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The development of amphibians

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ABSTRACT

This paper observes amphibian development. Fertilisation is mostly external. Oocytes are mesolecitic. Holoblastic and asynchronous cleavage. It also describes the processes of gastrulation, neurolation, organogenesis and metamorphosis in detail.

Keywords: amphibians, ontogenesis, blastula, cleavage, development of cells and organs

1. INTRODUCTION

Amphibians, and above all some frogs and newts, are a favorite subject of developmental biology. Representatives of this class of animals, easily accessible to the researcher, possessing rather large eggs, unpretentious in keeping, have become the object of various experimental, including microsurgical studies, which have made a significant contribution to the understanding of such fundamental problems of morphogenesis as embryonic induction and determination of animal axes [1].

The amphibians are represented by three orders, namely the Apoda, Urodela and Anura. The question of the monophyletic or polyphyletic origin of the Amphibians remains unsolved. The embryonic development of the Apoda is practically not studied. There are significant differences in the development of the Tailed and Tailless, although the basic development scheme is common to them. The African clawed frog Xenopus laevis and, to a lesser extent,

representatives of the genera Rana and Bufo became the model objects of developmental biology from the Tailless. Among the tailed beasts, the axolotl Ambystoma mexicanum, the Japanese newt Cynops pyrrhogaster, and the European newt Pleurodeles waltl are the most popular [16].

2. OOCYTE STRUCTURE AND DEVELOPMENT

The maturation of female germ cells in amphibians (oogenesis) lasts 3 years. During the first two years, a very slow increase in the size of the oocyte occurs, while in the third year of development, a rapid accumulation of yolk is noted, which leads to the formation of full-sized oocytes (Fig. 1). The maturation of oocytes occurs in batches - the first one immediately after metamorphosis (the tadpole is an adult frog), the second one a year later [4].

Figure 1. Stages of oogenesis of the clawed frog: 1 – secondary oocyte; 2 – primary oocyte; 3 – oogonia; 4 – ovarian epithelium

Amphibian oocytes belong to moderately telolecitic, mesolecitic cells. Most of the mature oocyte is pigmented due to the melanin it contains. The pigment part is located on top (animal pole). Such an arrangement guarantees maximum absorption of solar radiation and, as a consequence, the creation of optimal temperature conditions for the development of the embryo. In the middle latitudes, in the common frog, the color of the mature oocyte is black brown due to the low temperature of the water in which the eggs develop. Frogs of southern latitudes are characterized by a less intense, from yellow to light brown, coloration of the animal pole of the ovum [7]. The vegetative pole of the oocyte is filled with yolk. Yolk grains are large oval plates. Closer to the animal pole, the number and size of yolk plates decrease.

3. FERTILIZATION

All amphibians are characterized by monosperm fertilization, both internal and external. (In the vast majority of amphibians fertilization is external, it occurs in water. Internal fertilization is characteristic only of legless and some tailed amphibians. Mating occurs of amphibians with internal fertilisation. In water, the fertilized clutch increases in size due to swelling of the tertiary gelatinous shell of the egg.) The sperm penetrates the egg cell, usually, below the equator, after which the fertilization shell is immediately formed. Amphibian eggs are surrounded by a gelatinous shell. The method of laying fertilized eggs varies from species to species: some lay eggs one at a time, others form cords or chains of eggs, others form a single gelatinous mass, and some bear eggs in pocket-like invaginations on the back.

Fertilization causes radical movements of the oocyte cytoplasm, which plays a decisive role in the processes of cell differentiation during the subsequent development of the embryo. In this case, the correct spatial distribution of morphogenetic determinants, which is a decisive condition for normal development, plays a special role.

In many species of tailless amphibians, a single sperm can penetrate anywhere in the animal hemisphere. After this, the distribution of the cytoplasmic components of the egg changes. The most noticeable changes are in the pigmented surface layer of the cytoplasm. Initially, a layer of cytoplasm containing melanin covers the entire animal hemisphere. After fertilization, a cortical rotation occurs, during which the cortex of the egg rotates approximately 30° along the meridian passing through the point of entry of the sperm, which is located in the animal hemisphere [2].

As a result, a sickle-shaped, weakly pigmented area - a gray sickle - appears near the equator, opposite the site of sperm penetration.

Cortical rotation is a very important event in the development of amphibians, since it determines the movement and activation of a number of morphogenetic factors and predetermines the position of the embryo axes. The gray sickle corresponds to the area in which gastrulation is initiated in amphibian embryos. The mixing of the cytoplasmic masses, which occurs during such movements, initiates a chain of reactions that determine the position of the dorsoventral (dorsal-abdominal) axis of the frog embryo. With the appearance of the gray sickle, the future right and left sides of the embryo can also be determined. The plane of the meridian, which passes through the middle of the gray sickle, represents the sagittal plane of the embryo [9].

Thus, the initial organization of the oocyte cytoplasm undergoes profound changes, especially in the future dorsal region of the embryo. In this case, a part of the ovum's vegetative material is carried away to the animal pole of the zygote.

