrulation.*** During fertilization, the radially symmetrical unfertilized egg becomes bilaterally symmetrical.

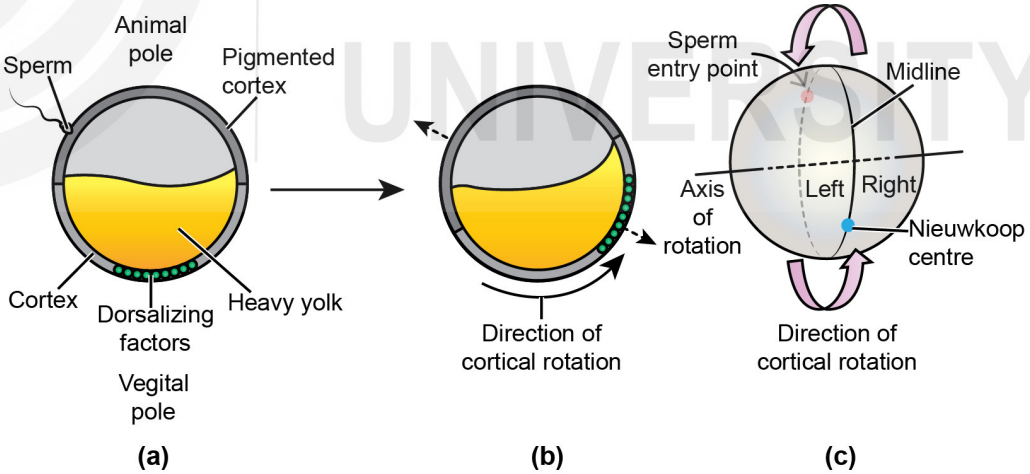


Fig.14.2: a) sperm entry point (SEP) and; b) cortical rotation that determines the (c) dorso-ventral axis of the embryo and establishment of Nieuwkoop Centre which is essential for normal development. Maternal factors are responsible for forming this organiser region in the egg.

14.2.2 Cleavage and Blastula Formation

The amphibian egg is moderately telolecithal. The yolk is concentrated in the vegetal hemisphere which creates hindrance in cleavage. The cleavage is radially symmetrical and unequal holoblastic (recall the various types of

cleavages from Unit 13). The first cleavage furrow is vertical and occurs along the animal – vegetal axis. It begins to form at the animal hemisphere and slowly extends towards the vegetal hemispheres. It divides the egg into two equal sized daughter cells and *also forms the prospective left and right sides of the developing embryo*. The cells derived from the cleavage division as you know, are called blastomeres. The first cleavage cuts through the site of the sperm entry and the Nieuwkoop Centre or the gray crescent in case of *Rana* species (see Fig.14.2 c and 14.3).

The second cleavage is also vertical along the animal – vegetal axis but at right angles to the first. It starts while the first cleavage is still going on in the vegetal hemisphere, producing four blastomeres (2 with the Nieuwkoop Centre that is the dorsal side and 2 without on the ventral side). The third cleavage is equatorial. This cleavage furrow does not pass exactly through the equator but is displaced towards the animal pole because of the vegetally placed yolk. It divides the embryo into four small animal blastomeres (micromeres) and four large blastomeres (macromeres). This way the animal and vegetal halves get separated (Fig.14.3).

If the 4 cell embryo is divided into two halves, one dorsal and one ventral, the half with the Nieuwkoop Centre develops fairly normally with only some ventral structures not developing. But the half without the Nieuwkoop Centre develops without dorsal or anterior structures. This shows that the Nieuwkoop centre is required for normal development and also explains Roux's experimental result where he had destroyed one cell at the 2 cell stage and got half and embryo.

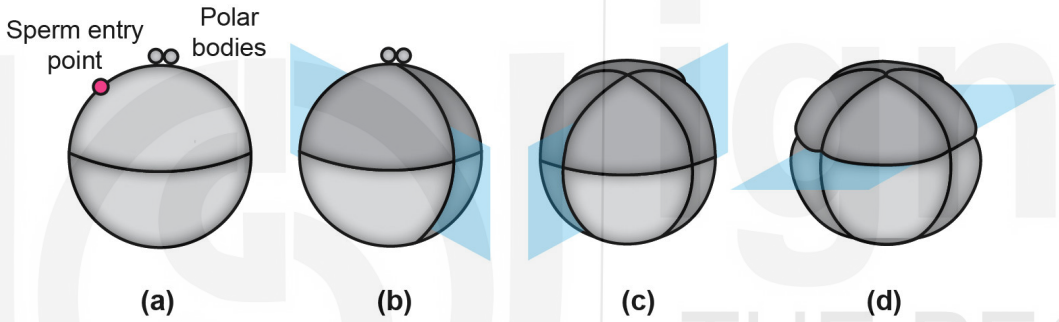


Fig.14.3: a) Fertilized egg and (b, c & d) first three cleavage divisions that separate the animal and vegetal valves.

The fourth cleavage is vertical, holoblastic and unequal. It produces sixteen blastomeres. Further divisions result in rapid multiplication of micromeres as compared to macromeres (Fig.14.4 a).

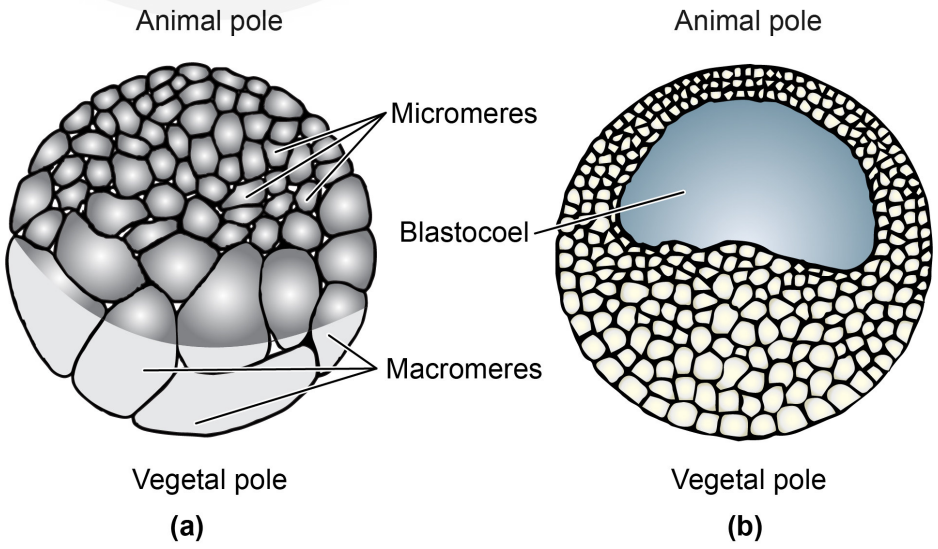


Fig.14.4: a) Rapid division in the animal half results in blastomere called micromeres and the slowly dividing blastomeres in the vegetal half are called macromeres; b) section of the blastula showing the blastocoel.

An amphibian embryo consisting of 16 to 64 cells is commonly called a **morula** (resembles the shape of mulberry). The continued cleavage results in formation of smaller and smaller blastomeres since there is no growth between cell divisions. Cells at the vegetal pole are larger than those at the animal pole as rate of division is slower in comparison to the cell division in the animal hemisphere. At the 128 cell stage a fluid filled cavity called **blastocoel** clearly develops in the animal region. The **blastocoel** is eccentric in position (see Fig. 14.4 b). The embryo at this stage as you know is called a **blastula.** The blastomeres adhere to each other strongly by cell adhesion molecules mainly by EP-cadherins. The mRNA for this molecule is already present in the maternal cytoplasm.

Blastocoel serves two major functions:

1. It permits cell migration during gastrulation
2. It prevents premature interaction of cells beneath and above it.

The amphibian egg at the end of blastula formation has gone through 12 cell divisions. At this stage the embryo consists of many thousand cells.

14.2.3 Mid-Blastula Transition

After the 12th cleavage division, the embryo enters the stage of mid blastula transition (MBT) during which it prepares itself for the next stage namely, gastrulation. The zygotic genes now begin to get expressed and prepare the blastomeres for movement. MBT demarcates the transition from maternal cell fate determinants like mRNAs and proteins (already present before fertilization) to the embryo producing its own mRNAs and proteins produced by the developing zygote.

The characteristic features of mid blastula transition (MBT) stage are the following:

1. Rate of cleavage slows down.
2. Synchrony of cell division is lost.
3. The embryo's own RNA synthesis starts and different genes are transcribed in different cells, e.g. Veg T protein is formed in the vegetal cells from the localized maternal m RNA. Vegetal cells under the influence of Veg T protein become endodermal cells. They begin secreting factors that induce the cells above them to become mesodermal.

