EMBRYOGENESIS OF FROG

The frog is an anuran amphibian of subphylum Vertebrata. It lives in freshwater ponds, swamps and has easy availability for embryological studies. The embryology of frog has been most extensively worked out and its development has fetched the attention of many embryologists because of following reasons : (i) An understanding of frog and other amphibian development is fundamental to the interpretation of chick and mammal development. (ii) Its early development furnishes an excellent transition between the corresponding stages in *Amphioxus* and in those animals which are more highly evolved. (iii) Its later development (*viz.*, metamorphosis of gill-breathing, aquatic fish-like tadpole larva into lung-breathing, amphibious, tetrapod-like adult animal) suggests a possible evolutionary transition from gill-breathing vertebrate type (*i.e.*, fish) to lung-breathing vertebrate type (*i.e.*, tetrapod). (iv) The eggs of frog and other amphibians are easily available and they may be studied in the laboratory from the moment of fertilization onward. (v) The amphibian embryos are relatively straight, not coiled like other vertebrates. This makes their description easier. (vi) In most experimental embryological studies, the frog eggs are generally used, having been either collected in abundance from freshwater ponds during early spring or obtained by means of induced ovulation, (*i.e.*, by injecting pituitary contents of female frog in a sexually mature female during non-breeding seasons) in all the seasons of the year.

17-1. REPRODUCTION

In frog, the sexes are separate. The sexual dimorphism is well marked, *i.e.*, the male frog has **copulatory** or **nuptial pads** on thumbs of the forelimbs and also possesses **vocal cords** for attracting female frogs for copulation during the breeding seasons. The gonads and gametogenesis of both sexes are of following types :

17-1.1. Testes and Spermatogenesis

The male gonads are testes which are paired, small-sized, white, and ovoid bodies. They remain located in the coelomic cavity near the upper end of the kidneys, where they are held in place on the dorsal body wall by a double sheath of peritoneal mesentery, the **mesorchium**. Histologically, each testis is composed of numerous fine convoluted tubules, the **seminiferous tubules**, which are lined by germinal epithelium which includes spermatogonial cells and Sertoli cells. The spermatogonial cells undergo spermatogenesis and produce spermatozoa, which have a typical structure of a flagellate spermatozoon of Chordata. The matured spermatozoa are conveyed from the seminiferous tubules to **vasa efferentia**. By ciliary movement of the lining of vasa efferentia, the mature sperms come in **vas deferens**. Just prior to mating they are stored in the **seminal vesicles** (Fig. 6.1).

17-1.2. Ovaries and Oogenesis

The female gonads of frog are two ovaries, which remain attached to the dorsal side of the body cavity in its anterior portion by a double peritoneal sheath called **mesovarium**. Unlike the testes, however, the ovaries always vary greatly in size and appearance, depending upon the time of year. During breeding season, *i.e.*, summer, they assume large size due to storage of numerous, mature eggs. After ovulation in spring, they become small-sized.

Histologically, each ovary consists of many compartments, whose outer walls are formed of connective tissue or **stroma**, and interior space is filled by **oocytes**. Each of these oocytes is surrounded by a single layer of flattened cells, the **follicle cells** which make the **ovarian follicle**. The oocytes undergo oogenesis and mature ova are released from the ovaries (*i.e.*, ovulation) into the coelomic space from where they are conveyed by the ciliary action on the peritoneum to the exterior by **oviducts**. Oviducts are long convoluted tubes whose size and convolutions are somewhat increased during breeding season. They open anteriorly into the coelom by a ciliated funnel, the **infundibulum**. Posteriorly, they open into the cloaca. The lumen of the duct is lined by tubular glands (which form gelatinous coats around the passing ova) and the ciliated epithelium for the extrusion of ova. At the posterior end, each duct widens and its walls become thinner and very elastic. These dilated regions, known as the **uteri**, serve for storing the ova just prior to extrusion (Fig. 6.2).

The pre-embryonic development of the egg or **oogenesis** of a frog or any other amphibian is a slow process.It requires three seasons during which time it passes through three phases : **multiplication, growth** and **retention**. Multiplication phase started from the tadpole stage and exists up to older adult stage. During this phase, the oogonial cells undergo mitosis and form **primary oocytes**. The primary oocytes enter the prophase of meiosis-I and before the completion of meiosis-I, they enter the most important phase of oogenesis, the growth phase. During this phase primary oocyte increases tremendously in its size. The chromosomes which are occurring in synapsis, become large-sized lampbrush chromosomes and large amount of rRNA, mRNA and tRNA are transcribed around the DNA of chromosomal loops. Certain proteins are also accumulated inside the nucleus. As a result, the volume of nuclear sap increases and nucleus of oocyte becomes inflated and is called **germinal vesicle**. The increased transcription of rRNA is related with number and size of nucleoli per nucleus, both of which are usually increased during this phase.

The volume of cytoplasm of the oocyte of frog also increases qualitatively as well as quantitatively. The amount of yolk platelets and glycogen, etc., increase with tremendous rate and a definite polarity is determined in terms of yolk distribution — the yolk tends to accumulate at one pole, the **vegetative pole** and occupies two-third (*viz.*, 2/3) space of egg, pushing the germinal vesicle eccentrically, which lies in a comparatively yolk-free cytoplasm at another pole of egg, the **animal pole**. Besides yolk, amount of glycogen, nucleic acids (DNA, mRNA, rRNA and tRNA), mitochondria, etc., gradually increase in the ooplasm. When, growing oocytes attain the size 1500 to 2,000 μm, follicle cells form a non-cellular **vitelline membrane** around the egg's plasma membrane.

The primary oocytes reach their full growth toward the end of the second season, but, they are not immediately laid. The third phase is, therefore, one of quiescent retention of these primary oocytes in the ovary until the next breeding season. Then, sensitized by hormones of pituitary and stimulated by the clasp of the male, the ripe ova erupt through the epithelium covering of ovary as the ovarian follicles break and enter the body cavity. This is called **ovulation**. The ovulation is accompanied by the dissolution of the germinal vesicle and the spreading of its liquid contents (nuclear sap) across the animal hemisphere. The **spindle** of meiosis-I forms and the first polar body is given off. As the egg enters the oviduct, a **spindle** of mieosis-II forms and second meiotic division progresses as far as the metaphase. At this stage, it pauses, and no further progress is made until and unless the ovum is fertilized.

Spawning. Each frog egg, as it comes from the cloaca of female, is spherical, unicellular, 1.75 mm in diameter, **mesolecithal** and **telolecithal.** It is surrounded by the vitelline membrane (primary egg membrane) and many layers called tertiary egg membranes of adhesive jelly which were added as it passed through the oviduct. The outermost jelly layer is absorptive and tends to hold the eggs together. A cluster of frog eggs which remain stick together is called **spawn**. A spawn of *Rana tigrina*, may have 3000 to 4000 ova. The spawn is laid, when during pseudocopulation or **amplexus**, male clasps the female and sheds a suspension of sperm over the eggs as they leave the cloaca.

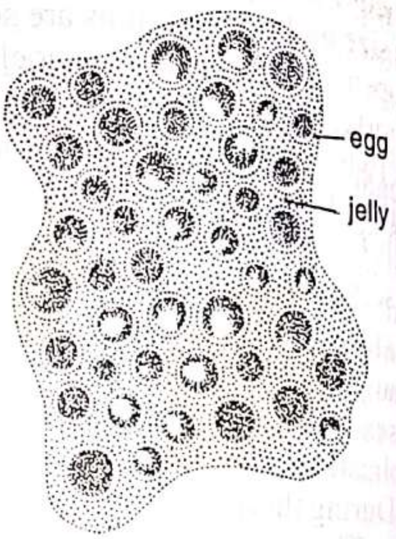

Fig. 17.1. Spawn of frog.

On reaching water, the jelly layers of fertilized eggs absorb water, begin to swell and lose much of their stickiness. These jelly layers of eggs serve various important functions — 1. They serve to attach the eggs to each other and to debris, so that they are not readily washed out. 2. They protect the eggs from the mechanical injuries and make the eggs unedible or distasteful to water snails and other aquatic creatures. 3. The jelly layers serve as insulators to protect the egg from the drastic effects of radiant energy and heat of the sun rays. 4. Being light in weight, jelly helps the ova to float on the surface of pond water. 5. Jelly contains entrapped air bubbles which may help in the respiration of ova.