4. CLEAVAGE

Cleavage of amphibians is holoblastic (complete), uneven and asynchronous. The first cleavage furrow is meridional, passing from the animal pole to the vegetative one. The upper blastomeres that separate are smaller than the lower vegetative ones. Since in amphibians the vegetative pole is overloaded with yolk, the closer to it, the lower speed of incision of the furrow becomes. It does not have time to reach the vegetative pole, as the second furrow begins to cleave (Fig. 2).
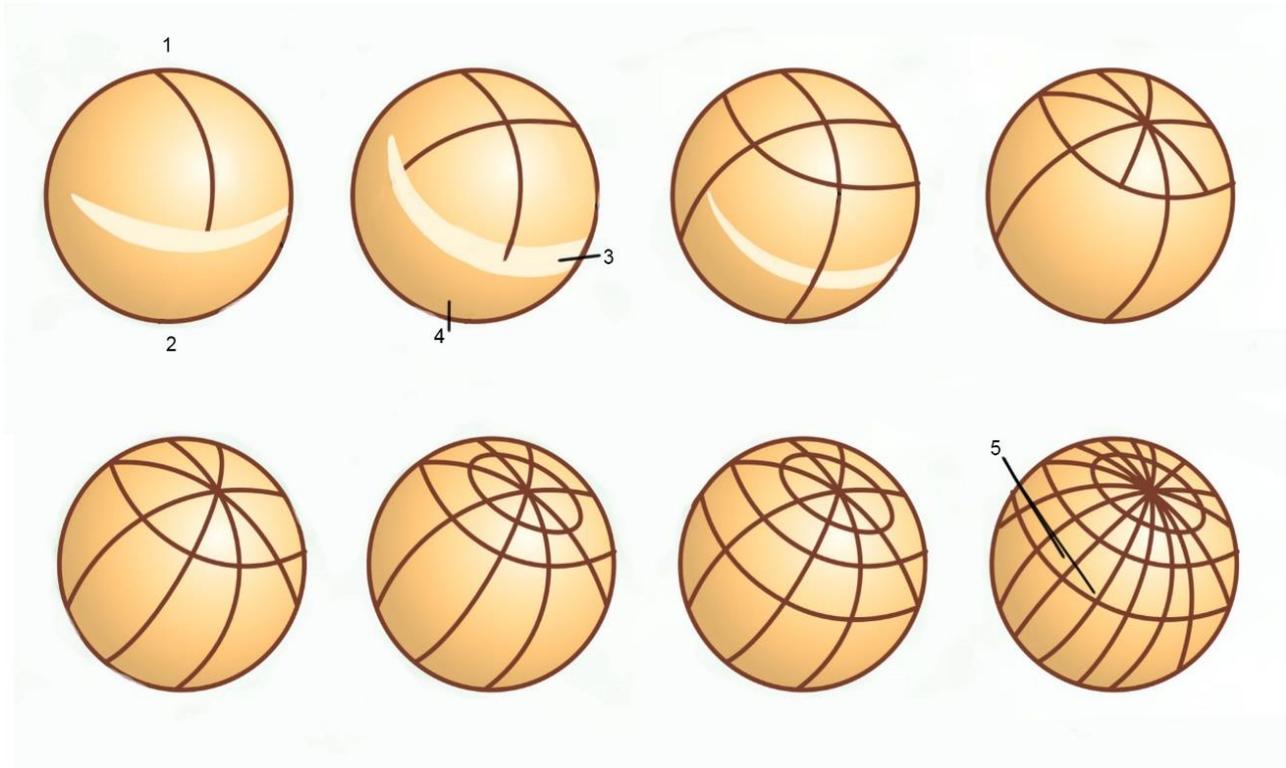
Figure 2. Cleavage of the zygote and cross section of the blastula of the frog (according to Gilbert, 1993): 1 – animal pole; 2 – vegetative pole; 3 – gray sickle; 4 – overload of yolk; 5 – blastoderm sells

The second furrow is also meridional, running perpendicular to the first. After passing the first furrow, two blastomeres are formed, after the second - four blastomeres. At the stage of 4 blastomeres, two animal blastomeres receive 1/2 of the gray sickle material each, and in 2 vegetative blastomeres there is no gray sickle material. The third furrow crushing is latitudinal, passing closer to the animal pole.

As a result of its passage 8 blastomeres are formed: 4 animal and 4 vegetative which essentially differ in the sizes. Next are two meridional furrows. Due to the difference in the size of blastomeres and the amount of yolk, first 4 animal (short-term stage 12 blastomeres) are divided, and then 4 vegetative blastomeres (stage 16 blastomeres) [5].

The next two furrows that occur simultaneously are latitudinal. Faster passage of the upper latitudinal sulcus causes a short-term stage of 24 blastomeres. Completion of the passage of the lower latitudinal furrow leads to the formation of 32 blastomeres. After the stage of 64 blastomeres, despite the fact that the sequence of furrows is preserved, the geometric sequence of the series (2–4–8–12–16–24–32–64) is broken.

In parallel with the passage of the meridional and latitudinal furrows of fragmentation, a tangential furrow is formed, as a result of which the embryo becomes multilayered. At the 128-cell stage, a well-marked blastocele appears and it is believed that at this time the embryo reaches the blastula stage, although the formation of blastocele can be traced from the very first division of fragmentation [6].

5. BLASTULA

Amphibian blastula (amphiblastula) is characterized by a number of special features (Fig. 3).
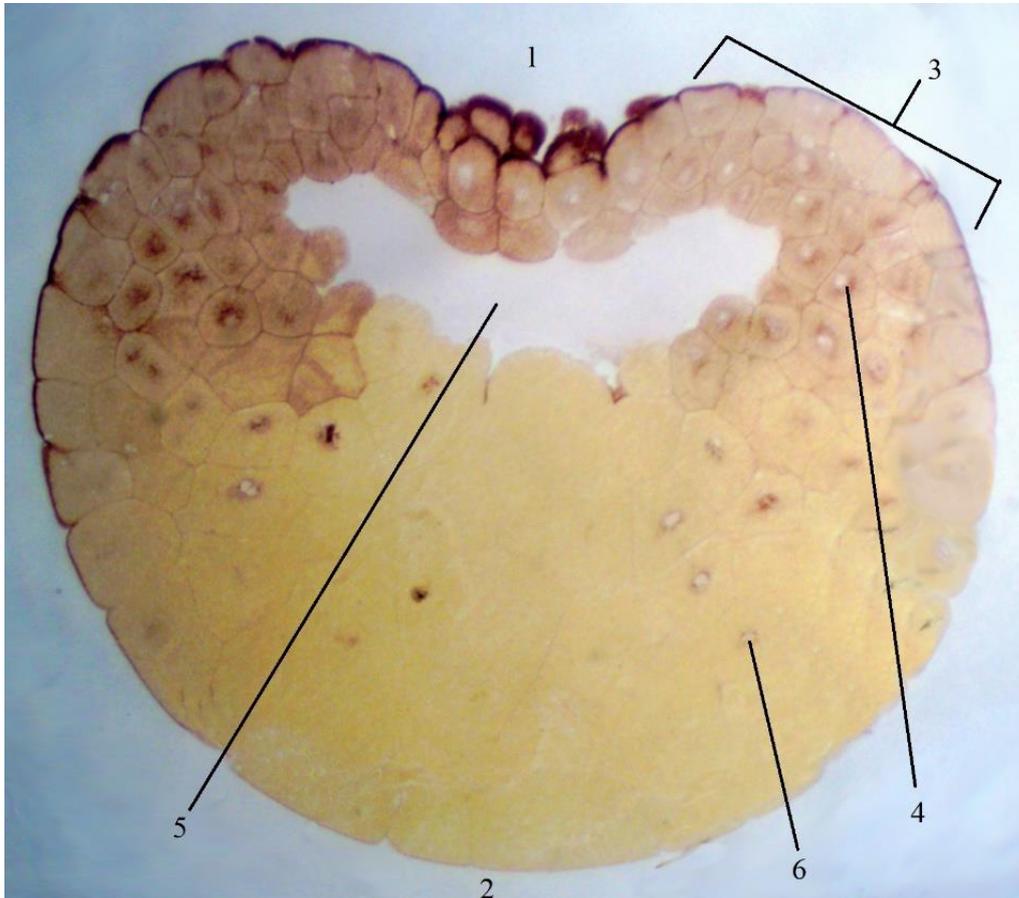
Figure 3. Blastula of a grass frog: 1 - a roof of a blastula; 2 - the bottom of the blastula;
3 - intermediate (marginal) zone of the blastula, 4 - micrometers of the animal pole;
5 - blastocele; 6 - macromers of the vegetative pole