SAQ 1

Fill in the blank spaces in the statements given below:

i) The Nieuwkoop Centre's role is to specify another signal centre the …….. which in turn leads to the development of embryo's ……

ii) Sperm entry results in the specification of the …… side, opposite the SEP.

iii) Egg structure determines the animal ……..…/…… ……..….. axis and the first cleavage also determine the prospective ……. and ……., ……. sides of the embryo.

iv) The cells of the ……... hemisphere divide at a faster rate than cells in the ……. hemisphere.

v) Mid blastula transition marks the expression of …… genes.

14.2.4 Fate Map of Amphibian Blastula

You already know that a fate map gives the information about regions of the blastula that will give rise to the future cells and tissues of the embryo. By studying the fate map, one can easily understand the complex morphogenetic movements involved in gastrulation. The external appearance of the blastula at 32 cell stage gives no indication of what those cell will become at later stage as the cells are largely unspecified. If some cells are removed during the blastula stage, others will be able to take over the fate of the missing cells. This implies considerable developmental flexibility at this early stage of development. The actual fate of cells is heavily dependent on the signals they receive from neighboring cells. The fate map of amphibian blastula had been first constructed by vital staining method of Vogt (1929). According to the fate map the surface of the amphibian blastula can be divided into three major areas or zones (Fig.14.5.) namely, presumptive ectoderm, presumptive mesoderm and presumptive endoderm.

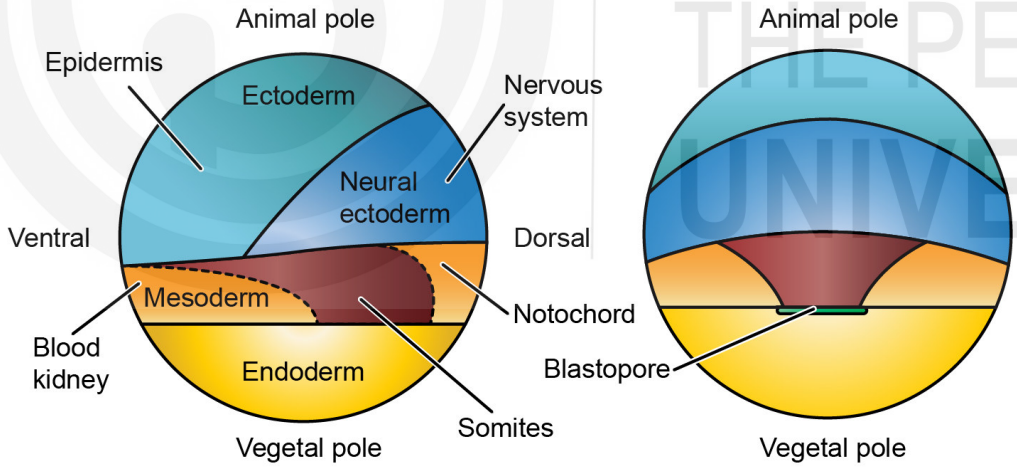


Fig.14.5: Fate map of late blastula just before gastrulation: a) lateral view in which blue denotes the animal zone with prospective ectodermal and neural ectodermal cells, yellow is the prospective endodermal cells in the vegetal zone and the mesoderm is derived from the superficial and deep cell layers in the marginal zone between the animal and vegetal zones; b) dorsal view showing the position of the blastopore where gastrulation will start.

1. **Presumptive ectoderm area:**

   The pigmented area around the animal pole forms the presumptive ectoderm including the neural and epidermal ectoderm.

2. **Presumptive mesoderm area**:

Beneath the presumptive ectoderm area is present a belt like area the **marginal zone** or gray crescent. This region develops into presumptive mesodermal cells that are induced from the prospective ectoderm by a general signal arising from the ventral vegetal region that operates around the circumference and induces the ventral mesoderm (Fig.14.6). A dorsal signal that arises from the vegetal cells of Niewkoop center induces the axial mesoderm (Spemann's organizer). The patterning of mesoderm along the dorso-ventral axis produces regions from dorsal to ventral, that, give rise to **chordamesoderm** which forms the notochord above the region of the foregut. **Somatic mesoderm** gives rise to somites, which are present on both sides of the notochord area. The lateral and ventral part of the marginal zone constitutes the **ventrolateral mesoderm** which gives rise to the circulatory system, kidneys and gonads etc.

3. **Presumptive endoderm area:**

The yolky non pigmented area around the vegetal pole is the prospective endoderm. The yolk provides the nutrition and is used up during development. In *Xenopus* but not all amphibians there is a thin outer layer of presumptive endoderm overlying the presumptive mesoderm in the marginal zone.

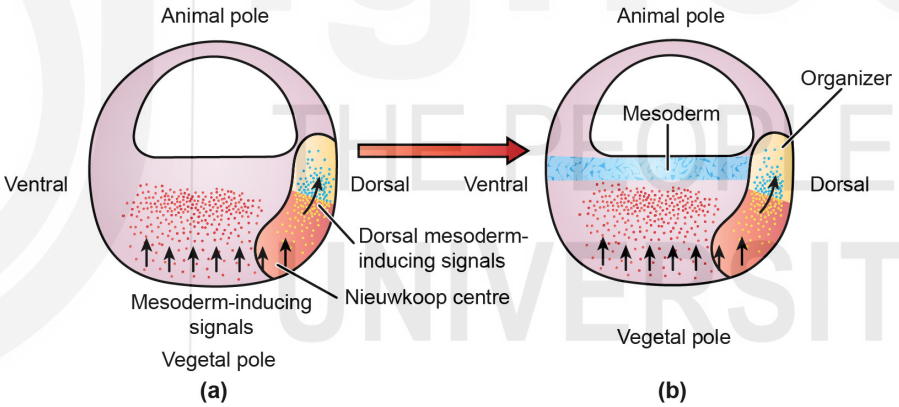


Fig.14.6: Signals involved in mesoderm induction in *Xenopus.* Signals from vegetal cells first induce mesoderm from prospective ectodermal cells of the animal zone. The organizer is specified on the dorsal most side where the inducing signal is strongest and of longest duration. Signals from the ventral zone of the embryo, pattern the rest of the mesoderm and the extent of their influence is counteracted by signals coming out of the Spemann's organizer.

SAQ 2

1) What is the dorsal most structure derived from the mesoderm?

b) What does the ectoderm give rise to?

c) What induces the formation of prospective mesoderm?

d) What is the prospective fate of the vegetal blastomeres?

14.3 GASTRULATION

The process of gastrulation in amphibians is quite complex. It involves various morphogenetic movements and rearrangement of cells. From the extensive studies done on amphibian gastrulation, it has emerged that the gastrulation movements are not uniform among the major amphibian groups. The process of gastrulation described below is based on studies chiefly done on *Xenopus laevis.* We know from Unit 13 that the main task of gastrulation is to bring cells from the surface of the blastula to the inside for forming the endodermal organs, to surround the embryo with cells capable of forming ectoderm and to place the mesoderm in its proper place between the external ectoderm and internal endoderm layers in order to give rise to organs derived from it. The main morphogenetic movements involved are chiefly – invagination, to initiate gastrulation which is followed by a variety of cell movements including convergence, divergence, involution and epiboly.

14.3.1 Initiation of Gastrulation

Gastrulation in frog is initiated on the future dorsal side just below the equator in the marginal zone i.e. the area of gray crescent where animal and vegetal hemispheres meet. Here the endodermal cells are not so large or yolky. The small endodermal cells in this region invaginate (sink in) to form a slit-like groove. The formation of the groove is the first external sign of gastrulation. The invaginating cells change their shape dramatically. They constrict at their apical surfaces and expand at their bases, but remain attached with the outside surface by their slender neck. These cells are called **bottle cells**. The change in shape of the cells into bottle shaped cells is associated with an inward pulling movement which results in the formation of a small groove. The initial groove is probably due to a coordinated contraction of the apical ends of "bottle cells" in a plane perpendicular to their long axis as occurs in the invaginating epithelia in general. The opening of this groove is called the **blastopore**.

14.3.2 Involution of Endoderm

Before the bottle cells are formed, there is an internal cell rearrangement that pushes the cells of the dorsal floor of the blastocoels made up of vegetal cells towards the animal cap. This rotation of the vegetal cells places the pharyngeal endoderm cells adjacent to the blastocoel immediately above the mesodermal cells that begin to move inside (Fig.14.7). Pharyngeal endoderm cells start migrating along the roof of the blastocoel towards the future anterior end of the embryo.