17-2. ORGANIZATION OF THE UNFERTILIZED EGG

An unfertilized egg of frog is spherical and has a well-marked **polarity** and **radial symmetry**. Its cytoplasm, *i.e.*, **ooplasm** remains differentiated into following two parts :

1. Cortex; 2. Endoplasm.

1. Cortex of the egg. The egg cortex includes the plasma membrane of the egg and adjacent highly viscous or gel-like peripheral layer of cytoplasm called **ectoplasm** of 2 to 3μm thickness. Its material basis is below the limits of resolution of the electron microscope, but chemically, it has been found to be a colloidal complex of mucopolysaccharides, and TP polymers like microfilaments, which requires calcium ions for its stability and physiological integrity. It remains thicker around the animal pole of the egg and becomes gradually thinner towards the vegetal pole of the egg (Fig. 17.3). The egg cortex of frog contains some membrane bounded, spherical bodies of 2µm diameter containing acid mucopolysaccharides and are called **cortical granules**. These cortical granules remain arranged in a layer close to the plasma membrane. They are manufactured by the Golgi apparatus and their mucopolysaccharide contents are fairly homogeneous, finely granular and floccular (*i.e.*, like a tuft of wooly hair). In the egg cortex of frog, just beneath the layer of cortical granules, there occurs a layer of dark brown pigment granules, due to which an unfertilized egg outwardly consists of a dark brown animal region which extends below the equator and a whitish vegetal region which has little pigment granules in the cortex.

The cortical layer of ooplasm remains fairly stable and is not readily displaced by cytoplasmic streaming (cyclosis) or in moderately strong centrifugation. Due to its stability, it plays the role of a fixture in the egg cell and has been found to remain in the same position for continuous period of growth and development. The larger cytoplasmic inclusions are either embedded or attached to the cortex by the help of cortical microfilaments (see **Lovtrup**, 1974) and so they remain in a fixed position. Recent work has found the cortex as a site of morphogenetic patterning relating to polar, bilateral (which arises during fertilization) and general organization of the developing egg.

2. Endoplasm of the egg. The internal portion of ooplasm is called **endoplasm**. It remains in the physical state of a suspension, with a continuous liquid phase (containing in solution various large and small particles, ranging in size from simple inorganic ions to protein molecules) and larger inclusions such as mitochondria and ribosomes dispersed more or less at random in the continuous phase. These inclusions are freely movable in natural conditions owing to cytoplasmic streaming or cyclosis. It consists of a cup-shaped mass of white yolk platelets called **vitelline cupola (Berrill**, 1971) at the vegetal pole. The vitelline cupola occupies most of the vegetal pole and its central concavity remains opened toward the animal pole. Its cavity is occupied by a somewhat pigmented mass of cytoplasm, while the endoplasmic region of the animal hemisphere is occupied by less pigmented cytoplasm. Yolk platelets are largest near the vegetal pole, smaller and less densely packed towards the animal pole. There is accordingly a polar axis, the **animal-vegetal axis** with egg constituents arranged radially around it and exhibiting a concentration gradient from pole to pole.

The polarity and concentration gradients of different chemical substances from pole to pole of the unfertilized egg of frog are revealed by following facts — 1. The germinal vesicle (egg nucleus) is located near the animal pole of the egg and it is here that the polar bodies are given off. 2. The yolk granules are few and small-sized in animal hemisphere, while they are larger and densely packed in the vegetal hemisphere. 3. The cortical layer along with pigment granules remains thick around the animal pole and remains thin around the vegetal pole. 4. Besides the concentration gradients of yolk, cortical gel and pigment granules, there exists a gradient in the distribution of the ribosomes.

17-3. FERTILIZATION

In frog, the fertilization is **external** and occurs in water outside the body of the oviparous female. It involves the penetration of one complete sperm into an ovum anywhere around the animal hemisphere. To recall, an egg or ovum is laid at the secondary oocyte stage at a time when the second maturation division has already reached halfway. Spermatic fluid is shed by the male, involved in amplexus (**insemination**) almost simultaneously with the **oviposition** (*i.e.*, release of ova, about 500 to 600 at a time, from the cloacal aperture of female) and no time is lost in bringing about the fertilization. In the case of delay, the jelly coats around the ovum become too thick for the sperm to pass through them and the ovum also starts to show degenerative changes with the loss of power of development.

Monospermy is the rule in mesolecithal ova of the frog. In the act of fertilization, the sperm becomes attached to the jelly coats of ovum due to fertilizin-antifertilizin reaction (see Chapter 8) and locally dissolve them by acrosomal lysins (enzymes) of sperm to create a passage for sperm entry up to the vitelline membrane of ovum. Recently it has been found that the vitelline membrane is made permeable for the sperm in the body of female, while **'coelomic eggs'** (*i.e.*, ovulated eggs present in the coelom) enter the uppermost section of the oviduct. In the middle part of oviduct, the eggs are enveloped by the jelly coats. Some substance contained in the jelly coats provides the necessary condition for spermatozoa to go to the egg (**Balinsky**, 1981). In fact, jelly layers of the frog's egg depend on the presence of disulphide bonds for maintaining their structure. It has been suggested that the lysins of broken acrosome of frog's sperm may utilize a disulphide bond as a substrate. According to **Gusseck** and **Hedrick** (1971), the fusion of plasma membranes of the sperm and the egg occur by sulphydryl-disulphide bond interchange (**J.B. Phillips**, 1975).

Further, as soon as the tip of the acrosome filament of sperm touches the oolemma of ovum, the latter forms a small crator-like projection called the **cone of reception** or **cone of fertilization** which engulf the sperm by a complex process resembling to phagocytosis. Sperm entry is followed by the following sequence of events (often a complex series of cortical and cytoplasmic rearrangements) :

1. Lifting of vitelline membrane and rotation of egg. The entry of sperm in the ovum has caused some cortical reactions in the latter due to which the vitelline membrane is get separated from the egg plasma membrane and a perivitelline space is established in between the two membranes. This perivitelline space is filled with the liquid substance that is released by exocytosis of cortical granules. This frees the egg to orient itself with respect to gravity, so that the darker animal pole faces upwards and the pale vegetal pole downwards. Such a rotation of fertilized egg is called **''rotation of orientation''**. This is first visible manifestation of fertilization.

2. Formation of fertilization membrane. Contents of burst cortical granules adhere to the vitelline membrane making it impermeable for late coming sperms (*i.e.*, it checks the polyspermy) (**Wolf** *et al.*, 1976). Such a transformed vitelline membrane is called the **fertilization membrane.**

3. Completion of maturation division of egg. The stimulus of the entrance of sperm inside the egg incites the germinal vesicle or egg nucleus which had paused in the metaphase, to complete its second maturation division. It gives off second polar body at the animal pole and becomes vesicular to become **egg pronucleus.**

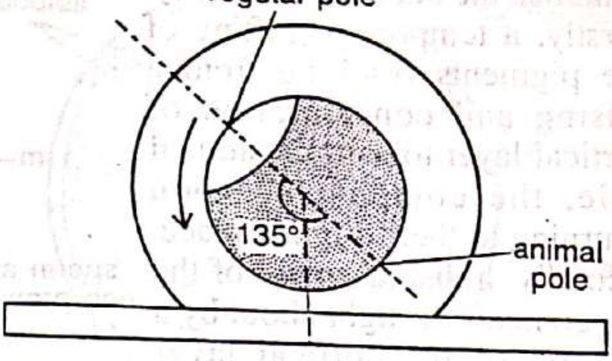

Fig. 17.2. Rotation experiment to determine the median plane of the frog's egg. An unfertilized egg in its jelly membrane if mounted on a glass plate; the egg axis forms at an angle of 135° with the vertical (after Berrill, 1971).