In the process of cleavage, a cavity is formed - a blastocoel. In amphibians, the blastocoel contains a liquid, the salt concentration of which is higher than the salt concentration in the environment. The blastocoel is located in the animal hemisphere. The roof of the blastocoel consists of several rows of cells. The vegetative hemisphere is formed by large, yolk-rich cells, among which there are also primary germ cells [20].

When considering the map of presumptive amphibian primordia, attention is drawn to its fundamental similarity with the corresponding maps of lamprey, lancelet, and ascidians. In the center of the vegetative region, there are also cells that, with further development, give rise to the rudiment of endoderm. In front of the cells of the endoderm there are located the cells of the future head gut and notochord. Farther ahead, in a zone located mainly in the animal hemisphere, there are cells that form a nerve primordium.

At the same time, it is easy to notice a significant difference between the map of presumptive rudiments of amphibians from the corresponding maps of cyclostomes and lower chordates. And this difference concerns, first of all, the presumptive neural primordium, most of which in amphibians moves to the animal half of the embryo. The shift of the neurogenic zone to the animal hemisphere, possibly, correlates with the progressive development of the nervous system in the series "lower chordates - lower vertebrates," and creates the prerequisites for the involvement of a larger number of cells in the process of neurogenesis. The ratio between the position of the neural primordium and the main axis of the embryo varies in the Tailed and Tailless: the axolotl's neurogenic zone extends almost to the animal pole itself, while the clawed frog's border of the neural primordium is about 45◦ from the animal pole [18].

The blastocele has a smaller volume compared to the celoblastula blastocele and is markedly shifted to the animal pole. The presence of a blastocyst ensures the preservation of the cells intended for the formation of the integumentary epithelium and the nervous system, their derivative properties. In amphiblastula there are 3 components: the roof, the edge (intermediate/marginal) zone and the bottom.

In the roof area there are 2–4 layers of the smallest cells (micrometers), the marginal (intermediate) zone consists of 5–7, and the bottom consists of 10 or more layers of medium (mesomers) and large (macromers) blastomeres, respectively. Within the marginal zone, the surface layer of cells is distinguished and the lower layer, the deep cells of the marginal zone, is located below. In the area of the roof of the blastula is the material of the epidermis and neuroectoderm, which gives, respectively, the beginning of the skin (epidermis) and the nervous system. The bottom of the blastula is represented by the material of the endoderm and prechordal plate. In the marginal zone, in the deep layer, there is a material of the chord, segmented and unsegmented mesoderm.

The area of the intermediate zone containing the future material of the chord corresponds to the dorsal, and the material of the unsegmented mesoderm - the abdominal side of the embryo. The line separating the roofing material of the blastula from the material of the marginal zone determines the boundary of the transition of the germinal material inward during gastrulation [3].

6. GASTRULATION

Cell preparation for gastrulation is carried out already during the cleavage period. In amphibians, at the stage of transition to the middle blastula, the cell cycle is rearranged - it slows down as a result of the appearance of the G1 and G2 periods. Cell divisions become asynchronous, cells acquire the ability to move, and for the first time in an animal's life, new mRNAs are transcribed in the cell nuclei. The purpose of gastrulation is to bring the areas intended for the formation of endodermal organs into the embryo, surround the embryo with cells capable of forming ectoderm and place mesodermal cells in the proper places between them [13].

In frogs, the vegetative half of the blastula is very massive and inert due to overload with yolk inclusions, and the blastocele is too small to accommodate the vegetative half. Together, these factors prevent intussusception of cellular material.

In the frog embryo, gastrulation begins with the concentration of the pigment in the form of a strip below the equatorial line in the area of the gray sickle, i.e. in the marginal zone near

the equator. The endodermal cells located here are not as large and do not contain as much yolk as most vegetative blastomeres. The cells in this area contract their outer apical surface and significantly increase the area of the basolateral membranes. They acquire a flask-like shape and begin to sink deep into the embryo, while maintaining a connection with its surface. As a result, a slit-like blastopore is formed on the future dorsal side of the embryo.

Its animal margin is called the dorsal lip of the blastopore. The slit of the blastopore continues to deepen and lengthen, enveloping the vegetative mass of yolk cells in an arc - the lateral or lateral lips of the blastopore are formed. The resulting cavity is called the gastrocoel (the cavity of the primary intestine or archenteron). When the blastopore closes into a ring on the ventral side of the embryo, the ventral lip of the blastopore is formed. The cells of the vegetative yolk mass inside the annular blastopore are called the yolk (rusconium) plug.