The initial shallow groove formed in the **dorsal blastopore lip** is the beginning of a groove or cavity known as **archenteron**. As the bottle cells which invaginate to form the groove leave the dorsal lip, their place is taken by new groups of cells derived from the more anterior regions of the marginal zone (gray crescent). They roll over the dorsal lip (involute) and continue to migrate inward deepening the groove. These endodermal cells that migrate from the superficial marginal zone form the roof of the deepening groove which becomes the tubular archenteron. This lining of cells is the future gut lining.

The superficial cells of the marginal zone that form the lining, migrate in passively because they are pulled along with the active migration force of the deep cells that are present under the superficial cells of the marginal zone and that also start to involute (you must remember that all the cells that are migrating inwards are doing so at the same time though we describe the movement step wise).

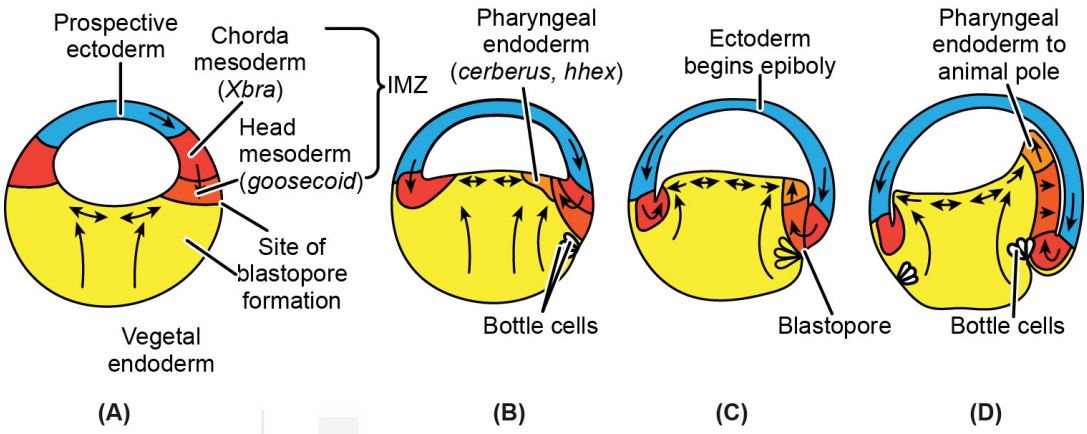


Fig.14.7: (A) At the beginning of gastrulation, the involuting marginal zone (IMZ) forms. Pink represents the prospective head mesoderm. Chordamesoderm is red; (B) Vegetal rotation (arrows) pushes the prospective pharyngeal endoderm (orange) to the side of the blastocoel. (C & D) The vegetal endoderm (yellow) movements push the pharyngeal endoderm forward, driving the mesoderm passively into the embryo and toward the animal pole. The ectoderm (blue) begins epiboly. (After Winklbauer and Schürfeld 1999)

Experiments show that endodermal lining along with the attached bottle cells is moved passively because it has been observed that removing the bottle cells that are attached to the tip of the archenteron, does not stop the migration. However it is seen that if the deeper involuting marginal cells are removed then the archenteron does not form. Continued inward migration of the involuted cells extends the archenteron into the blastocoel anteriorly until its tip reaches up to the inner surface of the animal pole region (Fig.14.7D).

As the archenteron expands the blastocoel cavity gets reduced and ultimately is obliterated. The large yolky cells of the vegetal zone also perform slow inward streaming movements so that the cells initially situated in the vegetal zone just below the initial groove reach the anterior part of the archenteron floor and those from the vegetal pole region come to be located in its posterior part.

Thus the first cells to undergo involution over the dorsal lip of the blastopore are the **pharyngeal endodermal cells**.

14.3.3 Involution of Mesoderm

The presumptive mesodermal cells lie in the deep marginal zone while a thin layer of endodermal cells lie in the superficial marginal zone. During early gastrulation, several layers of deep involuting marginal zone cells interdigitate

by a process known as radial intercalation to form a thin broad single layer. Further intercalation takes place as the cells involute into the embryo. This intercalation causes a convergent extension thereby, integrating several mesodermal streams to form a long narrow band (imagine on the road, several rows of cars are lined side by side and they all have to move forward to form one row of cars that will enter a single lane). The anterior part of this band migrates towards the animal cap followed by continued migration of mesodermal streams. The radial and mediolateral intercalations of the deep cells appear to be responsible for the continued movement of mesoderm into the embryo (see Fig.14.8 to understand this process).

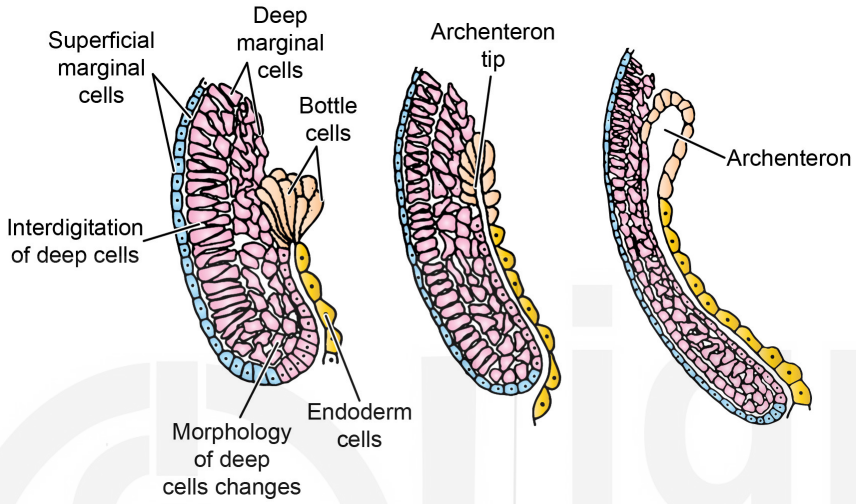


Fig.14.8: During early gastrulation the cells in the deep marginal zone show interdigitation to form a band of mesodermal cells and move in by involution. As they move in they drive the movement of surface endodermal cells with the bottle cells leading and the archenteron lengthening.

The extension of the dorsal mesoderm by convergent cell movement is due to several factors:

a. **Polarized cell cohesion:** in which the involuting mesodermal cells send out protrusions to contact one another which requires an extracellular fibronectin matrix under the ectodermal roof of the blastocoel.

b. **Differential cell adhesion:** genes encoding for axial protocadherin in notochordal cell and paraxial protocadherin in the somatic mesodermal cells ensure that proper cell sorting occurs so that only the trunk mesoderm undergoes convergent extension but head mesoderm does not

c. **Calcium flux:** also seems to regulate convergent extension by regulating the contraction of actin microfilaments. A surge of calcium ions across the dorsal tissue undergoing convergent extension causes contraction in the tissue. This was shown to be true because if calcium release from the intracellular store is blocked, no convergent extension occurs.

Involution of cells into the embryo follows a precise sequence. As explained earlier, the pharyngeal endoderm cells are the first to involute over the dorsal lip of the blastopore. As the cells move to the interior they are followed by

prechordal plate cells (precursor the of head mesoderm). The next cells involuting into the embryo through dorsal lip of blastopore are the **chordamesodermal cells** (precursor of notochord). Thus the cells that form the dorsal lip are constantly changing as one type of cells follows the previous type.

Meanwhile the blastopore lip also extends laterally forming the **blastopore crescent** with **lateral lips** (14.9 b &c). At the same time the ectodermal cells of the animal hemisphere begin to cover the embryo by epiboly to converge at the extending blastopore. As epiboly continues, a **ventral lip** of the blastopore forms and soon it becomes a complete circle (Fig.14.9 d).Invagination of cells is much more extensive around the dorsal lip of the blastopore than at the lateral and ventral lips. The yolky endodermal cells of vegetal hemisphere encircled by the blastopore form the yolk plug (Fig.14 d). By the end of gastrulation, blastopore is reduced to a narrow vertical slit. (Fig.14.9 e).