4. Penetration and copulation path. The whole spermatozoon usually takes a minute or two to get entirely inside the ovum. The sperm tail disintegrates, sperm nucleus becomes vesicular by absorbing water from the ooplasm and is called **sperm pronucleus**. It rotates so that proximal pair of centrioles come to lie at its forwarding end and develop asters. As the sperm pronucleus and centrioles travel (glide) centripetally to meet the egg pronucleus, they carry a trail of cortical pigment along with them. This is called **sperm track**. The straight sperm track is called the **penetration** or **entrance path**. However, in the case of an eccentrically placed egg pronucleus, the path followed by male pronucleus is first straight, then inclined at an angle. The inclined pigmented trail is called the **copulation path**.

5. Amphimixis. The sperm pronucleus along with its centrioles moves towards the egg pronucleus and ultimately both pronuclei fuse together to form **zygote nucleus.**

6. Bilateral symmetrization. During fertilization, another most important process called **bilateral symmetrization** is also occurred. During this process the radially symmetrical unfertilized egg becomes bilaterally symmetrical and it has been found that the bilateral symmetry of uncleaved fertilized egg remains intact during embryogenic development. Thus, the bilateral symmetry of the adult animal originates during fertilization.

First sign of the origin of bilateral symmetry in the fertilized frog egg is the appearance of heavily pigmented **sperm entry point (SEP)** on one side of the animal hemisphere, while the side opposite to SEP becomes lighter in pigmentation. The sperm-entry half of the egg eventually forms the **posterior** and **ventral structures** of the larva, with other half forming **head** (anterior) and **dorsal structures (Smith,** 1988).

Following fertilization, certain very significant changes occur in the egg cortex to establish the bilateral symmetry. Firstly, a temporary shifting of the pigments resulting from a raising and concentration of cortical layer toward the animal pole, the components soon returning to their original place, secondly, a displacement of the egg contents brought about by a rotation of the cortical layer around the underlying layers, with relative concentration of the pigmented layer towards a particular meridian. This is accompanied by a corresponding thinning of the cortex on the opposite side of the egg, thereby producing a crescent-shaped surface known as the **gray crescent**, which resembles the yellow crescent of the *Styela* egg. The plane passing through the centre of the gray crescent and the animal pole defines the median plane of bilateral symmetry in each case, *i.e.*, it coincides with the embryonic axis and is the only plane which separates the egg into two equivalent parts, each containing half the crescent material. In *Styela* the gray crescent is made visible by its contained yellow, mitochondrial granules. In the frog, the gray crescent represents material no longer covered by the pigmented cortical layer, and it is gray because it lacks reflective yolk platelets.

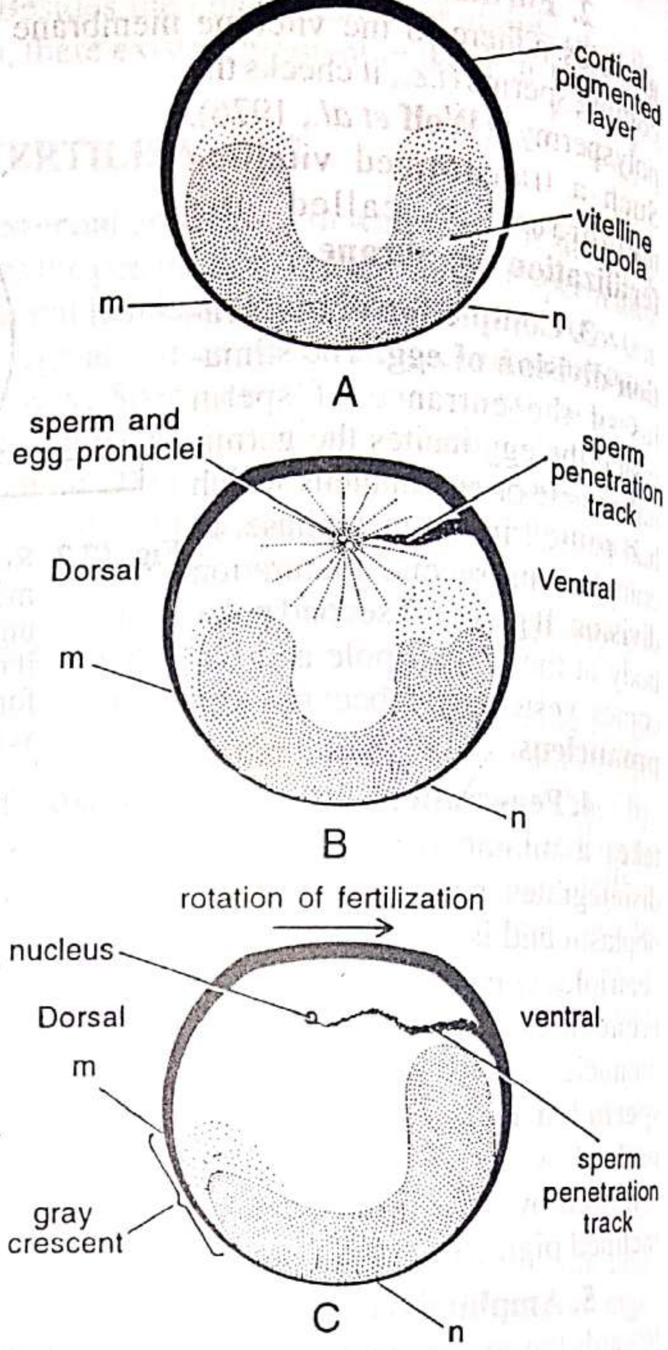

Fig. 17.3. A—Diagrammatic axial section of an unfertilized egg of *Rana fusca,* m-n representing the interior limit of the cortical pigment layer; B—The reaction of the egg to the spermatozoon when it enters. Note in particular the 100 inclination of m-n, and that the vitelline horn has moved closer to the future dorsal side; C—The rotation of symmetrization of cortex and formation of gray crescent (after Berrill, 1971).

According to **Pasteels** (1964), who has worked intensively in this field, two periods should be recognized between fertilization and the first cleavage : (1) The labile period between fusion of the pronuclei and the appearance of the gray crescent and (2) the time when bilateral symmetry is irreversibly fixed.

Thus, due to slight rotation of pigmented cortex around the endoplasmic mass, the median axis of the bilateral symmetry is established and becomes irreversibly fixed. Such a cortical movement which fixes the median axis of the bilateral symmetry is called the **"rotation of symmetrization."**

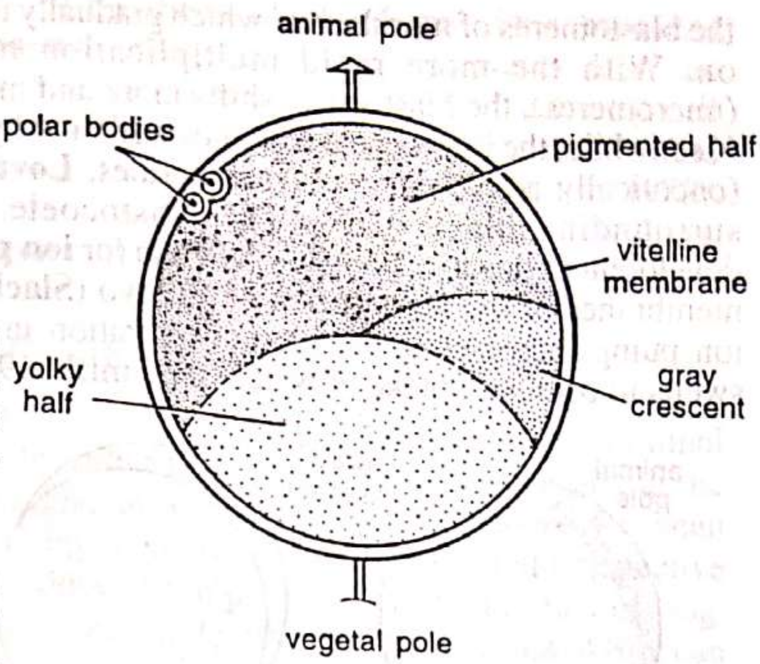

Fig. 17.4. A fertilized egg of frog (after Huettner, 1949).