The next phase of gastrulation includes tucking (involution) and migration of cells in the marginal zone of the embryo. The first flask-shaped cells on the dorsal side of the embryo, having plunged inward, begin to actively move in the animal direction, dragging along with them the cells of the marginal zone, including the flask-shaped cells that form in the region of the lateral lips (Fig. 4). The cells of the animal region at this time spread due to intensive cell division to the blastopore (epiboly).
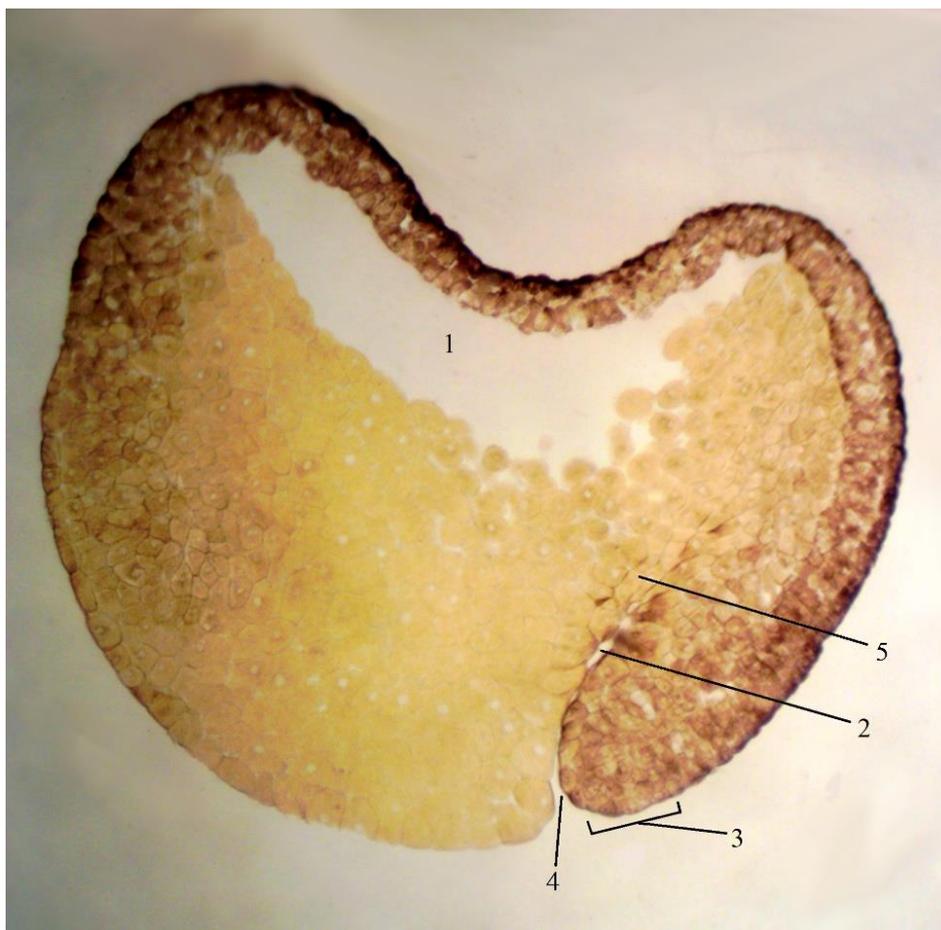
Figure 4. Gastrulation in amphibians: 1 – blastocoel; 2 – gastrocoel; 3 – dorsal lip of the blastopore; 4 – blastopore; 5 – flask-shaped cells

The more active animal half of the blastula begins, as it were, to grow, to stretch over the relatively inert vegetative half. Epibolia does not go immediately along the entire circumference, but at first only in a certain area on the border between the gray sickle and the vegetative pole, spreading laterally in both directions and completing the formation of the blastopore. The main mechanism of epiboly is an increase in the number of cells through division and the simultaneous integration of several deep layers of cells into one. During gastrulation, the cells have time to go through the mitotic cycle three times. As a result, their number increases by about 8 times and, accordingly, their size decreases. By the end of gastrulation, deep cells of the blastocoel roof, as well as subsurface cells of the marginal zone from layers containing 3-4 layers of cells, are integrated into a single layer of a larger area.

The cells of the surface layer and subsurface cells of the marginal zone first move in the vegetative direction to the blastopore slit, and upon reaching it, turn inward and continue to move as a single mass in the animal direction along the inner surface of the blastocoel roof.

The first cells found in the dorsal lip of the blastopore are endodermal, representing the material of the prechordal plate, which invaginate form the leading edge of the archenteron. These cells later become cells of the pharyngeal forehead. The next cells that screw through the dorsal lip of the blastopore are called chordomesoderm cells. These cells will form the notochord, a temporary mesodermal spine, which is essential for initiating the differentiation of the nervous system [8].

As new cells move into the embryo, the blastocoel is pushed aside to the side opposite to the dorsal lip of the blastopore. Meanwhile, the blastopore itself is displaced into the vegetative region and expands, forming the lateral lips and, finally, the abdominal lip, through which additional mesodermal and endodermal progenitor cells pass inward. Large endodermal cells still remain on the surface (yolk plug). Epiboly in combination with convergent extension (see below) of the dorsal side of the embryo, as well as involution of the yolk plug cells, lead to a gradual reduction in the diameter of the annular blastopore (the lips of the blastopore are closed). As a result, all the precursors of the endoderm end up inside the embryo, the ectoderm surrounds its surface, and the mesoderm is located between them.

Although the flask-shaped cells are possibly responsible for the formation of the initial depression of the blastopore, the driving force of involution is, apparently, in the deep layer of the marginal cells. In addition, they probably determine the ongoing migration of cells into the embryo. Apparently, involutional precursor cells of the mesoderm migrate to the animal pole along the fibronectin network secreted by the cells of the blastocoel roof. The factor or factors that initiate gastrulation are initially contained in the deep cytoplasm and not in the cortex of the gray sickle. It is known that in the Xenopus embryo at the stage of 64 blastomeres, the three most dorsally located vegetative blastomeres are able to induce the formation of a dorsal blastopore lip and a complete complex of axial structures.

This small group of vegetative blastomeres is responsible for the appearance in the adjacent marginal cells of the ability to invaginate and form the dorsal mesodermal structures of the embryo. From the stage of the middle gastrula (formation of an annular blastopore), an additional direction of movement of the layers appears. The cells of all layers of the marginal zone begin to shift from more lateral areas (right and left) to the dorsal medial plane of the embryo (lateral-medial migration) and here they integrate between each other (convergent extension). This lateral-medial displacement and intercalation on the dorsal side of the embryo leads to significant stretching of all layers in the longitudinal (animal-vegetative direction), also contributing to epiboly by the cells of the animal pole of the vegetative bottom of the blastula.