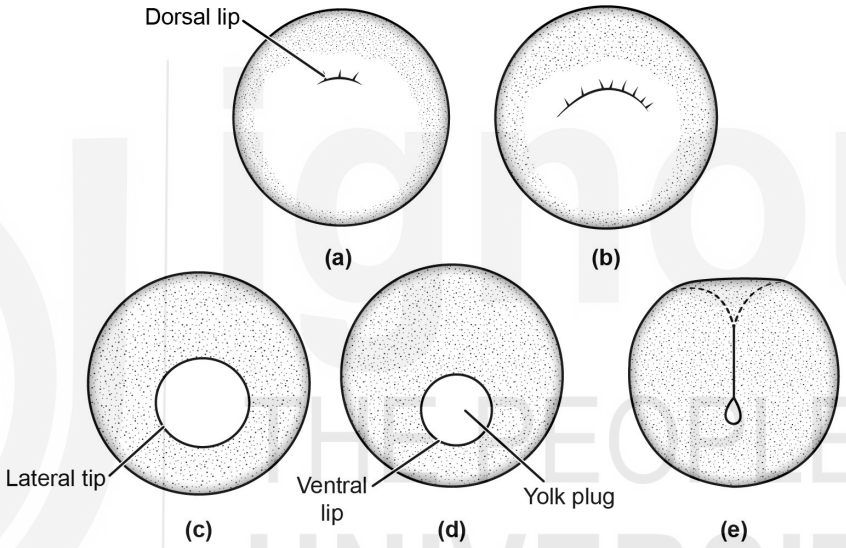


Fig.14.9: Formation and closure of the blastopore. a) formation of blastopore dorsal lip; b) elongation of blastopore slit laterally; c) and d) completion of blastopore with dorsal, lateral and ventral lips enclosing the yolk plug and; e) end of gastrulation in which the blastopore is a narrow vertical slit.

Later the prechordal plate becomes a part of archenteron roof in front of the anterior end of the notochord. The anterior part of the notochord extends along the dorsal side of the archenteron and occupies the mid-dorsal region of the archenteron roof. Meanwhile, the presumptive posterior notochordal cells involute over the dorsolateral lips of blastopore. Later they join the anterior notochordal cells. Thus those mesodermal cells that enter over the dorsal lip form the central dorsal mesoderm (notochord and somites) and majority of the mesoderm consisting of presumptive somitic mesoderm and ventrolateral mesoderm involute by rolling over the lateral and ventral lips of blastopore. Once the involution of the mesoderm is over, these cells move from the posterior (blastopore) side towards the anterior side as a single layer called **chordamesodermal mantle**. It occupies a space between the ectoderm and endoderm, except for a small part at the anterior end of the gastrula. This part does not contain mesoderm and later forms the mouth. The point where the endoderm and ectoderm meet at the blastopore becomes the anus.

14.3.4 Epiboly of Ectoderm

From the time of the first appearance of the blastopore slit on the dorsal side, the presumptive ectoderm, including epidermal and neural, expands to cover the whole embryo by a process known as epiboly. As gastrulation proceeds the cells of the animal cap and non involuting marginal zone cells (NIMZ) expand by epiboly to cover the entire embryo. This process helps in the formation of the circular blastopore and in the enclosing of all the yolky endodermal cells inside the embryo. These presumptive ectodermal cells form the surface ectoderm. The expansion of ectoderm is a very active process. There are various mechanisms to explain the process of epiboly. One such mechanism involves i) increase in cell numbers by cell divisions and cell flattening in the superficial layer ii) intercalation of several deep layers into one layer by radial intercalation. Second, mechanism of epiboly involves assembly of fibronectin fibrils by the blastocoel roof. This fibrillar fibronectin plays an important role in migration of vegetal cells and the animal cap cells and also in enclosure of the embryo.

As a result of epiboly the ectoderm covers the whole embryo after the mesoderm and the endoderm have disappeared into the interior of the embryo (Fig.14.10).

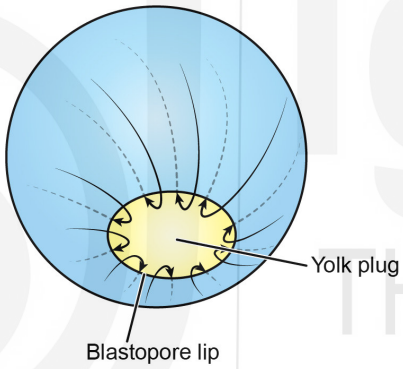


Fig 14.10: Epiboly of ectoderm to enclose all the prospective endodermal cells. The arrow indicate involution of vegetal cells through the narrowing blastopore which also shows the yolk plug.

See Fig 14.11 that summarizes the cell movements in gastrulation. At the end of gastrulation the following features are seen in the developing zygote:

1. Radially symmetrical monoblastic blastula is converted into triploblastic gastrula having anterior–posterior axis with the head in at one end and the future tail at the other.
2. Externally gastrula is covered by ectoderm. Mesoderm and endoderm occupy the internal position. Thus the three germ layers move into positions in which they will develop into structures of the larva/adult.
3. The ectoderm forming the mid dorsal band is the presumptive nervous system and the rest of the ectoderm forms epidermis.
4. The gastrocoel or archenteron is the presumptive lumen of the gut
5. The roof of archenteron is the chordamesodermal mantle and the floor and sides are formed of endodermal cells

The gastrula then undergoes a process of neurulation and becomes the neurula.

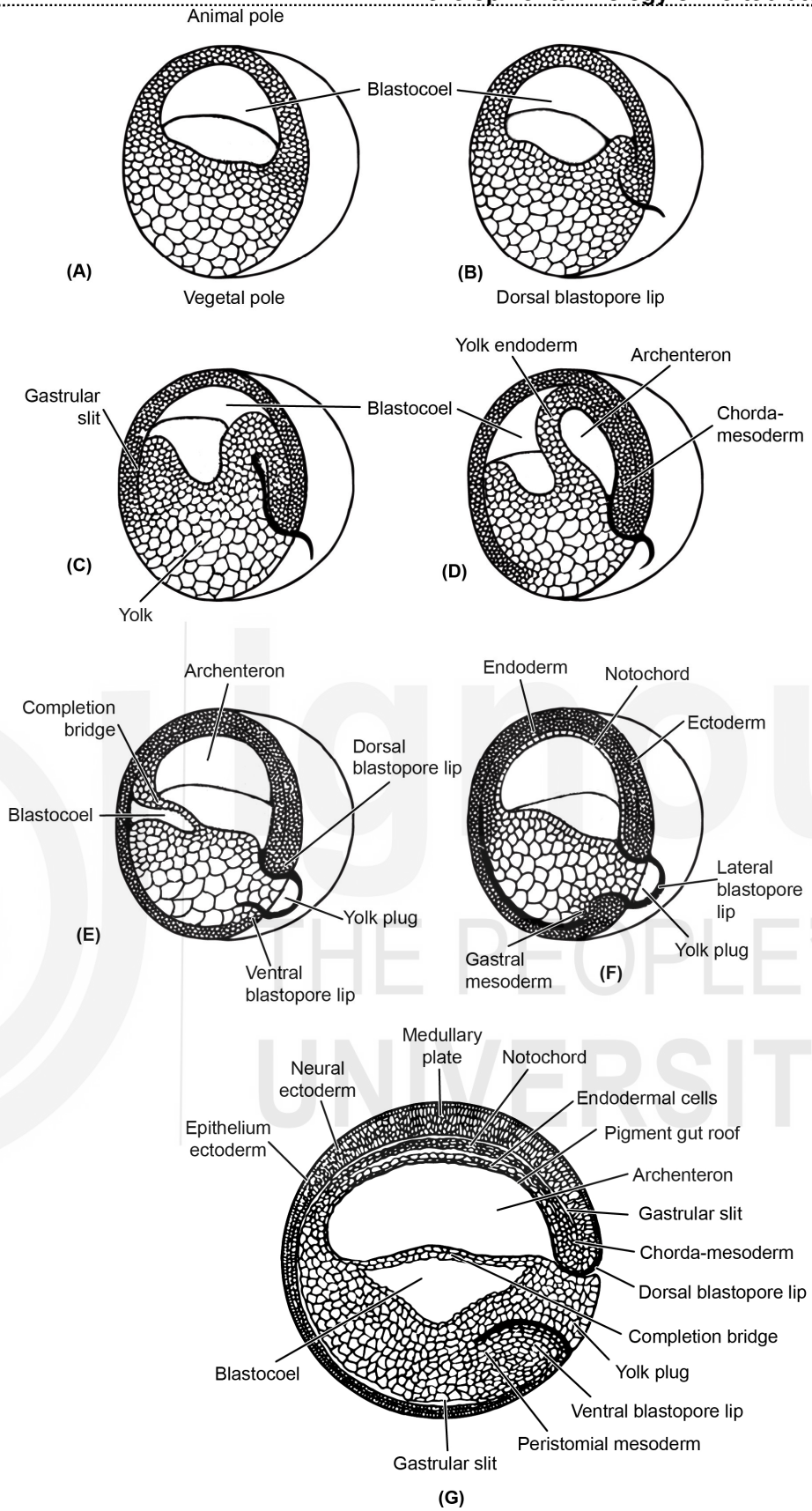


Fig.14.11: Cell movements in gastrulation: (A to G) drawings of section cut through the middle of the embryo. (B) shows the formation of blastopore lip on the dorsal side. (C&D) Archenteron forms by involution of endoderm and displaces the blastocoels, the cells migrate from the lateral and ventral lips of the blastopore. (E&F) the blastocoels is reduced and the embryo is surrounded by ectoderm while mesoderm is placed between the ectoderm and endoderm that is internalized. (G) Section of gastrula at the end of gastrulation.

