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Exercise 8: Development of a 10mM Frog Embryo

Introduction

One basic and crucial fact is that an embryo is a highly dynamic organism that is in constant flux in time and space. As such, the embryo undergoes progressive changes that yield different views of the same structures in successive sections, more so in the succeeding stages of development. This is especially true in the study of the 10 mm frog embryo and chick embryo which is to be monitored from 18-hour of incubation up to the third day of incubation.

As purely morphological study, microscopic analysis of prepared slides of embryos is a must. However, the thrill of monitoring the progressive development of the embryos is somehow dimmed by its two dimensional view and confusion arise due to positional changes in some embryos. These can be offset by studying 3-D models and CD viewing for a panoramic view before microscopic study. The proper use of some anatomical terms must be also clarified to have a proper perspective. The following are used here with their usual meaning.

Body Axes	Body Part Referred to	In the direction of
Dorsal	Back surface	Upward/anterior
Ventral	Front surface	Downward/posterior
Cephalic/Cranial	Head end	Toward the head/anterior
Caudal	Tail end	Toward the tail
Median	In the midplane	
Medial	Near the midplane	
Lateral	Farther from the midplane	To the right or left
Proximal	Near the central part of the body	
Distal	Away from the central part of the body	

Since we would be using different types of sections of embryos, a review of these terms is in order. A *transverse section* is a plane which is perpendicular to the long axis (cranio-caudal) of the embryo. *Longitudinal or frontal section* is one that cuts the embryo parallel to its long axes and divides it into dorsal and ventral halves. *Sagittal section* is a plane parallel to the midplane and divides the embryo into right and left parts. *Parasagittal section* is a cut to the right or left of the midline. A set of *serial transverse sections* is a collection of all transverse sections from the most anterior to the most posterior part, i.e., the very first section to the last section of the body of the embryo. Hence, if the total number of sections from the most anterior to the most posterior end of the embryo is 100, the first 20 sections mounted on the first slide may represent the most anterior part, the second and the third slides would contain the 21st to the 60th section which maybe the middle parts and the fourth and fifth slides, the remaining 40 sections, thus, maybe considered as posterior parts. The sections are arranged sequentially from *left to right*, with a few rows of sections per slide. The slide of a transverse serial section would look like this:

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1	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20

Slide No. 1

21	22	23	24	25	26	27	28	29	30
31	32	33	34	35	36	37	38	39	40

Slide No. 2

In reality, there may be two to four rows of sections per slide and more than four slides in a set of serial sections. It is for this reason that certain sections are selected, drawn and identified or labeled. These are the so-called “*sections through the level of the heart*” or some other organs. They are chosen because they represent specific portions of the embryo’s body containing many important organs in a particular stage of development. Lastly, the interpretation of the sections is such that spaces/cavities identified as certain organs are actually the lumen or central space surrounded by the walls of the organs.

Exercise 8.1

Mid Sagittal Section of a Tadpole

Introduction

Organogenesis occurs mainly via differentiation of mesoderm and this continued in the tadpole. A vertical longitudinal section of the embryo will be studied first so as to get an overview of the entire embryo. Schumway's chart of frog development gives a complete record of events. The length in millimeters of a tadpole is use for easy reference to the photographed stages of development by Schumway.

From 4 to 7 millimeters frog embryo, only the somites, gills, and tail have developed externally but rapid growth has caused it to be elongated. The general appearance of a 10 mm tadpole is similar to a small fish with prominent tail. The most striking external features include the gills, ventral suckers, horny jaws, lips and papillae, sense organs and myotomes.