Thus, the dorsal side of the embryo is elongated, the archenteron is greatly elongated in the animal direction. Gastrulation is completed when the blastopore ring contracts to a point, the entire yolk plug sinks into the embryo, and the cells of the animal hemisphere cover the entire surface of the vegetative. Thus, the cells in the blastopore lips are constantly renewed. Tucking and migration are more active on the dorsal side of the embryo. The gastric invagination is also significantly deeper on the dorsal side. The material of the future notochord is tucked through the dorsal lip of the blastopore. Together with it, through the lateral lips, the material of the segmented mesoderm is rolled up. Intussusception of the unsegmented mesoderm occurs through the ventral lip, which closes the blastopore ring. Since the endoderm is unable to move completely inside the gastrula due to the large amount of yolk, it closes the entrance to the gastrocoel in the form of a yolk plug [20].

Ectoderm cells, multiplying, gradually overgrow the endoderm, as a result of the endoderm material is found inside the gastrula. As a result, only the darkly pigmented material of the animal part of the blastula remains on the outside, which actually forms the outer germ layer - the ectoderm.

The inner germ layer (endoderm) is formed by the material of the gray sickle (roof of the primary intestine) and the material of unpigmented cells of the former ventral lip (bottom of the primary intestine).

During gastrulation in frogs there is a separation of the middle embryonic leaf (mesoderm). Turning through the dorsal lip of the blastopore, the material of the medial part of the gray sickle folds into a dense cylindrical strand of cells - the rudiment of the dorsal string (chord). The lateral lips narrow to the end of the gray sickle, are turned inward through the lateral lips, which are not part of the primary intestine, and from the beginning grow into the space between the ectoderm and the wall of the primary intestine. This is the middle germ layer - the mesoderm.

After separation of the chordal cord and mesoderm, another material of the primary intestine closes under the chordal cord, forming the rudiment of the intestinal tube (epithelium of the secondary intestine). Its material originates from the massive vegetative half (bottom of the blastula) and represents the endoderm. Thus, amphibians differentiate the endoderm into yolk and intestinal parts [10].


7. NEUROLATION AND DIFFERENTIATION OF THE MESODERM

The early frog's gastrula has a spherical shape, the late one stretches slightly in length, acquires an oval shape due to the formation of nerve (medullary) rollers. These are paired thickened crest-like folds of the ectoderm that arise on the sides of the dorsal side of the body of the embryo. Nerve rollers limit the broad dorsal band of the ectoderm, called the neural plate (Fig. 5).

The nerve plate is the rudiment of the spinal cord and brain, representing the neuroectoderm. The rest of the ectoderm becomes the cutaneous (epidermal) ectoderm. Growing higher, the nerve rollers begin to converge with each other, grow together and form an odd ganglion plate or nerve crest. At the same time the nerve plate folds into a tube.

The neural tube and ganglion plate are immersed under the cutaneous ectoderm, the latter closes over them in a continuous layer and differentiates into the epidermis. After the fusion of the cutaneous ectoderm, the ganglion plate is clamped between it and the neural tube (Fig. 6).

As in the lanceolate, in tailless amphibians during the growth of nerve rollers at the posterior end of the body, the ectoderm approaches the blastopore and closes it. As a result, the neural tube connects to the intestine through the neuro-intestinal tract. In tailed amphibians, the ectodermal rollers grow so that the blastopore remains open and the intestinal tract is not formed [8].
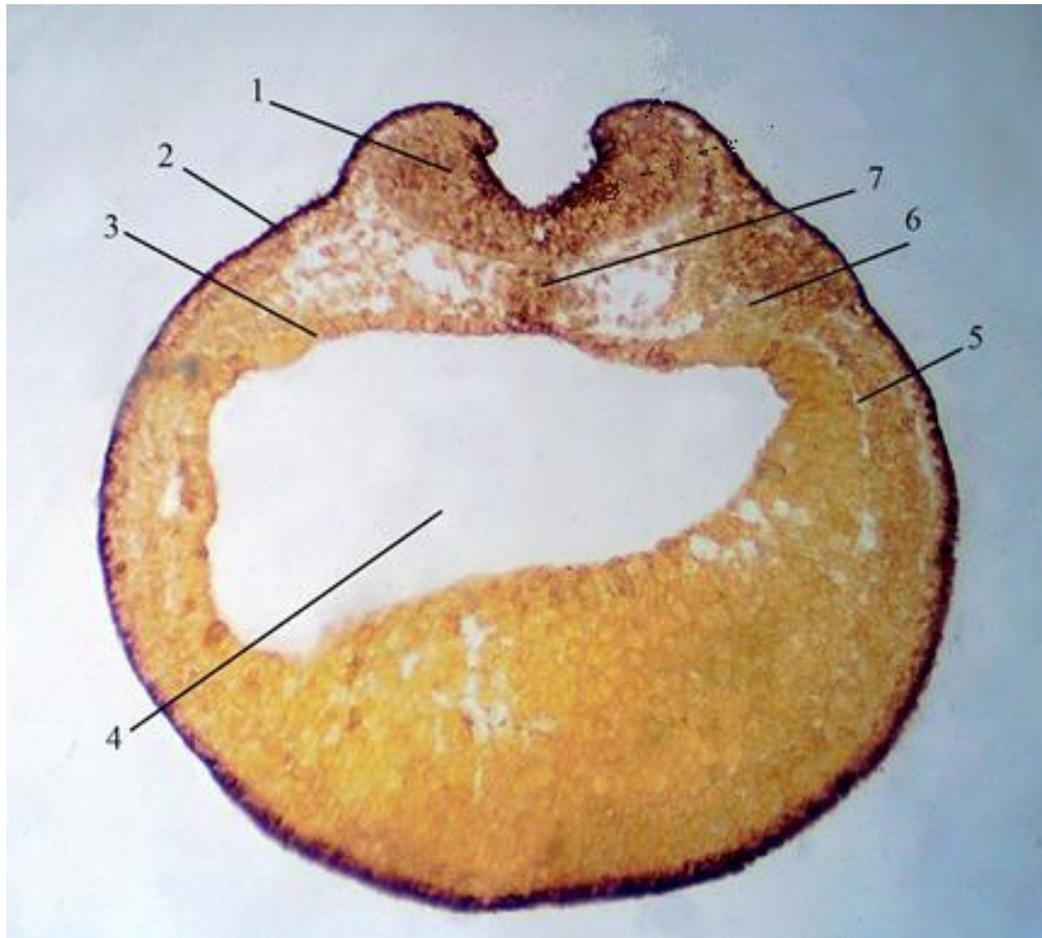