SAQ 3

Match the following given in A with the terms given in B.

	A		B
a	The first cells to involute over the dorsal lip of blastopore	i	mouth
b	Cells carried passively at the tip of the archenteron	ii	chordamesoderm
c	Drives the involution of cells inside the embryo and carries other superficial cells with its force	iii	Lateral blastopore lip
d	The axial mesoderm that forms notochord	iv	Archenteron
e	Cells invaginating to form blastopore slit, located on dorsal side	v	Vegetal cells below grey crescent
f	Mesodermal cells that form part of archenteron roof, anterior to notochord	vi	Chordamesoderm mantle
g	Place for involution of presumptive somatic mesoderm	vii	Pharyngeal endoderm
h	Mesodermal cell sheet that moves from the blastopore side towards the anterior side	viii	Deep mesodermal cells
i	The anterior end of embryo where there is no mesoderm	ix	Prechordal plate
j	Presumptive lumen of gut	x	Bottle cells

14.4 NEURULATION

You have learnt that the dorsal mesoderm or the organizer region involutes to form the prechordal plate just ahead of the notochord. This will give rise to the ventral head mesoderm and the notochord that lies just behind it is formed from the chordamesoderm. When gastrulation is near completion the notochord becomes flanked on both sides by blocks of somatic mesoderm which start from the anterior to posterior direction. As this happens, the presumptive area of the nervous system becomes differentiated from rest of the ectoderm. This region of the embryo is called **neural plate**. The process by which this tissue forms a neural tube (rudiment of central nervous system) is called **neurulation.** An embryo undergoing such changes is called a **neurula.**

During neurulation, the original ectoderm is divided into following three sets of cells:

1. Outer ectodermal cells forming the epidermis of skin.
2. Neural ectodermal cells giving rise to central nervous system.
3. Neural crest cells that give rise to peripheral nervous system and a variety of other cell types which later migrate to distant regions as the embryo develops.

14.4.1 Neural Tube Formation

As mentioned earlier, neurulation begins with the formation of a **neural plate**. The presumptive area of the nervous system becomes differentiated from rest of the ectoderm and the cells become flat and thick in the form of neural plate. Cells of the neural plate change in shape, become elongated and arrange themselves into a columnar epithelium layer. During this process, the embryo lengthens along the anterio-posterior axis, narrowing itself so that subsequent bending will form a tube. In both amphibians and amniotes, the neural plate lengthens and narrows by convergent extension, intercalating several layers of cells into a few layers.

At the same time the edges of the neural plate become thickened and become raised above the general level as ridges called **neural folds**. Neural folds become higher and consequently there is formation of the neural groove, which extends throughout the length of the embryo. Neural folds meet each other in dorsal midline and fuse to form neural tube. At the time of rolling of the neural plate to form the neural tube, the neuroepithelial cells at the lateral folds of the neural plate develop constrictions at their apical edges. This changes the shape of columnar epithelial cells into pyramidal cones. As the surface area of apical end of neural epithelial cells becomes smaller relative to the basal surface, the rolling up of the neural plate and formation of neural tube takes place. The neural tube separates from the surface ectoderm and forms a closed cylinder. The epithelial cells at the surface form a continuous layer to cover the neural tube. The embryo at this stage is called **neurula**. Thus the change in the shape of cells in the neural tube results in the regionalisation of the neural tube. In the cephalic end, i.e. the end which will differentiate into brain, the wall of the tube is broad and thick and a series of swellings and constrictions develop that define the various divisions of brain. Caudally, towards the posterior end, the tube is simple and narrow, extending into the tail while the neural tube overlying the notochord will develop into spinal cord (Fig.14.12).

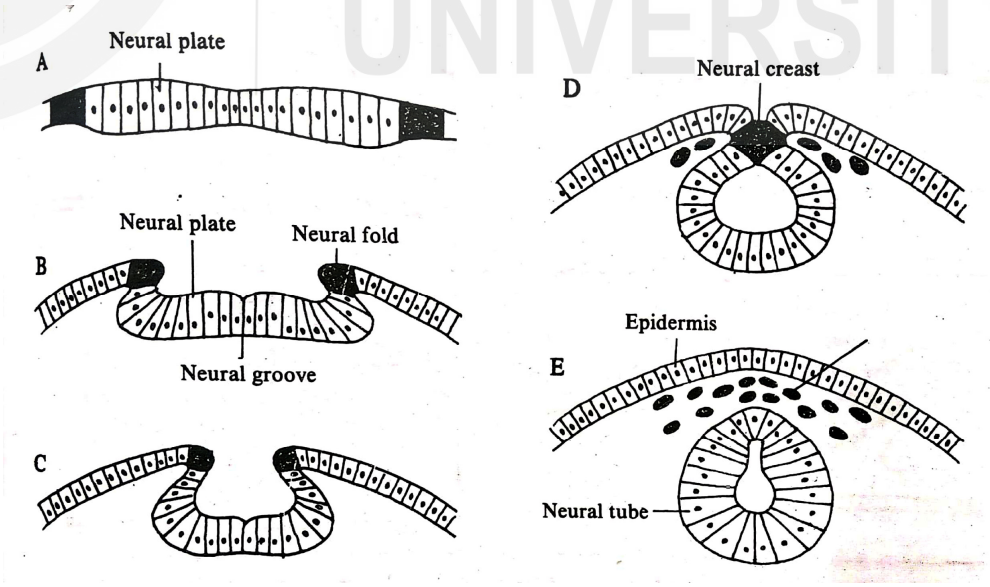


Fig.14.12: Stages in neurulation In frog: (A) thickening of dorsal ectoderm to form neural plate; (B) formation of neural folds and neural groove; (C) deepening of neural grooved) fusion of neural folds and formation of a neural tube; (D) neural tube has separated and is covered over by epidermis; (E) Neural crest cells have separated from neural folds.

The neural plate is induced both by early signals in the ectoderm and from signals from the mesoderm that comes to lie below the prospective neural plate ectoderm during gastrulation. Studies show that the notochord is necessary for neural plate formation. In *Xenopus* the organizer region is crucially important for inducing the anterio-posterior axis as well as the neural tissue from the ectoderm.

Separation of the neural tube from surface ectoderm is brought about by the expression of different cell adhesion molecules (recall cell adhesion and different cadherins from Unit 11). The cells of the surface ectoderm synthesize cell adhesion molecule, E – Cadherin. The ectodermal cells forming the neural tube which initially synthesize E-Cadherin, stop producing this protein and instead synthesize N – Cadherin and N – CAM. Because of the different cell adhesion molecules expressed, the neural tissue separates from the surface ectoderm. It has been observed that separation of neural tube from the surface ectoderm fails to occur if N – Cadherin m RNA is injected into the surface ectoderm of early *Xenopus* embryo.

To summarize, the process of neurulation can be divided into three distinct stages:

1. Formation and shaping of neural plate.
2. Bending of neural plate to form neural groove and neural folds.
3. Closure of the neural groove to form a neural tube.

14.4.2 Neural Crest Cells

Neural crest cells are induced at the borders of the neural plate. They rise up at neurulation to form the neural folds and form the dorsal most portion of the neural tube. These cells lie between the dorsal part of the neural tube and dorsal epidermis and run the full length on both sides of the neural tube(Fig. 14.12).Neural crest cells undergo extensive migration and form various types of cells. For example, neural crest cells give rise to glia, and neurons of the autonomic nervous system, the glial cells (Schwann cells) of the peripheral nervous system, pigment cells of the skin, adrenal producing cells of the adrenal glands. They also give rise to bone, cartilage, muscles of the facial region- cell types that would commonly be of mesodermal origin! The induction of neural crest cells in *Xenopus* is a multi-stage process that starts in the early gastrula stage and continues till the closure of the neural tube.