Methodology

1. View CD of a 10 mm tadpole (L. S.)
2. Study 3-D models of a 10 mm tadpole
3. Focus prepared slide of a 10 mm tadpole
4. Identify the following parts:
 - A. Epidermis – layers of darkly- stained cells surrounding the embryo.
How many layers are there?
 - B. Lip – upper and lower border of the mouth which arose from the extension of the skin. It is seen as a downward curve of the skin ectoderm on the latero-anterior part of the head of the tadpole.
 - C. Mouth – anterior opening of the digestive tract on the ventral side.
This structure appears as oval space beneath the lips.
 - D. External Nares – circular opening of the nasal passage to the exterior. It is found beneath the anterior region of the head opposite the lips.
 - E. Internal Nares – inner opening of the nasal passage seen as a *dot* mediolateral to the external nares.
 - F. Optic Cup – C-shaped organ composed of:
 - a. Retina –thick dense layers of cells partly enclosing the lens. It is located dorsolateral to the pharynx.
 - b. Lens – a dense spherical mass of cells flanked by the retina and the head ectoderm.
 - G. Pharynx – a large chamber of the foregut on the ventral region which forms the gills. This structure is visible as elongated and clear region below the mouth.
 - H. Thyroid –a short diverticulum of the pharynx visible as dense mass of cells

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lateral to the pharynx.

- I. Heart –oval chamber in the mid-ventral region of the embryo.
- J. Otic Vesicle –large spherical structure below the optic cup. It is quite transparent in contrast to optic cup.
- K. Liver – large mass of cords of cells surrounded by the coiled intestine below the heart.
- L. Pronephros – cluster of small tubules or oval structures lateral to the intestine.
- M. Intestine –long and coiled tubular organ which occupy most of the body cavity.
- K. Stomach– a saccular organ in the middle of the coelom. It is continuous with the intestine.
- L. Spinal Cord – a dense tubular structure dorsolateral to the posterior intestine.
- M. Cloaca – enlarged posterior region of the intestine.
- N. Anus– posterior opening of the digestive tract derived from proctodeum.
- O. Notochord – a clear tubular organ between the spinal cord and the myotomes.
- P. Myotomes – segmented blocks of mesoderm flanking the notochord.
- Q. Tail Fin – dagger-shaped extension of the tail of embryo. It appears translucent beneath the ectoderm.

5. Draw and label the parts of a longitudinal section of 10 mm tadpole.

Questions:

1. What parts of a 10 mm tadpole are critical for its aquatic existence? Which of them were first used by the embryo upon hatching?

2. What organs of the embryo are critical for its future terrestrial life? Give their functions.

3. What organs are unique to a tadpole in contrast to other vertebrate embryos? Explain their significance.

Exercise 8.2 **Development of a 10 mm Tadpole (Serial Transverse Sections)**

Some of the sections you will observe may not be exactly as they are described here because sections vary according to level and exact age of the embryo. An intermediate stage may be seen due to a difference in the temperature in which the embryo was reared. A constant temperature of 18^o C was used in Schumway's chart. Be patient in following the serial sections to get a proper perspective of a particular level.

Whereas the external morphology of the frog embryo seemed to develop quite slowly, the development of the internal morphology is at full blast. Rudiments of various organs in different systems of the body began to emerge and closely assume the definitive organs in the adult animal. The forerunners of respiratory, digestive and excretory organs started to appear and differentiate in preparation for their roles in the terrestrial environment. A longitudinal section of the embryo must be at hand to aid in understanding the location of the different organs of embryo.

I. Level of the Olfactory Placode

- A. Epidermis – double layers of cells covering the embryo. Note that it consists of two layers, the outer pigmented and inner nervous layers.
Which layer of cells developed as placodes?

- B. Telencephalon – small paired organ in the dorsomedian region of the head.
Recall that it is actually the fused anterior neural folds. The paired cavities are the *first and second ventricles* or the *lateral ventricles*.
- C. Olfactory Placode – large paired organ on either side of the telencephalon.
 - c.1. Olfactory Membrane– the thick median layer of cells partly enclosing a cavity.
 - c.2. Olfactory Tube – choanal space or canal leading to the internal nares. It is enclosed by the olfactory epithelium.
 - c.3. Jacobson's Organ – glandular mass lining the choanal canal.
What is the function of this organ?