Figure 5. Neurolation in frogs (early): 1 – neural plate; 2 – ectoderm; 3 – intestinal endoderm; 4 – blastocoel rudiment; 5 – gastrocoel; 6 – mesoderm; 7 – notochord

The mesoderm, which first forms a homogeneous layer of cells between the ecto- and endoderm, then differentiates into dorsal areas - dorsal segments (somites), segmental legs (nephrotomies), located ventrally lateral plates (splanchnot). Somites and nephrotomes are segmented, dividing into an increasing number of paired metameric sites. The splanchnotoma of the right and left sides of the body fuses with each other under the intestinal tube, so that the coelomic cavity becomes common to all segments and both sides of the body.

The visceral leaf grows to the intestine and other internal organs and forms a serous membrane. The parietal leaf grows from the inside to the body wall, forming the parietal peritoneum. Part of the cells of the mesoderm, evicting in the intervals between embryonic rudiments, forms a loose cell mass - the mesenchyme.

The segmented mesoderm is divided into a scleroth adjacent to the chord and neural tube, a dermatome adjacent to the epidermal ectoderm, and a myotoma located between them. From the sclerotomum develops the axial skeleton and all types of connective tissue, from myotomes - muscle tissue, from dermatomes - the skin itself. Nephrotomy is a material of the urogenital system. From the mesoderm there is an eviction of cells that form the mesenchyme, which fills the space between all the tabs.
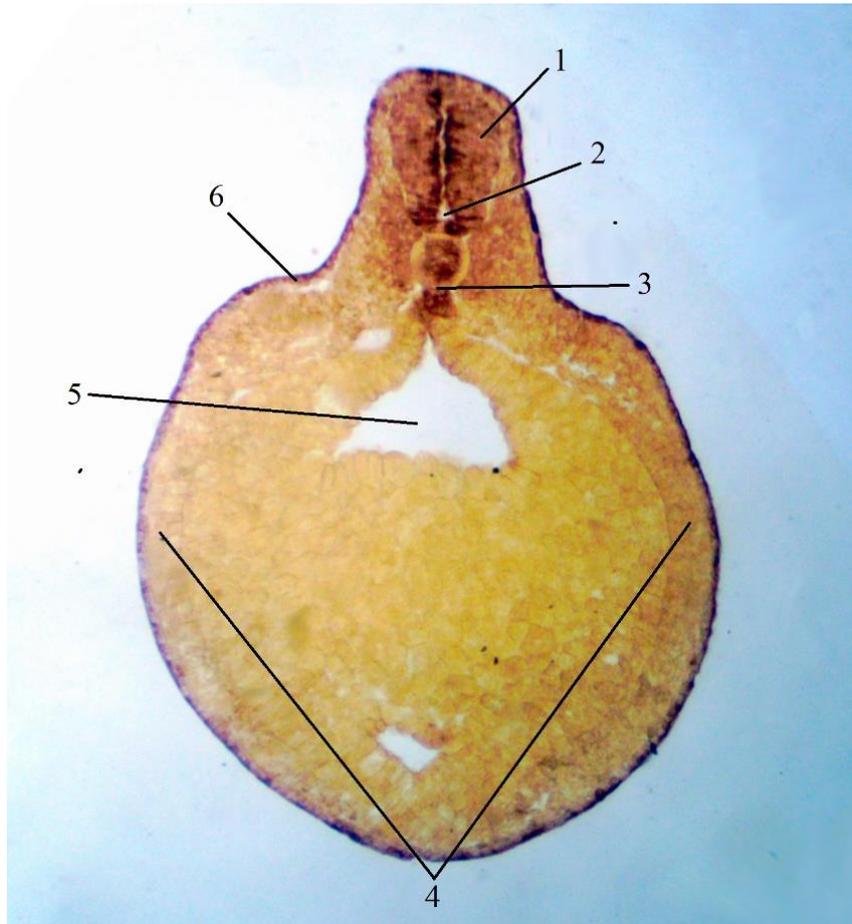
Figure 6. Neurolation in frogs (late): 1 – neural tube; 2 – neurocoel; 3 – notochord;
4 – mesoderm lateral plates; 5 – gastrocoel; 6 – ectoderm

The ganglion plate is divided into two parts, which lie on the sides of the neural tube. The cells that form them migrate in different directions, and then the two parts grow back into one common plate.

From the ganglion plate are formed spinal ganglia (spinal nodes), ganglia of the autonomic nervous system, chromaffin tissue (brain substance of the adrenal glands). In the main department, the offspring of nerve crest cells are found in the bones, cartilage and smooth muscles of the face, middle ear bones, odontoblasts of the teeth, thyroid and salivary glands, differentiated into peripheral neurons and glia. In the trunk, they form pigment cells of the skin (melanocytes).

At the posterior end of the embryonic body cutaneous ectoderm remains both on the posterior neuropore and on the blastopore, which is strongly narrowed and covers both of these openings. Under the cutaneous ectoderm, which fused above them, neuropores and blastopores are connected to each other by the intestinal tract. It does not last long and soon overgrows. At the posterior end of the embryo, a dense outgrowth of poorly differentiated cells is formed - the caudal bud, which is the rudiment of the tail.

At the anterior end of the body, a blindly closed endodermal intestinal tube is adjacent to the cutaneous ectoderm. At this point, the ectoderm and the anterior end of the intestinal tube become thinner, ruptures and an oral opening is formed. In the same way at the basis of a caudal bud there is a breakthrough and formation of an anal opening. The embryo begins to form individual organs and systems of the body (organogenesis) [3].