SAQ 4

Indicate whether the statements given below are **true or false** and correct the false statements.

i) The term neurulation refers to the partitioning of ectoderm, and the formation and inward displacement of neural tube. (T/F)

ii) During neural plate formation, the neuroeptithelial cells change in shape from columnar to pyramidal ones. (T/F)

iii) Neural cells are ectodermal in origin and migrate in the embryo to form various other tissue. (T/F)

iv) Cells of the notochord induce the formation of neural tube. (T/F)

14.5 METAMORPHOSIS

Metamorphosis is the post embryonic transformation of a larva of an organism into a juvenile adult. The changes during this period of development are dramatic and rather rapid. The phenomenon of metamorphosis is well established in amphibians, specially anurans (tail less amphibians) e.g. frogs and toads. They show dramatic metamorphic changes during their development, affecting nearly all organ systems and major functions. Amphibians are the first class of vertebrates to show transition from aquatic to terrestrial mode of life. Therefore, the metamorphic changes are associated with the change in their habitat. A number of features distinguish metamorphic changes from early embryogenesis and other post embryonic changes. The signals that bring about metamorphic changes are usually hormonal unlike the short term signals during embryogenesis that are typically protein growth factors. The synthesis of these hormones is directed by the central nervous system in response to environmental cues and there is a complex feedback mechanism that controls their production as well.

Figure 14.13 shows metamorphosis in frog. You can see that the egg develops into a larvae which is strikingly different from the adult in morphology. Apart from the appearance there are other significant changes in the physiology and biochemistry in the larva. Larva of frog is called tadpole. It is free swimming, and possesses a tail. It has three pairs of external gills covered by operculum for respiration. Locomotion is brought about by tail and chordal fin. The alimentary canal is long and coiled to aid herbivorous mode of digestion. Brain of tadpole is quite small and simple. Heart is two chambered consisting of auricle and ventricle.

14.5.1 Metamorphic Changes

The transformation of aquatic tailed tadpole larvae to a terrestrial adult frog involves major metamorphic changes. These can be classified as:

1. Ecological with respect to change in habitat.
2. Morphological with respect to change in morphology.
3. Physiological / Biochemical with respect to change in different bodily functions.

Ecological Changes:

The habitat of the tadpole larvae is fresh water whereas adult frog is land dwelling. Tadpole is herbivorous. Its horny beak and teeth help in rasping away the plant tissues. Frog is carnivorous feeding on insects and worms.

Morphological changes:

The morphological changes during metamorphosis are of three types; a) regressive changes, b) progressive changes and c) remodeling of existing structures in larvae.

a) Regressive changes: The organs or structures necessary during larval life, but redundant in adult are gradually reduced and ultimately

disappear in adults. Resorption of the long tail, chordal fin, external gills and ventral suckers take place. The gill clefts are closed. Peribranchial cavities are lost. Horny teeth and horny lining of jaw are shed. The cloacal tube gets reduced. Lateral line of the larva disappears. Aortic arches get modified due to reduced branchial arteries.

b) Progressive changes: Some organs develop and become functional only during and after metamorphosis. These involve the development and differentiation of fore limbs and hind limbs; the middle ear from the first pharyngeal pouch, tympanic cartilage and tympanic membrane; and hyoid apparatus from the pharyngeal arch to support the tongue which develops from floor of the mouth. The eyes protrude on the dorsal surface of the head developing eyelids and nictitating membranes.

c) Remodeling of existing larval structures: Some structures which function both in larval and adult form but undergo remodeling during metamorphosis. These involve basically skin, intestine and brain. The skin thickens and becomes multilayered with moucus and serous glands so that it remains moist. The skin acquires a characteristic color and pattern of pigmentation. The outer layer becomes keratinized. The long and coiled intestine shortens. Mesonephric kidney develops from pronephric kidney. Heart becomes three chambered from the earlier two chambered heart. Liver and pancreas become functional. Brain gets highly differentiated.

Biochemical / Physiological changes:

Along with morphological changes, certain biochemical / physiological changes take place during metamorphosis as explained below:

a) Tadpole larva is ammonotelic, excreting nitrogenous waste as ammonia which can be easily disposed off by diffusion in the aquatic medium. After metamorphosis, adult frog becomes ureotelic, excreting urea. This change occurs when the liver starts producing appropriate enzymes for the urea cycle.

b) The larval eye pigment porphyropsin is replaced by rhodopsin.

c) The respiratory pigment, larval haemoglobin is replaced by adult haemoglobin having more oxygen carrying capacity.

d) The site of erythropoiesis changes from liver to bone marrow and spleen.

e) Various digestive enzymes and hydrolytic enzymes are secreted.

f) Amoeboid macrophages by phagocytosis bring about autolysis of larval organs like gills and tails etc.

g) Shrinkage of body occurs because of suspended feeding and loss of some body parts like tails and gills. As a result degrowth (reduction in body mass) occurs. Head and trunk become small in adult.

Some important metamorphic changes seen in frog are summarized in Table 14.1

Table 14.1

System	Larva	Adult
Locomotory	Aquatic; tail fins	Terrestrial; tailless tetrapod
Respiratory	Gills, skin, lungs; larval hemoglobins	Skin, lungs; adult hemoglobins
Circulatory	Aortic arches; aorta; anterior, posterior and common jugular veins	Carotid arch; systemic arch; cardinal veins
Nutritional	Herbivorous; long spiral gut; intestinal symbionts; small mouth, horny jaws, labial teeth.	Carnivorous; short gut; proteases, large mouth with long tongue.
Nervous	Lack of nictitating membrane; porphyropsin, lateral line system, Mauthner neurons	Development of ocular muscles, nictitating membrane, tympanic membrane; rhodopsin; lateral line system lost, Mauthner neurons degenerate
Excretory	Largely ammonia, some urea (amoniotellic)	Largely urea; high activity of enzymes of ornithine-urea cycle (urotelic)
Integument	Thin, bilayered epidermis with thin dermis; no mucous or granular glands.	Stratified squamous epidermis with adult keratins; well-developed dermis contains mucous and granular glands secreting antimicrobial peptides

Three distinct stages of Metamorphosis:

Morphologically as seen in the Figure 14.13, the typical frog metamorphosis is divided into three stages.

1. Premetamorphosis – it is the first stage of metamorphosis when the tadpole has no limbs. This is the initial period of extensive growth but little developmental changes. Size of tadpole increases.

2. Prometamorphosis – during this stage developmental changes occur such as shortening of tail, gills becoming internal and growth of hind limbs take place.

Metamorphic climax – emergence of forelimbs marks the beginning of metamorphic climax. It is a brief period in which morphological changes occur very rapidly in quick succession including full limb growth and tail resorption.

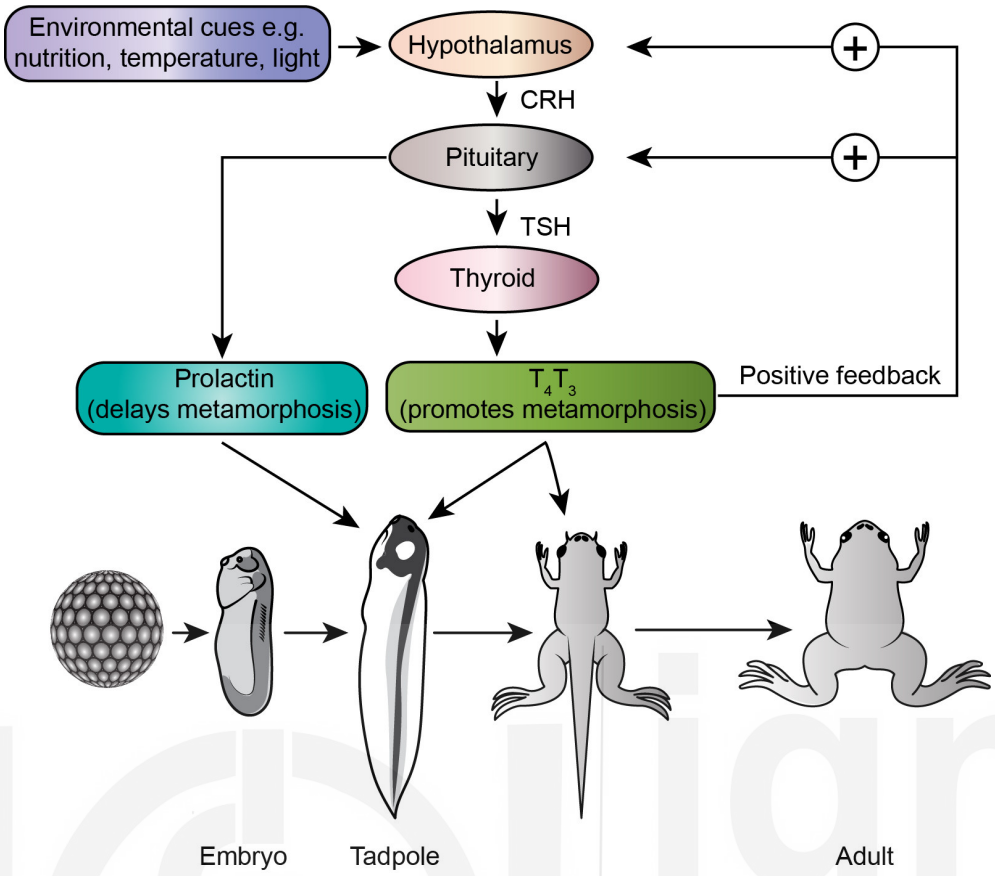


Fig.14.13: Environmental and nutrional cues cause the initiation of metamorphosis by secretion of corticotrophin stimulating hormone (CRH) from the larval hypothalamus which acts on the pituitary gland to release TSH. This in turn acts on the thyroid stimulating hormone (TSH) to release T4 and T3 that cause metamorphosis to happen. The thyroid hormones also act on the hypothalamus and pituitary to maintain the secretion of CRH and TSH.