17-4. CLEAVAGE AND BLASTULATION

The cleavage of a fertilized and telolecithal frog egg is of **holoblastic** and **unequal** type. The **first cleavage plane** is **meridional**. This cleavage furrow first appears near the animal pole and progressively extends towards the vegetal pole of the egg. It cuts the egg through its median animal-vegetal polar axis and results in two equal-sized blastomeres. The **second set** of cleavage divisions is likewise holoblastic and equal, and the cleavage planes are **meridional**, but at the right angles to the first plane. But with the **third set** of divisions, the unequally distributed yolk makes its influence felt. The mitotic spindles orient parallel to the polar axis, and displaced as they are toward the animal pole. The four blastomeres not only cleave **latitudinally**, but unequally. The eight-cell stage, therefore, consists of four small-sized, yolk-poor, animal cells, the **micromeres** and four large-sized, yolk-rich, vegetal cells, the **macromeres**. By so doing, thus, third set of cleavage is set up a differential. The **fourth set** of cleavage planes are **meridional, holoblastic**, but, **unequal**. They divide yolk-poor micromeres more rapidly, than yolk-rich macromeres. The micromeres divide rapidly, due to the fact that they are free from the burden of cleaving through a yolk-dense region. The macromeres divide slowly, since their cytoplasm is now loaded with yolk throughout. Yolk platelets have the double effect of conferring physical inertia on the dividing mass and diluting the active cytoplasm with metabolically inactive inclusions. The fourth set of cleavages, thus, producing a 16-cell stage.

The blastomeres in the early cleavage stages tend to assume a spherical shape like that of the egg before cleavage. Their mutual pressure flatterns the surfaces of the blastomeres in contact with each other but free surfaces of each blastomere remain spherical, unless these outer surfaces are also compressed by the vitelline membrane. At this stage the whole embryo acquires a characteristic appearance reminiscent of a mulberry and so it is called **morula**.

It is about the fourth and fifth cleavage stages that a small space, the **blastocoele**, appears between the blastomeres of morula. Actually, this blastocoelic space first may be represented just by narrow crevices between the blastomeres of morula, but, which gradually increases as the cleavage goes on. With the more rapid multiplication of the animal blastomeres (micromeres), the blastocoele shifts more and more towards the animal pole. Meanwhile, the blastocoele becomes infiltered by water and albuminous fluid (oncotically active muco-polysaccharides, **Lovtrup**, 1974), secreted by the surrounding blastomeres. The blastocoele enlarges througout early development due to a **Na$^+$-K$^+$- ATPase** (or **ion pump**) present in the internal membranes of the blastomeres of embryo (**Slack** and **Warner**, 1973). This ion pump increases the solute concentration in the blastocoele which then swells to osmotic uptake of water (see **Smith**, 1988).

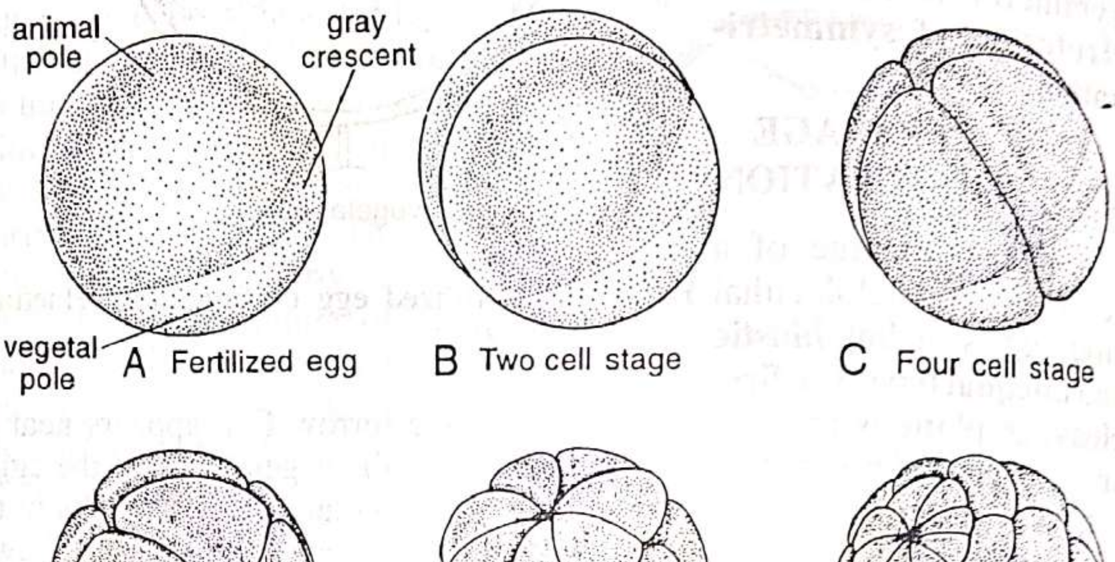

Fig. 17.5. Cleavage and balstulation of frog (after Torrey, 1971).

As cleavage proceeds, the adhesion of the blasomeres to each other increases and they arrange themselves into a true epithelium called **blastoderm**. This blastoderm remains two-cell thick towards the animal pole of the egg and here it forms a sort of roof of the blastocoele. The sides and floor of the blastocoele are occupied by multilayered blastoderm of large yolky blastomeres. The resultant embryo having fluid-filled blastocoele and blastoderm is the **blastula** of frog.

In frog (*e.g.*, *Xenopus*), it has been observed that 11 subsequent cleavages take place **synchronously** every 35 minutes. However, after the 12th cleavage or when embryo (blastula) containing about 4096 cells, the cell cycle becomes longer and **asynchronus,** the cells become motile and RNA-synthesis (*i.e.*, transcription) starts (**Newport** and **Kirschner**, 1982). This turning point in development is called **mid-blastula transition** (**Gerhart**, 1980). The process of gastrulation starts when there are about 20,000 cells (Smith, 1988).

17-5. FATE MAPS OF BLASTULA OF FROG

In the blastula, the blastomeres which have to form different germinal layers and different organs of the adult frog, have their representation at the external surface of the blastula. The fate of each type of blastomeres has been observed by artificial-vital staining methods of **Vogt** (1925) and prospective organ region maps or fate maps have been prepared for the blastula of frog. According to the fate map studies, the whole surface of the blastula of frog can be divided into followed three areas :

1. **Prospective ectoderm area.** The entire pigmented area on and around the animal pole is the prospective **ectoderm**. The **neural ectoderm**, which develops into the central nervous system, occurs largely on the future dorsal side of the blastula, while the **epidermal ectoderm**, which develops into the skin epidermis of the embryo, occupies the antero-ventral side of the blastula. The material for the sense organs is also contained in these two areas. Inside the neural ectoderm area occurs a small sub-area which develops into the eye of the embryo. Likewise, inside the epidermal ectoderm area, there occur the sub-areas for nose, sucker, ears and ectodermal part of mouth.

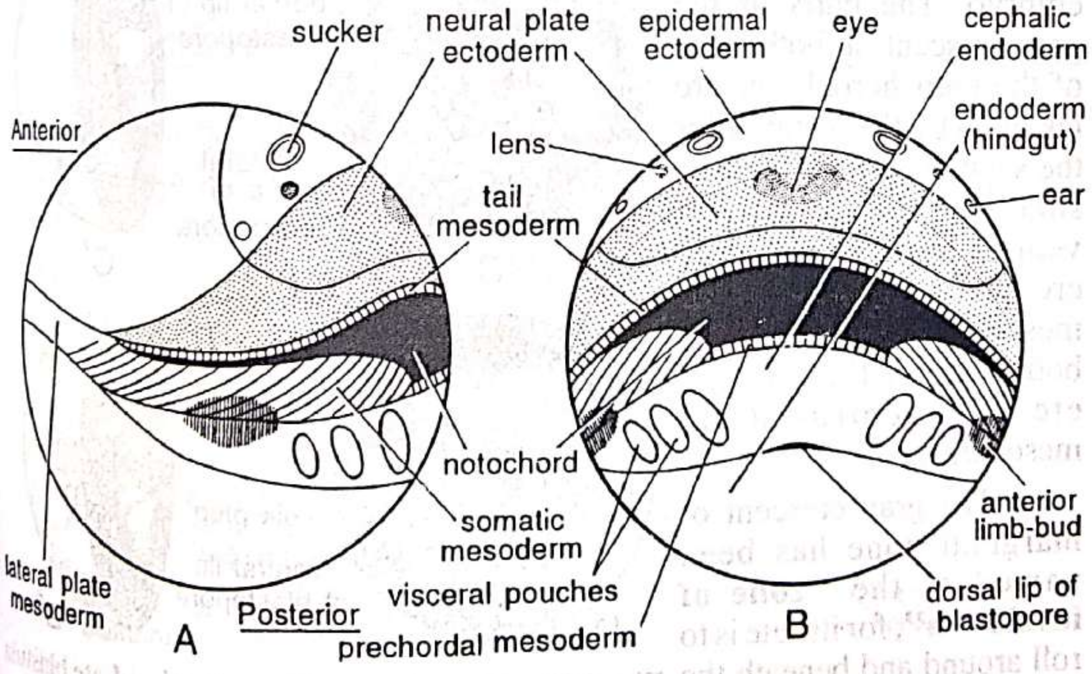

Fig. 17.6. Fate maps of balstula of frog. A—Viewed from the lateral side; B—Viewed from the dorsal side (after Huettner, 1949).