8. ORGANOGENESIS

The neuroectoderm of the dorsal region is represented by columnar cells that form the neural plate lying directly above the notochord anlage. In tailed amphibians, neural plate cells bordering the epidermis are strongly elongated in height, while contracting at the tops. The edges of the neural plate rise in the form of folds. These folds grow in the sagittal direction and close along the midline of the embryo. In the area of closing of the folds, the epidermal layers of the left and right sides of the embryo are connected into a continuous layer of the integumentary epithelium, and the neuroectoderm of the folds is closed into a neural tube. In this case, part of the material of the nerve folds is isolated in the form of a neural crest, the cells of which have wide potencies and, migrating to different parts of the body, differentiate into various structures. In tailless, the neural plate consists of the surface layer and the inner one, the sensory layer. Their neural tube is formed only by the cells of the sensory layer [11].

The formation of nerve folds begins in the head region and sequentially spreads in the posterior direction. The speed of convergence of the nerve folds is different along the anteroposterior axis of the embryo. The maximum speed is typical for the middle region. Due to this, the closure of the neural tube first occurs in the middle part of the body and from this area spreads back and forth. As in the lower chordates, in amphibians, a neuropore forms at the anterior end, connecting the cavity of the neural tube with the external environment, and the posterior ends of the closing nerve ridges form a vault that covers the blastopore, which thus turns into a neurointestinal canal.

The mechanisms of neural plate lengthening of Tailed and Tailless, apparently, are also different. In any case, under the conditions of organ culture, the monolayer neural plate of *A. mexicanum* grows in length only in the presence of the primordium of the notochord, whereas in *X. laevis*, the lengthening of the multilayered neuroectoderm also occurs in the absence of the notochord due to the mechanism of convergence and extension [16].

The emergence of mechanisms for the formation of a head with a separate brain, sensory organs and jaws - organs that made active nutrition possible and intensified many times over - became a major milestone in the evolution of animals. The emergence of these mechanisms, naturally, in combination with other transformations of ontogeny, opened up new paths for the evolution of vertebrates, which led to the appearance of fish, amphibians, reptiles, birds and mammals. Evidently, an indication of the evolutionary novelty of craniogenesis may be the fact that the concept of the development of the head differs markedly from the development of the

trunk. The brain in amphibians arises from the wide anterior part of the neural plate. Along the constrictions, the brain is divided into three main divisions - the anterior (prosencephalon), middle (mesencephalon) and posterior (rhombencephalon) brain. The forebrain, soon after its separation, is divided into two regions - the telencephalon lying in front and the diencephalon located immediately behind it, which borders the midbrain behind. The telencephalon is subdivided bilaterally, forming symmetrical left and right hemispheres and the olfactory lobes lying in front of them. In amphibians, the cerebral hemispheres are poorly developed. The diencephalon gives rise to the ocular vesicles, the hypothalamus and the neural part of the pituitary gland, this most important organ of internal secretion [14].

The development of the amphibian eye became a classic model of experimental embryology after Spemann discovered the dependent development of eye structures due to inductive interactions. The eye rudiments appear in the form of eye vesicles - symmetrical outgrowths associated with the diencephalon by the eye stem. Initially, the short and wide eye stalk becomes long and narrow as the eye develops. The eye vesicles grow distally and reach the ectoderm of the embryo. Ectodermal cells in the area of contact with the eye bladder become columnar and form the so-called lens placode (from the Greek πλακουζ - a cake, a pie). The wall of the optic bladder facing the placode invaginates into the primary cavity of the eye and forms a two-layer cup - the optic cup. The inner layer of the optic cup is the rudiment of the retina, and the outer layer is the rudiment of the pigment epithelium. The lens placode also invaginates and forms a vesicle, which is then detached from the integumentary ectoderm.

In the process of formation of the eye cup, the primary cavity of the eye disappears, since its leaves are closely closed, although they do not grow together with each other. Outside, the optic cup is surrounded by mesenchymal cells, which subsequently give rise to the choroid and sclera of the eye. The skin epithelium in the region of the eye rudiment, after the separation of the lens material, gives rise to the cornea.

Of great importance in the development of vertebrates are neural crest cells, an amazing population of cells of ectodermal origin, which gives an unusually wide range of derivatives. Arising at the junction of the epidermal and neural epithelium, these cells, at the moment of closing of the nerve folds and the formation of the neural tube, lose their epithelial structure and actively migrate, sometimes over very long distances. Among the derivatives of the neural crest, there are various kinds of pigment cells, spinal ganglia, ganglia of the autonomic nervous system, glial cells, Schwann cells, endocrine gland cells, mesenchyme and connective tissue, chondroblasts, odontoblasts [15].

A special role is played by the cells of the neural crest of the anterior neural tube, which gives rise to the brain. Indeed, they differentiate not only as elements of the sensory nervous system (trigeminal, facial, glossopharyngeal, vagus ganglia), not only as parasympathetic ganglia, but also as skeletal elements, due to which most of the skull is built. After the closure of the neural tube, there are three main streams of migration of neural crest cells - mandibular, hyoid and branchial. This migration is so intense that at the late neurula stage, the mass of migrating cells can be observed in vivo by the swelling of the ectoderm. The most anterior (mandibular) flow is formed by the cells of the ridge located in the midbrain. The mandibular flow bends around the eye rudiments and rushes forward, surrounding the mouth opening.

These cells form the anterior part of the neurocranium and the cartilage of the jaws. The hyoid flow forms in the area in front of the otic vesicle and forms the cartilage that underlies the floor of the mouth. In the area behind the auditory vesicle, there is a migration of cells that form the branchial arches. In the tadpole, four gill slits are formed, through which water is

removed from the pharynx to the outside. The branchial arches are associated with the formation of external gills, which are retained in tailed amphibians, and in tailless ones are replaced by internal gills.

The wide participation of cranial neural crest cells in the formation of the cartilaginous skull is considered as evidence in favor of the hypothesis that the vertebrate head arose as an evolutionary neoplasm that supplemented the protochordat body plan. The implementation of this neoplasm, as can be assumed, became possible due to the fact that the cells of the cranial neural crest, contrary to their seemingly ectodermal nature, acquired the ability to become a new source of cartilage, which replaced the traditional source of skeletal mesoderm, somites, which is absent in the cranial region [14].