These morphologically defined stages are influenced by different hormones which regulate the process of metamorphosis.

SAQ 5

i) Fill in the blanks with suitable words:

a) In anurans, metamorphosis is usually associated with a transition from …… to …… mode of life.

b) The three distinct stages of metamorphic changes in anurans are ……... and ……..

c) The three categories of morphological changes occurring during the metamorphosis of amphibians are ……, ……. and …… changes.

ii) Indicate the following changes that occur during metamorphosis in amphibians either as progressive or regressive or remodeling

a) The development of middle ear in connection with the pharyngeal pouch.

b) The change in the shape of the mouth and the shortening and reduction of the cloacal tube.

c) Disappearance of lateral line organs of skin and reduction of blood vessels.

d) The differentiation of brain.

e) The changes in the portal system and the changes in the vascular system to supply blood to the lungs.

f) The shortening and straightening of intestine.

g) The conversion of the heart into a three chambered one.

h) The development of fore and hind limbs.

i) Closing of gill clefts and loss of horny teeth.

j) Development of tongue from the floor of the mouth.

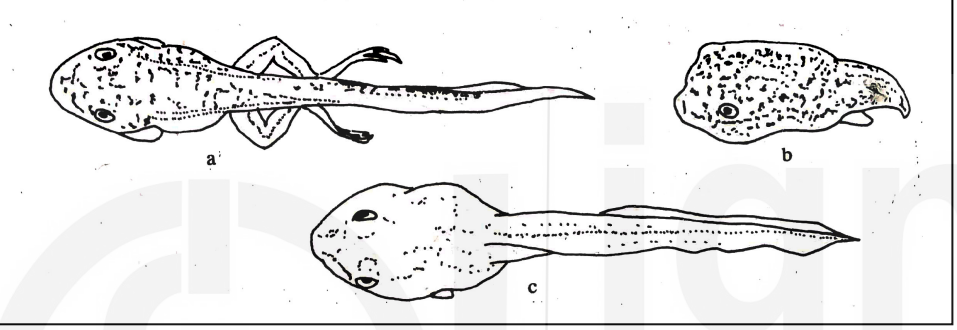
14.5.2 Hormonal Regulation

In amphibians, nutritional status and environmental cues such as, light and temperature, cause the neurosecretory cells of the hypothalamus to release **corticotrophin- releasing hormone (CRH)**. This hormone acts on the pituitary gland to release **thyroid stimulating hormone** (**TSH**) which in turn causes the release of thyroid hormones. This action of corticotrophin releasing hormone is peculiar to non-mammalian vertebrates and occurs in *Xenopus* only at the tadpole stage. In the adult frogs the release of thyroid stimulating hormone is due to **thyrotropin stimulating hormone**. The principal hormones which regulate metamorphosis in frog are **thyroid hormones** (TH) secreted by the thyroid gland (see Box 14.1). **Prolactin** and thyrotropic hormones released from the pituitary along with thyroxin from thyroid gland regulate metamorphic events in a sequential and coordinated manner (see Fig. 14.13).

The thyroid hormones exist as thyroglobulin in the thyroid follicles. These large proteins consists of tri-iodothyronine (T 3) containing three iodine atoms attached to tyrosine and tetra – iodothyronine (T 4) containing four iodine atoms attached to tyrosine. T 3 and T 4 are released during metamorphosis from the thyroid gland. T 4 is the precursor while T 3 is the active form of hormone. T 3 causes metamorphosis in thyroidectomized tadpoles in much lower concentration than T 4. Hypothalamus receives the exogenous signals such as temperature, light and nutrition etc. to release neurosecretory hormones. Pituitary also secretes another hormone, prolactin which is antagonistic to T 3. It inhibits metamorphosis and promotes growth in the larva. The action of prolactin is suppressed by dopamine produced by hypothalamus. Hypothalamus is the controlling centre for maintaining a balance between thyroid hormones and prolactin. During premetamorphosis, prometamorphosis and metamorphic climax the concentration of thyroid hormones circulating in blood vary, resulting in metamorphic changes.

Box 14.1

The role of thyroid hormone in metamorphosis was first demonstrated by Gudernatsch in 1912. He discovered that the tadpole when fed with powdered sheep thyroid glands, metamorphosed prematurely. Allen in 1916 showed that when thyroidectomy (removal of thyroid gland) was done on early tadpoles, the larvae failed to metamorphose and grew into giant tadpoles. If these tadpoles were fed dried powdered thyroid gland or immersed in iodine solution, they underwent metamorphosis. Subsequent studies by E.W.Etkin (1968) demonstrated the important role played by various hormones during metamorphosis. In the diagrams given below (a) shows normal metamorphic stage; (b) shows a tadpole exposed to thyroxin in the earlier stage undergoes premature metamorphosis and (c) shows the effect of removal of thyroid or pituitary gland which results in inhibition of metamorphosis



During premetamorphosis thyroid gland is still developing therefore, the **TH/Prolactin ratio is very low**. Prolactin secreted by pituitary gland stimulates growth of larval body. As hypothalamus develops in premetamorphosis, it produces CRH which causes rise in the level of TSH. As a response to this, T 3 and T 4 begin to rise causing premetamorphic changes. It also exerts positive feed back effect on the pituitary. As pituitary develops further, it causes increased flow of CRH between hypothalamus and pituitary. Hypothalamus releases prolactin inhibitor, causing increase in T 3 and T 4 and decrease in the level of prolactin. **In prometamorphosis, TH / prolactin ratio is intermediate.** When the level of T 3 and T 4 reaches the threshold, rapid metamorphic changes take place. *At this stage, TH/Prolactin ratio becomes very high*. High concentration of T 3 and T 4 exert a negative feed back on thyroid and hypothalamus causing decrease in CRH. Consequently thyroid gland partially degenerates.

Conversion of T 4 to its more active form T 3 takes place in the target tissue by the enzyme De – Iodinase II. Thyroid hormone acts on target cells by crossing the plasma membrane and interacting with thyroid receptors (TRs). T 3 binds to the nuclear thyroid receptors which has higher affinity than T 4. There are two types of thyroid hormone receptors; namely **TRα** and **TRβ**. Expression of thyroid receptors is under developmental control. Studies on *Xenopus* have shown the presence of TRα in all the tissues even before development of thyroid gland in the organism. The level of TRβ in the target tissue increased with high level of thyroid hormone as metamorphosis progresses. The thyroid receptors activate gene expression during

metamorphosis in the presence of TH. **In prometamorphic** tadpoles, the receptors are unliganed and so gene expression is repressed in the absence of TH to prevent metamorphosis, thus ensuring proper tadpole growth period. However, in the presence of TH, transcription is activated. At **metamorphic climax** the level of TR β reaches maximum due to which, rapid metamorphic changes take place.

In summary, metamorphosis in frog is regulated hormonally in a sequential manner as follows:

1. The thyroid gland secretes hormone thyroxin, T 4 into the blood.
2. T 4 reaches the target tissue and is converted to by enzyme Deiodinase II T 3 which is a more active form of the hormone.
3. T 3 binds to the nuclear thyroid hormone receptors. T 3 has a higher affinity to bind to thyroid receptors (TRs) than T4.
4. T 3-TR complex binds with retinoid receptor forming a complex which activates transcription of genes responsible for metamorphic changes.

Tissue reactivity and competence:

As described earlier, TH brings about diverse metamorphic changes. The hormone acts directly on the target tissue. This was demonstrated by implanting a pellet of inert material absorbed with T 4 on one side of the snout of frog. This caused widening of the mouth on that side only where the pellet was implanted. This clearly indicated that the hormone acts directly on the responding tissue.