Gilchrist (1968) has called the zone of prospective ectoderm as **"zone of expansion"**, because, during gastrulation this zone expands downward and then converges towards the vegetal pole. At the end of gastrulation, the ectodermal zone gives rise to the entire outer layer of gastrula.

2. Prospective notochord and mesoderm area. The pigmented animal hemisphere (the prospective ectodermal area) is followed by a crescentic area of gray colour, the **marginal zone**, which goes all along the equator of the blastula, and has blastomeres for the formation of **notochord** and **mesoderm** of the frog embryo. The notochordal cells occupy the large area of dorsal side of the gray cresecent. Beneath the notochordal area and toward the vegetal pole there lies a narrow strip of cells which form the pre-chordal connective tissues of the embryo and, hence, this strip is called **pre-chordal plate**. At the upper side of notochordal area and toward the animal hemisphere, lies another narrow strip of cells which develops into the tail mesoderm of the frog embryo. The parts of the gray crescent on both sides of the notochordal area are taken up by the material for the segmental muscles, *i.e.*, somites. The lateral and ventral parts of the gray crescent give rise to the mesodermal lining of the body cavity, the kidneys, etc., (ventro-lateral mesoderm).

The gray crescent or marginal zone has been named as the **"zone of involution"**, for its fate is to roll around and beneath the lips of the blastopore (**Gilchrist**, 1968).

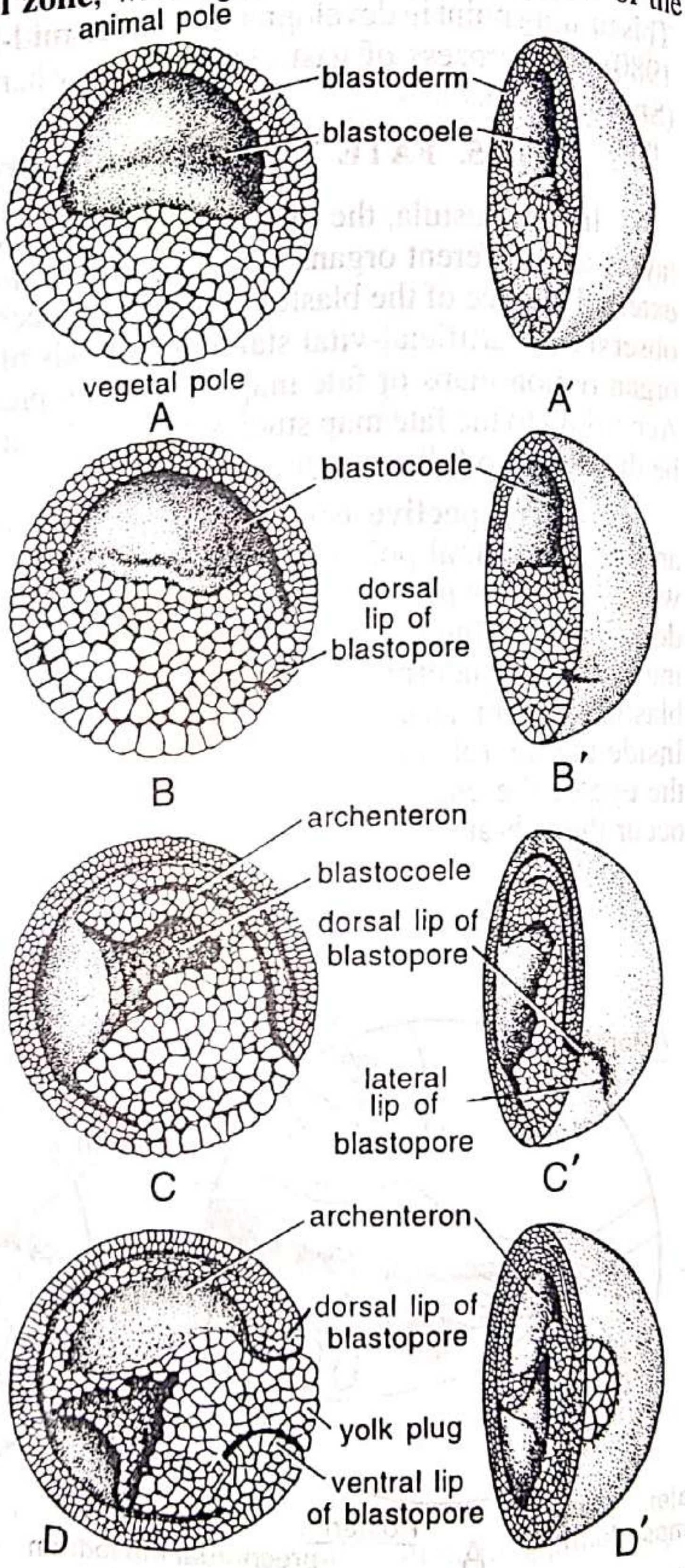

Fig. 17.7. Gastrulation of frog. A,A′ — Late blastula stage; B,B′— Beginning of gastrulation; C,C′— Middle gastrula stage; DD′ — Late gastrula stage; A to D are median sections of the embryos and A′ to D′ are dorsal views of respective sections (after Balinsky, 1970).

3. Prospective endodermal area. The entire non-pigmented area on and around the vegetal pole of frog blastula is the prospective **endoderm**. Just beneath the pre-chordal plate area of mid-dorsal part of gray crescent, occurs the endodermal cells for the anterior parts of the alimentary canal—the endodermal lining of the mouth, gill region and pharynx. Rest of the endodermal area includes the materials for mid-gut, hind-gut and other organs such as liver, pancreas, urinary bladder and certain endocrine glands.

Gilchrist (1968) has called the prospective endodermal zone, the **"zone of invagination"**, because, during gastrulation, it sinks and glides into the blastula of frog.

Pregastrulation. In the late blastula, shortly before the appearance of the blastopore, certain pregastrulatory activities have been observed by **Vogt** (1929). Thus, pre-gastrulation includes three processes—displacement of the marginal zone, 'contraction' of the vegetal area and inclination of the animal-vegetal axis.