 - c.4. Internal Nares – narrow cavity leading to the pharynx. It is defined by paired layer of cells above the pharynx. It is also called the *choanae*.
 - c.5. External Nares – large opening to the exterior. It is also called the *olfactory pits*.
 - c.6. Lateral Appendix – small lateral outgrowth near the epidermis and external nares.

- D. Notochord – spherical mass of vacuolated cells below the telencephalon. It is surrounded by a fibrous sheath.
- E. Parachordal Cartilage – paired circular structures flanking the notochord.
Scattered within this are the nuclei of the chondrocytes which appear

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basophilic.

- F. Head Mesenchyme – undifferentiated cells visible as tiny granules loosely distributed all over the head. These cells are migratory in behavior. Focus under HPO and find out the shape of the cells.
- G. Melanophores – darkly pigmented cells that form an irregular layer lateral to the pharynx. ***What is the shape of the cells?***
- H. Pharynx – a broad chamber with tapering end. It appears as large space in the center of the head.
- I. Mandibular Cartilages – irregular bands of cartilage lateral to the pharynx. These are the *primordia* of the right and left jaws.
- J. Tooth Germ – horny epidermal papillae attached to the jaw cartilages. Visible as dense convex structures at the posterior end of the pharynx.
- K. Lip – fingerlike outgrowth ventro-lateral to the teeth. Dorsal lip developed 3 rows of teeth while the ventral lip developed 4 rows of teeth that are deciduous.
- L. Mouth – narrow opening flanked by the teeth and lips leading to the pharynx.

II. Level of Optic Cup

- A. Diencephalon – large oval region of the brain with a central narrow cavity that later enlarged. Its cavity is called the ***III ventricle or diocoel***.
 - a.1. Anterior Choroid Plexus – the roof of diencephalon that is convoluted and supplied with capillaries. This may be seen also in telencephalon since it developed at the junction of the two brain vesicles.
 - a.2. Infundibulum – a V- shaped pocket on the floor of diencephalon with a thin roof and thick walls.
 - a.3. Hypophysis – a round mass of pigmented cells immediately below the infundibulum. ***From what organ did it originate?***
- B. Optic Cup – more defined but similar components as described earlier.
 - b.1. Retina – double layered part medio-lateral to the lens which appears as C-shaped structure.
 - b.2. Lens – translucent spherical organ partly enclosed by retina. It usually disappears earlier than the other parts.
 - b.3. Cornea – epidermis cells partly enclosing the optic cup.
 - b.4. Optic Nerve – transverse band of nerves extending from the retina to the posterior end of the diencephalon.
- C. Anterior Cardinal Vein – blood vessel seen as round structure between utricle and epidermis.
- D. Trabecular Cartilage – visible as slender or round mass of chondrocytes ventral to the diencephalon.
- E. Pharynx – seen here as elongated space below the diencephalons and optic cup.

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- F. Ceratohyal Cartilage – a pair of elongated and curved structures below the pharynx. It bends upward halfway beside the pharynx.
- G. Basibranchial Cartilage—triangular structure flanked by ceratohyal cartilage.
- H. Mandibular Muscles – bands of cells lateral to the hyoid cartilage. The cells are differentiated from the cartilage by their fusiform nuclei and greater staining affinity.
- I. Hyoid Muscle – transverse layer of muscle below the ceratohyal cartilage.
- J. Thyroid Bodies – small dense round structures below the hyoid cartilage.
- K. Lymph Sinus – concave space partially enclosing the pharynx and other structures below it.
- L. Ventral/Oral Suckers – paired adhesive organs seen as round structures attached to the posterolateral part of the head.