Diversity of tissue response to T 4 is very striking i.e. different tissues respond to the hormone differently. The muscles of tails and fins etc. regress whereas, the tissues of limbs differentiate and grow as a response to the hormone. As you have learnt before in Unit 11, this difference in the reactive ability of different tissues to thyroid hormone is known as Competence and only cells that are competent to respond to TH do so.

Threshold and rate of response to thyroxin hormone:

Different tissues are sensitive to different doses of thyroxin level. This is known **as threshold value.** Experiments have shown that different tissue have different threshold values e.g. forelimbs require a high concentration of thyroid hormone to develop than hind limbs which are sensitive to low threshold value. For resorption of tail much higher concentration of the hormone is needed during metamorphic climax (that is why tail is reabsorbed only after the limbs grow). Thus each tissue type is responsive to a certain threshold for reactivity. Once this threshold is reached, the tissue responds.

When a very high dose of hormone is supplied to a young tadpole, all the metamorphic changes take place at once. This results in a chaotic situation leading to its death. Thus, developmental events during metamorphosis are sequential with progressively increasing threshold of the hormone.

SAQ 6

Indicate whether the following statements are true or false:

i) CRH secreted by the pituitary gland activates the thyroid gland to secrete thyroid hormone. (T/F)

ii) Tetraiodothyronine (T4) is a less potent hormone and is the precursor of triiodothyronine (T3). (T/F)

iii) Prolactin has an antagonistic action to thyroxine, and so promotes larval growth and inhibits metamorphosis. (T/F)

iv) The reactivity and responses of target tissues of the tadpoles to the thyroxine hormone are extrinsic and non-specific. (T/F)

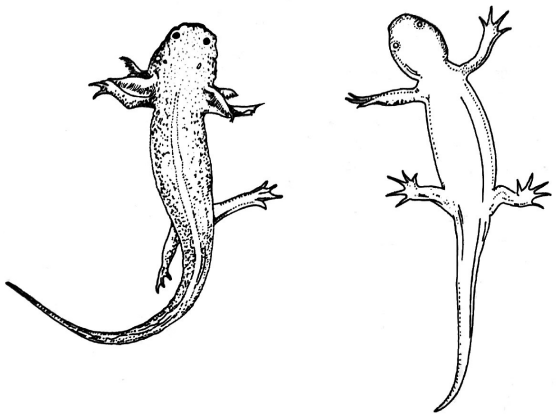
v) According to the threshold concept, different tissues of the tadpole larva are sensitive to different concentrations of the thyroxine. (T/F)

vi) Expression of thyroid hormone receptors in cells is under development control and not dependent on release of thyroid hormone. (T/F)

14.6 NEOTENY

Neoteny is referred to as retention of larval characters in sexually mature adult animals. Many urodeles (tailed amphibians) exhibit this interesting phenomenon of neoteny.

In some Salamanders, juvenile characters are retained by retarded body development while the gonads achieve maturity at the normal time. *Ambystoma mexicanum* (Mexican axolotl) does not undergo metamorphosis in nature. Sexually mature form has external gills and a long tail with dorsal and ventral fins and an reproduce (Fig.14.14 a). Failure of metamorphosis in the larva is because of absence of TSH from the pituitary gland. Axolotl can be made to metamorphose into an adult form when it is administered with either a thyroid hormone or thyrotropin (fig.14.14 b). This indicates that Axolotl larvae do have functional thyroid hormone receptors as they respond to the artificially administered thyroid hormone.



(a) (b)

Fig. 14.14: The sexually mature aquatic Mexican axolotl retains larval features like gills but reproduces. If the neotenic form is administered thyroxine which it does not produce, it metamorphoses to become the terrestrial salamander.

Some species of *Ambystoma* like *A. tigrinum* fail to metamorphose until environmental conditions change. The aquatic form *A.tigrinum* remain neotenic. However, if provided with terrestrial environment it metamorphosis into a land dwelling adult, tiger salamander. Some urodeles, e.g. *Necturus* and Siren exhibit permanent neoteny in which the tissue fail to respond to even artificially administered TH. Though the functional TH receptors are present in these, they do not activate those genes that initiate metamorphosis.

SAQ 7

Match the statement on the right side (A) with the species on the left (B).

A	B
a) No metamorphosis in nature, retention of all larval characters but sexually mature.	i) *Ambystoma tigrinurn*
b) Metamorphosis occurs only if environmental cues are provided. Otherwise neotenous larvae successfully reproduce	ii) *Necturus*
c) Administration of thyroxine or TSH results in the metamorphosis of the otherwise neotenous larva into an adult	iii) *Ambystoma mexicanum*
d) Administration of thyroid hormone has no effect on the larva as the larval tissues have lost their capacity to respond to thyroid hormone. These forms show permanent neoteny	

14.7 SUMMARY

In this unit you have learnt:

- Amphibian cleavage is holoblastic, but it is unequal because of the presence of yolk in the vegetal hemisphere. The mature unfertilized egg of frog already has a distinct animal-vegetal axis laid down by maternal genes in the egg during its development in the ovary. The dorsal-ventral axis is specified by the site of sperm entry and the resulting cortical rotation brings the maternal factors opposite to the SEP on the prospective dorsal side.
- The blastula is composed of thousand of cells with micromeres in the animal hemisphere and macromeres in the vegetal hemisphere with an eccentric cavity called blastocoel. The fate map of blastula shows presumptive ectoderm in the animal zone and endoderm in the vetal zone with a belt like marginal zone, induced by the underlying vegetal cells and the dorsal organizer region.
- Amphibian gastrulation begins with the formation of blastopore on the dorsal surface, just below the grey crescent by invagination of the bottle cells. This is followed by the coordinated involution of the mesoderm and the epiboly of the ectoderm. Vegetal rotation plays a significant role in

directing the involution. The driving forces for ectodermal epiboly and the convergent extension of the mesoderm are the intercalation events in which several tissue layers merge and migrate into the embryo.

- The result of gastrulation is the formation of ectoderm including epidermal and neural ectoderm, covering the surface of gastrula with the internalisation of endoderm with the placement of mesodermal cells between the two. The blastocoels get obliterated by the formation of a new cavity, the archenteron. The blastopore becomes the anus and the mouth develops as a new cavity on the ventral surface.
- Gastrulation is followed by the process of neurulation in which the neural plate is induced in the ectorderm followed by formation of neural tube and the neural crest cells. Differential cell adhension molecules are mainly responsible for separation of neural tissue from the surface ectoderm.
- In frogs the embryos develop and hatch as a larval stage tadpole which is free swimming and undergoes a process of metamorphosis before it is transformed to the young adult. Metamorphosis entails morphological, biochemical and physiological changes in the larva that can be catergorised into progressive, regressive or remodelling events regulated by thyroid hormones.
- Some amphibians (urodeles) retain their larval characters though they attain sexual maturity by a process called neotany. Some species showing neotany can be induced to undergo metamorphosis by exposure to thyroid hormones or environmental cues, while some may show permanent neoteny.

14.8 TERMINAL QUESTIONS

1. How are the body axes determined in frog?
2. Describe the process of internalisation of mesoderm in frog. What are the end results of the gastrulation process?
3. Make a flow chart to show the events in metamorphosis.
4. Describe the process of neurulation in frog.
5. Why is mid-blastula transition stage significant?

14.9 ANSWERS

Self Assessment Questions

1. i) organizer; dorso-ventral axis; ii) dorsal;
   iii) vegetal; left; right; iv) animal; vegetal;
   v) zygotic.
2. i) notochord;
   ii) epidermis and nervous tissue;

iii) paracrine signals from endodermal cells of vegetal pole and signals from organizer;

iv) endoderm.

3. a) vii; b) x; c) viii; d) ii; e) v;
f) ix; g) iii; h) vi; i) I; j) iv

4. i) T ii) F iii) T iv) T

5. i) a) aquatic, terrestrial;
b) premetamorphosis; prometamorphosis and metamorphic climax;
c) regressive, progressive and remodelling.

ii) a) progressive; b) regressive;
c) regressive; d) remodelling;
e) progressive; f) remodelling;
g) remodelling; h) progressive;
i) regressive; j) progressive

6. i) F ii) T iii) T iv) F v) T vi) ….

7. a) matches iii); b) matches i);
c) matches iii); d) matches ii).

Terminal Questions

1. Refer to section14.2
2. Refer to section 14.3.3
3. Refer to section14.5
4. Refer to section 14.4
5. Refer to section 14.2.3