17-6. GASTRULATION

The process of gastrulation of frog is somewhat more complicated than *Styela* and *Amphioxus*. It includes three kinds of morphogenetic movements, namely **invagination of endoderm, involution of chordamesoderm** and **epiboly of ectoderm.**

17-6.1. Invagination of Endoderm and Formation of Blastopore

In frog, the gastrulation is inaugurated by the deformation of certain prospective endodermal cells in a circumscribed area below the equator or in

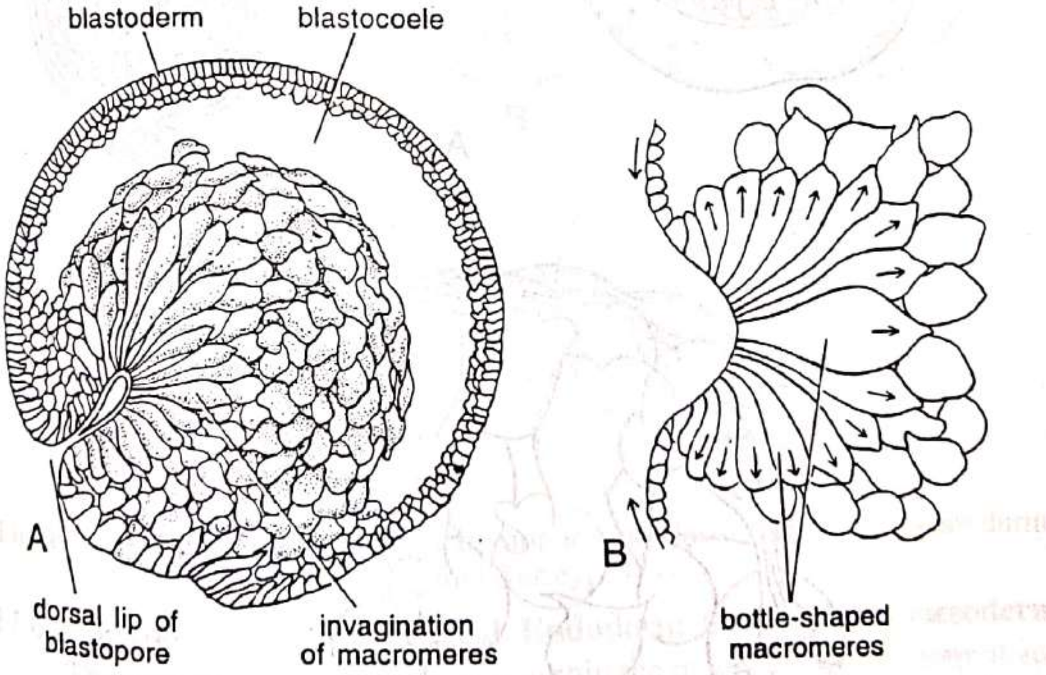

Fig. 17.8. Invagination of endodermal cells in an amphibian egg. A—Slightly schematized section through an advanced gastrula; B—The mechanics of gastrulation, showing active streaming of endodermal cells by bottling process (after Berrill, 1971).

the borderline of the vegetal region and exactly below the mid-dorsal point of gray crescent of blastula. These cells assume the elongate shape of a bottle and move toward the interior of the blastula. Their steadily elongating necks remain attached to the surface of the blastula with the outermost cementing layer which is discovered by **Holtfreter** (1943) and is found to lack in any electron-dense material, hence, remains unrevealed by electron microscopic studies of **Balinsky** (1960), but, recently confirmed to occur (see **Lovtrup**, 1974). Therefore, as the bulky-cell bodies move inward, a pull exerted along their attenuated necks creates an indentation at the surface. With continued multiplication and attenuation of bottle cells, the indentation or **invagination** deepens. Because, by internal movements to be described shortly the invagination will be expanded to form the cavernous **archenteron** or **gastrocoel**, the original indentation is called the **blastopore**, because, it forms a external opening for the gatrocoel. We have already seen that the region of the animal pole is the future anterior end of the embryo, so the blastopore marks

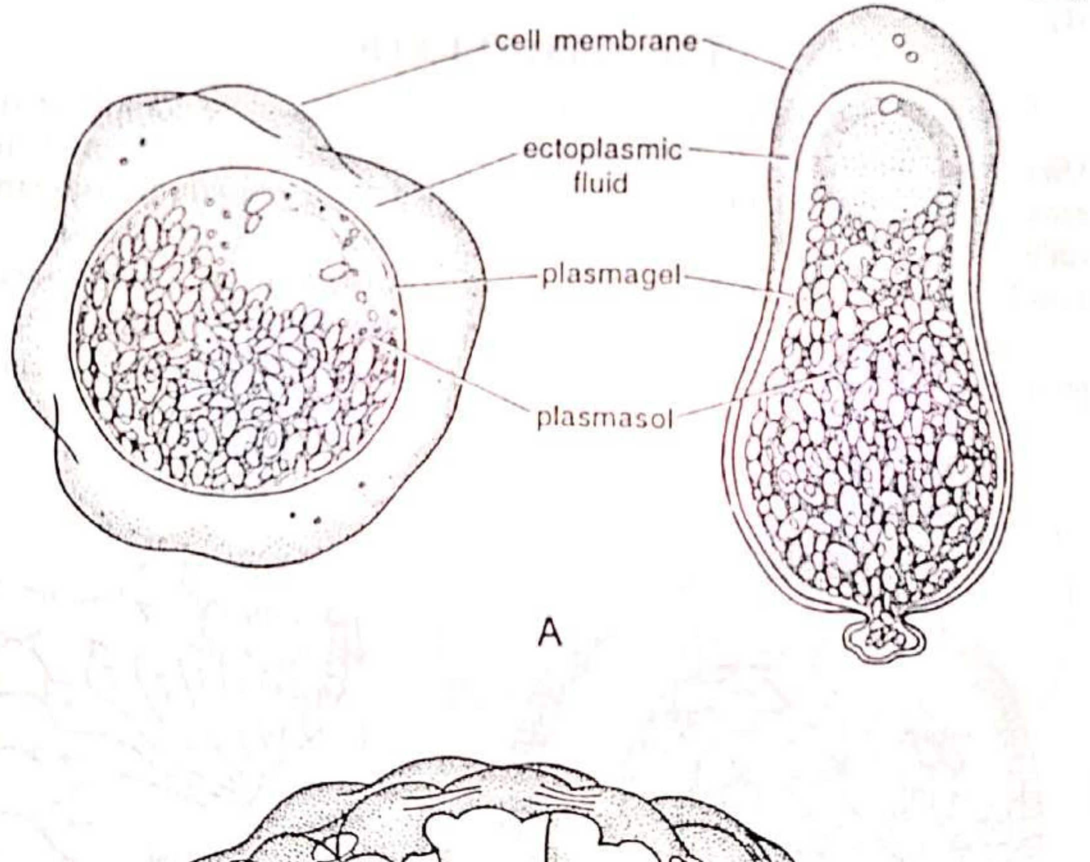

Fig. 17.9. A—Protoplasmic structure of a globular and an elongate cell from an amphibian gastrula; B—A fragment from an early balstula showing dark surface coat and filiform and knob-like processes inter-connecting the blastomeres (after Berrill, 1971).

the posterior end. And because the prospective notochord and neural ectoderm identify the dorsal side of the embryo, the area immediately above the blastopore may be termed as the **dorsal lip** of the blastopore.

Gradually, the blastoporal invagination extends circulolaterally on each side, so that the blastopore becomes crescentic, then horse-shoe-shaped and finally circular. Accordingly, the materials bounding the blastopore on the lateral sides represent its ''**lateral lips**'' and the material on the ventral side, the ''**ventral lip**''. Obviously, however, the terms dorsal, lateral and ventral refer only to the topography of a continuous circular lip.

It should be noted here that different parts of endoderm perform different kinds of morphogenetic movements to migrate inside the gastrula. Thus, the part of the endoderm lying in the marginal zone (the prospective fore-gut area) is mainly absorbed into the original pit-like invagination of the blastopore, but, some of endoderm of fore-gut involute over the dorsal lip along the chorda-mesoderm. The rest of the prospective endoderm of vegetal region passes into the interior of the embryo more or less passively and there it comes to lie in the floor of the gastrocoel. Actually, it is gradually covered up by the epibolic growth of ectoderm.