III. Level of the Heart

- A. Myelencephalon – has thin roof and thick walls with a V-shaped cavity called the *rhombocoel* or *IV ventricle*.
- B. Otic Vesicle – rudiment of the inner ear seen as large irregular sac lateral to myelencephalon.
 - b.1. Utricle – appears as small round cavity dorsal to the saccule and in contact with myelencephalon.
 - b.2. Anterior Semicircular Canal -- more anterior and vertical subdivision of the utricle.
 - b.3. Saccule – large round cavity ventral to the anterior semicircular canal.
 - b.4. Endolymphatic Duct – small round structure mediolateral to myelencephalon.
- C. Ganglion VIII – large dense mass of neurons mediolateral to otic vesicle.
- D. Anterior Cardinal Vein – minute round structure below the otic vesicle.
- E. Pharynx – seen as a narrow transverse slit at the center of the head.
- F. Velar Plate – lateral projection on the floor of the pharynx. This prevents clogging of gill slits with the ingested food.
- G. Gill Rakers – comblike structures ventrolateral to the pharynx. These serve to prevent passage of solid particles through the gill slits and for mechanical sifting of water.
- H. Aortic Arch – branchial vessels seen as round spaces between pharynx and bulbus arteriosus.
- I. Heart – differentiated into :
 - i.1. Bulbus Arteriosus –tubular extension of the ventricle appearing as oval structure below the aortic arch. First to be seen in transverse sections.
 - i.2. Atrium – anterior region of the heart with thin walls. This and ventricle are both seen in later sections. Also called *auricle*.
 - i.3. Ventricle – posterior chamber of the heart with thick walls that appear undulating or net like.

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- J. Pericardial Cavity – space where the heart is found.
- K. Opercular Chamber -- space lateral to the heart where gills are found.
- L. Internal Gills --fingerlike projections inside the opercular chamber. It is derived from branchial arches III, IV and V.
- M. Gill Filaments – cluster of tiny spherical structures loosely lying within the opercular chamber. There are two rows of these ventral to the branchial arches.

IV. Level of Glottis

- A. Rhombencephalon – the hindbrain.
 - a.1. Posterior Choroid Plexus – downward growth of the roof of myelencephalon containing capillaries. It is seen as coiled tubules hanging from the roof of the brain.
 - a.2. Rhombocoel – large, central V-shaped cavity of the myelencephalon.
- B. Notochord – round vacuolated structure below the rhombencephalon.
- C. Parachordal Cartilage – paired mass of chondrocytes lateral to the Notochord and apposed to the somites.
- D. Dorsal Aorta – large vessel mediolateral to the notochord.
- E. Internal Carotid Artery – elongated vessel lateral to dorsal aorta.
- F. Glottis – a narrow cavity below the pharynx and shaped like an inverted V.
- G. Sinus Venosus – large transverse chamber below the glottis. Inside its lumen are numerous blood cells.
- H. Duct of Cuvier – paired short vessels directly connected to sinus venosus. This is also called the *common cardinal vein*.
- I. Intestines – oval structures with long lumen lying within the coelom. The epithelium lining may be thin or irregularly thick and undifferentiated. *What type of epithelium surrounds the lumen?*
- J. Ventral Mesocardia – short band of cells attached to the posterior region of ventricle and lateral walls of mesocardia.

V. Level of Lung Buds

- A. Esophagus – “winglike” organ below the notochord.
- B. Lung Buds – paired oval structures with a cavity located on either side of esophagus.
- C. Pronephros – coiled tubules appearing as cluster of round or oval structures dorsolateral to the lung buds.
 - c.1. Nephrostome – funnel shaped structure between the pronephric tubule

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and glomus. Note the cilia lining this organ. The removal of larval waste products is accomplished via ciliary motion.

- c.2. Glomus – short branch/radice of dorsal aorta with a tuft of capillary. It is closely adjacent to the nephrostome.
- c.3. Pronephric Chamber – space where the pronephros is found.

- D. Spiracle – tiny opening to the outside which serve for the egress of water. Located on the posterior left margin of the operculum.
- E. Intestines – coiled tubules appearing as two to five round or oval structures lined with simple epithelium and with a large lumen. Some parts of the intestine are still undifferentiated and have much yolk-laden cells.
- F. Stomach – oval organ with thick walls and wavy inner lining adjacent to the liver.
- G. Gall Bladder – a small round organ with a thin lining and a central cavity. It is located adjacent to the stomach and liver.
- H. Liver – cords of cells which ramified and aggregated into one or two masses.
- I. Peritoneal Cavity – large space where the viscera are located.