After the completion of neurulation, amphibian embryos acquire a plan of body structure common to all vertebrates, characteristic of the pharyngula stage, i.e. the stage at which the formation of gill slits occurs, lying on both sides of the pharynx (pharynx).

Below the epidermal epithelium is the neural tube, which is converted to the spinal cord, and the neural crest of the trunk. In contrast to the cranial region, the cells of the neural crest of the trunk are not capable of chondrogenesis. Under the neural tube is a notochord, which runs along the entire body, a support rod formed by vacuolated cells. Ventral to the notochord is the aorta. On either side of the neural tube and notochord is the paraxial mesoderm. In the course of development, this mesoderm is segmented into metamerically located somites. Each somite is subdivided into sclerotome, dermatome, and myotome. Sclerotome cells differentiate as chondroblasts and give rise to cartilage of the axial skeleton of the animal. The dermatome serves as a source of development of the connective tissue of the dermis. The miotome is the rudiment of the cross-striated muscles.

Ventral to the myotome, on both sides of the endodermal gut, there are mesodermal lateral plates. The lateral mesoderm forms a coelomic body cavity and is subdivided into the outer, lying under the integumentary epithelium, the parietal leaf (somatic mesoderm) and the inner, bordering the intestine, visceral leaf) splanchnic mesoderm. The parietal leaf, together with the epidermis, is called the somatopleura, and the visceral leaf, together with the endodermal epithelium, is called the splanchnopleura. The parietal leaf is the source of the cross-striated muscles, and the visceral leaf is the source of smooth muscles and mesentery. An intermediate mesoderm (mesomer) is located between the somites, which represent the dorsal part of the mesoderm (epimer), and the lateral plate (hypomer). The intermediate mesoderm is the source of the formation of the reproductive and excretory system, which in amphibians is represented at different stages by the pronephros and, later, by the mesonephros [9].

8. 1. Limb development

As in all Tetrapodia, the limbs in amphibians are laid in the form of four primordia. The first sign of an anlage of a limb is an accumulation of mesenchyme, which is formed due to the expulsion of individual cells from the parietal leaf of the lateral mesodermal plate. Soon, this accumulation becomes visible from the outside in the form - at first - a small swelling, which gradually, as the mesenchyme accumulates, increases in size and becomes the bud of the limb. Somites are another source of the limb kidney mesenchyme.

The mesenchyme that is evicted from the somites subsequently forms the muscle elements of the limb, while the mesenchyme of the parietal leaf gives chondrogenic cells. Finally, neural crest cells are the third source of mesenchyme. The descendants of these cells give rise to the connective tissue of the limb [17].

8. 2. Development of the heart

The heart is formed from the mesoderm, which at the gastrula stage lies on both sides of the prechordal plate. The heart is laid on the ventral side of the embryo in front of the hepatic outgrowth of the intestine. The inner layer of the heart, or endocardium, is formed from the cells of the mesenchyme, as an element of the circulatory system, which is formed by converting the mesenchyme into the endothelium of blood vessels. The tubular endocardium in front passes into arterial, and behind into venous vessels. The ventral edges of symmetrical left and right splanchnotomes grow towards each other and grow together along the medial line, forming a common cavity.

The visceral sheet of the splanchnotome forms a kind of groove along the medial line, into which the endocardial tube is immersed. Later, the upper edges of this groove are closed over the endocardium. This mesodermal layer external to the endocardium, with further differentiation, forms the heart muscle or myocardium. The parietal leaf of the splanchnotome transforms into the pericardium, the outer lining of the heart that limits the pericardial cavity [15].

8. 3. Metamorphosis

The overwhelming majority of amphibian species develop indirectly. In the process of embryogenesis, a larva is formed, leading an aquatic life. Later, however, the larva transforms into an animal that is able to live not only in the aquatic environment, but also on land. This radical change in the morphology and physiology of the animal is called metamorphosis.

Metamorphosis affects all organ systems. In tailless amphibians, the main organ of movement of the larva is resorbed - the tail with its powerful muscles; in tailed amphibians, changes in the tail are less pronounced. In the process of metamorphosis, there is an increased growth of the limbs. The gills, gill slits and operculum are reduced. In an adult animal, the lungs and skin take over the respiratory function. Of the branchial slits, only the first pair is preserved, which is transformed into the middle ear (tympanic cavity). There is a restructuring of the skeleton, during which the cartilaginous elements are replaced by bone. The growth of the limbs is accelerated. The upper and lower jaws are enlarged. Serious changes occur in the digestive system: the relative length of the spirally folded intestine of the herbivorous tadpole decreases, while the intestine changes its differentiation, providing the carnivorous nature of the diet of an adult animal. During the metamorphosis of the larva, the eyes shift from a lateral position to an anterior one, which creates the preconditions for binocular vision [20].

Metamorphosis is an extremely interesting model of developmental processes that combines the processes of destructive and constructive morphogenesis. Apoptosis and phagocytosis play an important role in the destruction processes.

A complex and correlated set of changes occurring during the period of metamorphosis is initiated by thyroid hormones - triiodothyronine and, to a lesser extent, thyroxine, the secretion of which is under the control of the hypothalamus, and also depends on the pituitary hormone - prolactin, the presence of which inhibits the production of triiodothyronine [19].

In tailed amphibians, the larvae are more similar to adult organisms in lifestyle. Their metamorphosis is less long, without a radical restructuring of the internal organization of the animal. Some species of tailed amphibians are characterized by developmental delay at the larval stage, in which the reproductive organs develop.

This phenomenon is called neoteny, or larval reproduction. It is characteristic of American ambistomas, the larvae of which are called axolotls [12].

9. CONCLUSIONS

Summing up, the individual development of amphibians has been studied well by scientists all over the world. The mechanisms of development of amphibians from the beginning of fertilization up to the formation of organs are known in details.

However, certain issues remain poorly studied, such as neurulation of tailless amphibians. Also, further researches can be directed to study molecular biological mechanisms of morphogenetic events of metamorphosis.

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