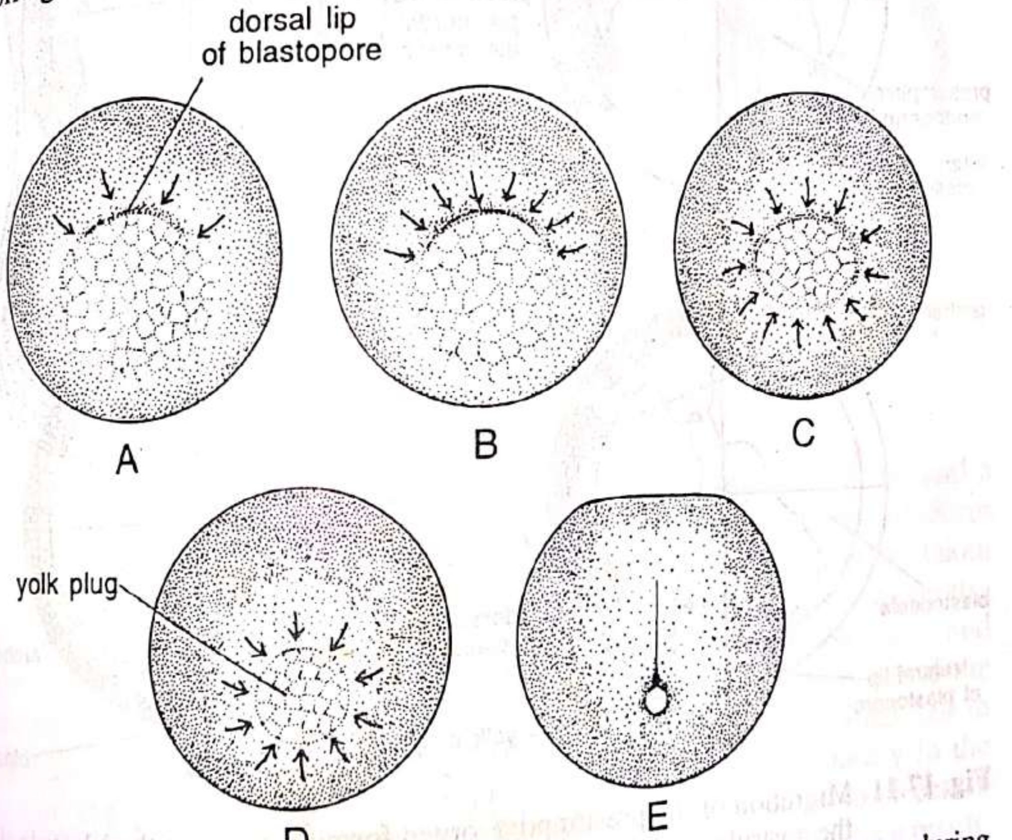

Fig. 17.10. Changes in shape of the blastopore and closure of the blastopore during gastrulation in a frog (after Torrey, 1971)

17-6.2. Involution of Pharyngeal Endoderm and Chorda-mesoderm

The initiation of the blastopore within the prospective endodermal zone sets in the most important gastrular morphogenetic movements, the **convergence** or flow of surface material towards the lips of blastopore and the **involution** of that converged material inside the blastoporal lip. To recall, the endodermal cells abuting the dorsal lip of the blastopore form the prospective pharyngeal endoderm, which is followed by pre-chordal plate, notochord and tail mesoderm. When the dorsal lip is formed, the pharyngeal endodermal cells are the first cells which "turn the corner" or undergo involution over the dorsal blastoporal lip. These cells move to the interior and are followed by prechordal material which converges in to replace it. Soon, pre-chordal material also involutes and is trailed by the anterior part of the prospective notochord and tail mesoderm. As these materials flow inward around the dorsal lip, they become considerably narrowed and in the process of forward flow, greatly elongated. The prospective pharyngeal region later forms the most anterior part of the advancing gastrocoel. In the later stages of gastrulation, the oral and the pharyngeal endoderm expands so as to form the spacious **foregut** whose lateral, ventral and anterior walls consist of a thin layer of endoderm. Only part of the dorsal wall of the foregut is taken up by the prechordal plate and the anterior tip of the notochord.

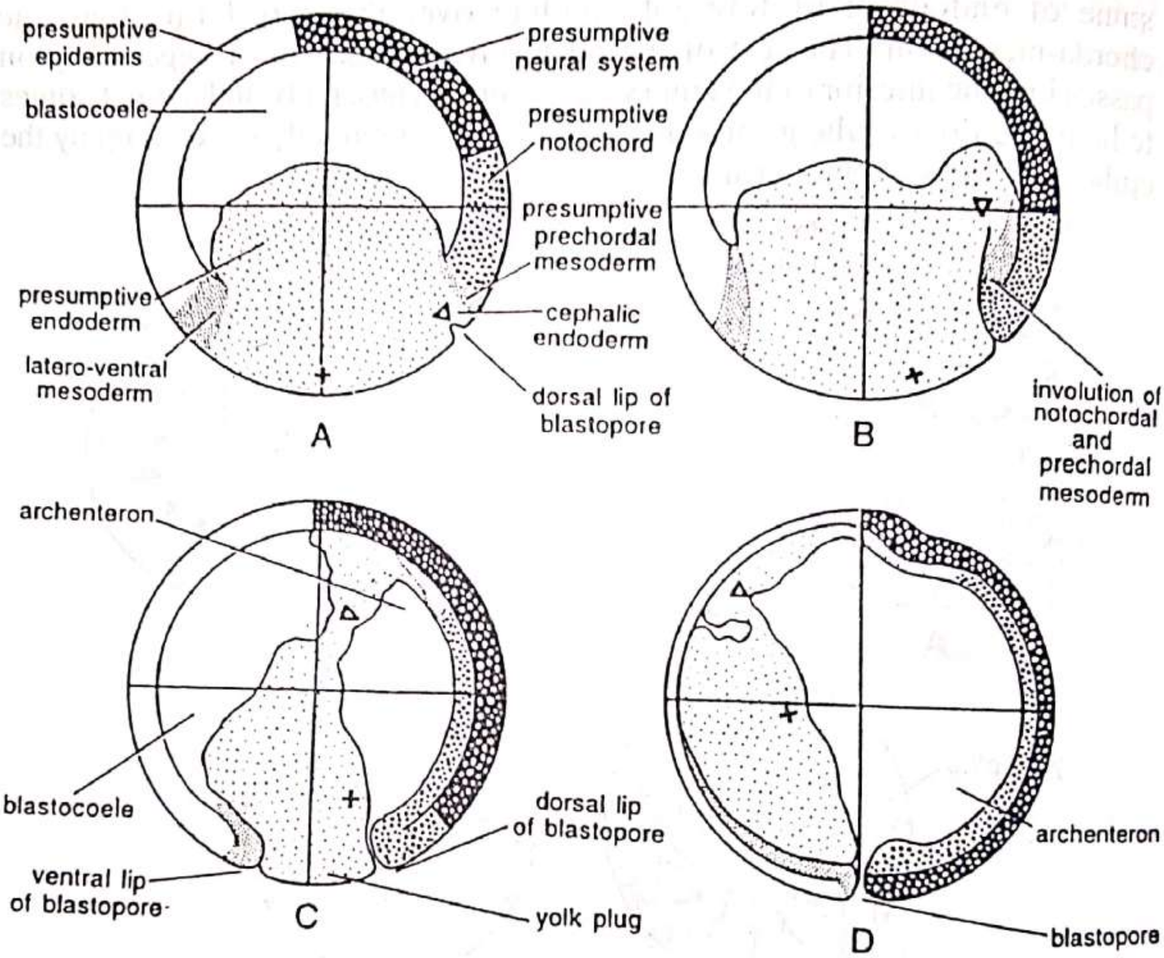

Fig. 17.11. Migration of the presumptive organ forming areas of the blastula during the gastrulation in the Amphibia. The triangle mark the endodermal cells which start invagination and form the foregut. The cross mark the position of endodermal cells which were at the vegetal pole when gastrulation began and which are covered by epibolic growth of ectoderm (after Balinsky, 1970).

Inside the developing gastrula, the prechordal plate becomes a part of the archenteron roof in front of the anterior end of the notochordal material. The anterior part of notochord stretches along the dorsal side of the archenteron, forming the mid-dorsal strip of the archenteron roof. Later it is joined by the cells of posterior part of the notochord which involutes or rolls over the dorso-lateral lips of the blastopore. Eventually, the presumptive notochord undergoes a very considerable elongation in the transverse direction. It becomes concentrated on the dorsal side of the embryo. The tail mesoderm remains near the blastopore, which marks the posterior end of the embryo.

Most of the mesoderm (*i.e.*, trunk somites and ventrolateral mesoderm) invaginates into the interior by rolling over the lateral and ventral lips of the blastopore. Once inside the embryo, the whole kinds of mesoderm namely, notochord, prechordal mesoderm, somites and ventrolateral mesoderm move from posterior side (blastoporal end) towards the anterior side as a single unit called **chorda-mesodermal mantle**, penetrating between the ectoderm on the outside and the endoderm on the inside. Eventually, the mesoderm occupies whole place between the endoderm and ectoderm except a small portion at the anterior end of the embryo which remains free from the mesoderm and it represents the place at which the mouth is formed, during later developmental stage.

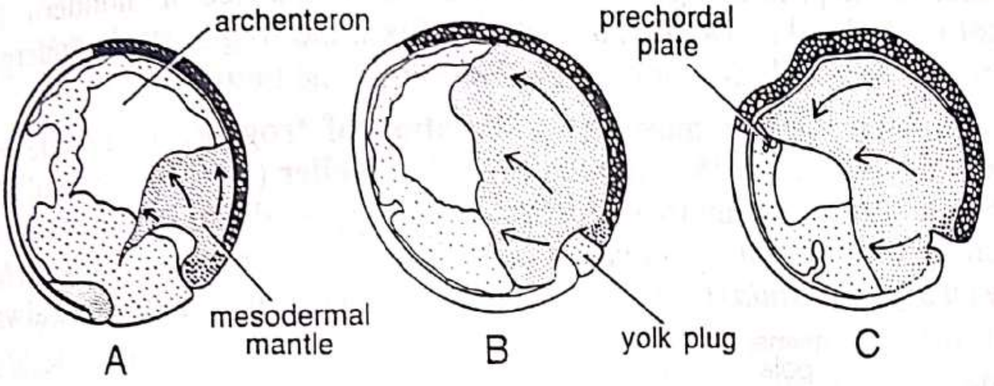

Fig. 17.12. Internal streaming of the mesodermal mantle during the later phases of gastrulation of an amphibian (after Torrey, 1971).

17-6.3. Epiboly of Ectoderm

Throughout gastrulation the embryo retains its spherical shape and a uniform size. This means that as soon as the prospective pharyngeal endoderm and chorda-mesodermal mantle move out of the surface, their places are taken by ectoderm. To accomplish this both neural and epidermal ectoderm greatly increase their surfaces. The expansion of ectoderm is an active process, and the increase of surface area goes on at the expense of a thinning out of the epithelial layer. The presumptive epidermis expands in all directions, but in the case of the presumptive nervous system the expansion is mainly in the longitudinal direction, *i.e.*, toward the blastopore. In the transverse direction on the other hand, the presumptive nervous system area contracts. As a result, the whole nervous system area changes its shape and becomes oval, elongated in an antero-posterior direction.

17-6.4. Closing of the Blastopore

It is in consequence of the removal of endodermal and mesodermal materials from the surface and the compensating spreading of the ectoderm that ectoderm finally arrives at the circular lip of the blastopore, that is, the **yolk-plug** (white coloured endodermal cells as seen from the blastopore) comes to be bounded by ectoderm. Meanwhile, due to forward movement of early invaginated pharyngeal endoderm, and its extension in the antero-posterior direction following changes occur in the gastrula : (1) The gastrocoel gradually deepens and it eliminates completely the blastocoele. (2) As the endodermal mass accumulates on the future ventral side, the centre of gravity is shifted and the embryo rotates so as to bring its dorsal side uppermost. (3) The protruding yolk plug gradually withdraws to the interior, and as it does so, the diameter of the blastopore steadily contracts until at the end of gastrulation, the blastopores recognizable only as a narrow slit.

17-6.5. Gastrula

Thus, gastrulation converts the radially symmetrical monoblastic blastula into a spherical, bilaterally symmetrical triploblastic gastrula having a head-to-tail axis. It is clothed externally by ectoderm and containing endoderm and mesodermal components internally. The part of the ectoderm that lies lenghtwise as a broad, mid-dorsal band is the fore-runner of the nervous system ; the remainder is the potential epidermis. The gastrocoel (or archenteron) represents the lumen of the gut to come. For the present, the walls and floor of the primitive gut or archenteron are composed of endoderm, but, its roof is of chorda-mesodermal material. Soon the frog gastrula undergoes the process of **tubulation** or **neurulation** to become **neurula**.

Modern views regarding gastrulation of frog. Recently fate map studies of **Lovtrup** (1975) with urodels and of **Keller** (1975, 1976) and **Dale** and **Slack** (1987) with anuran *Xenopus*, have suggested that in contrast to the prevailing view, *the prospective mesodermal cells never occur at the surface of the embryo (blastula)* (Fig. 17.13). During the gastrulation, they are always beneath the prospective endodermal sheet which is at the surface prior to its invagination and involution, and between the involuted endoderm and superficial ectoderm after involution. Moreover, the prospective mesodermal cells begin moving into the interior before the appearance of the blastoporal depression in a kind of **ingression** (*i.e.*, mesodermal cells may sink inside as individuals). During ingression, mesoderm acts as a flowing cell stream and

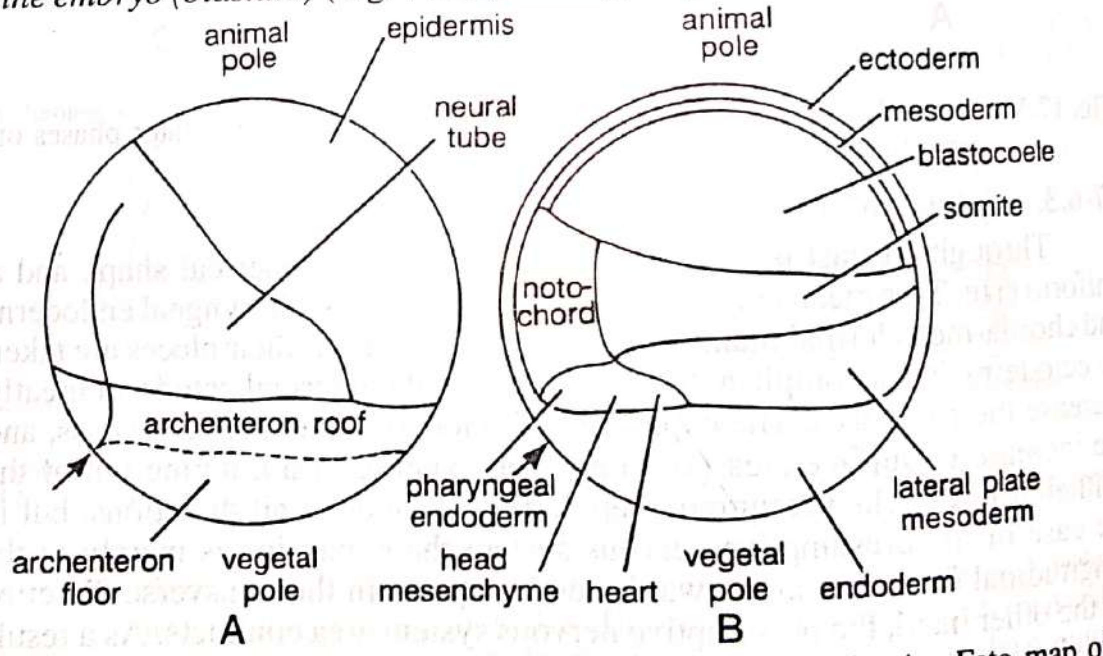

Fig. 17.13. Fate map of the *Xenopus* late blastula–early gastrula. A—Fate map of superficial layer; B—Fate map of deep layer of cell (after Smith, 1988).

it initiates the process of **invagination** and **involution**. This advance (*i.e.*, ingression) toward the newly formed blastopore (convergence) and as the mesodermal cells enter the zone of involution (*i.e.*, lip of blastopore), they lose their regular arrangement, become motile and move actively into the interior, forming the mesodermal mantle of the wall of newly forming archenteron. Since the cells of mesodermal mantle are also motile, they continue this movement forward. Since both the overlying prospective ectoderm and endoderm are attached to the moving underlying mesoderm, both of them are moved passively downward toward the blastopore, where the endoderm undergoes involution because of this continuing attachment (**Trinkaus**, 1984). Such a working hypothesis, therefore, accounts both for the *epiboly of the prospective ectoderm* and *convergence and involution of prospective endoderm*.