F. Level of Mesonephros

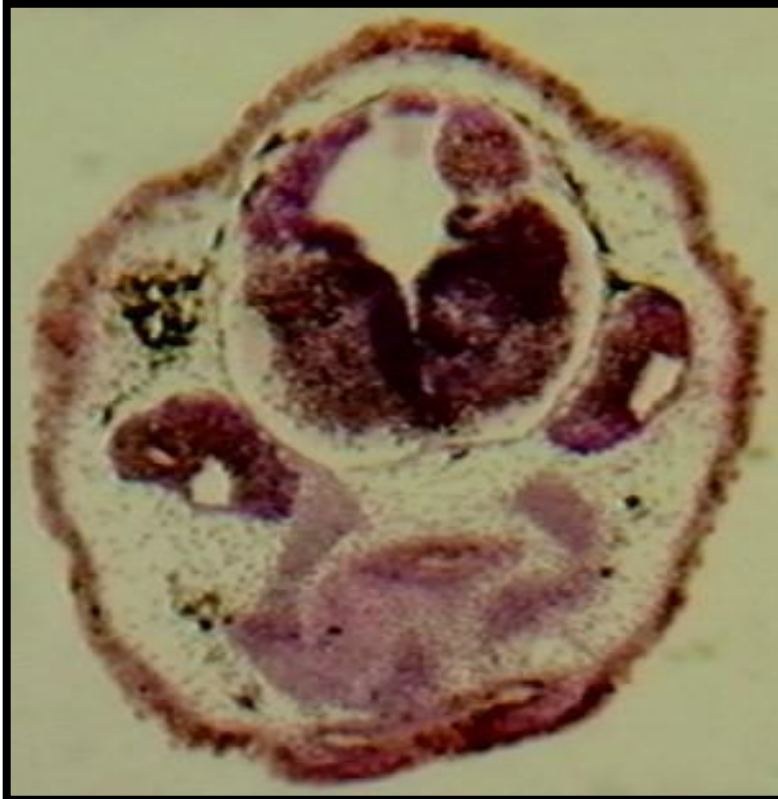
- A. Spinal Cord – tubular organ seen as small round structure with thick lateral walls and thin roof and floor. Its cavity is a narrow slit called the *central canal*.
- B. Myotomes – bands of cells appearing as oval masses apposed to the notochord and later encompassing notochord and spinal cord.
- C. Mesonephric Duct – short tubule seen as round structure medial to the mesonephros. Also called the *Wolffian Duct*.
- D. Mesonephros – small mass of coiled tubules ventral to the somites VII to XII.
- E. Gonad Primordium – round mass of cells attached to the dorsal mesentery and hanging into the coelom.
- F. Hindgut – tubular organ seen as round with folded inner lining and lumen. It is found directly below the mesonephros.
- I. Intestines – varied in size and shape filling the peritoneal cavity.

G. Level of Cloaca

- A. Dorsal Tail Fin – anterior extension of the tail bud.
- B. Spinal Cord and associated organs – as described previously.
- C. Hindleg Bud – median swelling on the posterior region of the embryo.
- D. Blood Vessels
 - d.1. Posterior cardinal vein – paired vessels seen as round structures below dorsal aorta.
 - d.2. Caudal Artery – blood vessel just below the notochord.
 - d.3. Caudal Vein – blood vessel just below the caudal artery.

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- E. Cloaca – posterior chamber of the hindgut with elongated lumen and simple lining of cells.
- F. Ventral Tail Fin – posterior extension of the tail bud. Both dorsal and ventral fins contain nerves and blood vessels and are derived from the posterior growth of the myotomes.
- G. Proctodeum – narrow groove of the ectoderm ventral to the cloaca.
- H. Anal Plate – fused ventral endoderm and ectoderm at the region of proctodeum.



Level of olfactory placode

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Level of the optic cup



Level of the heart

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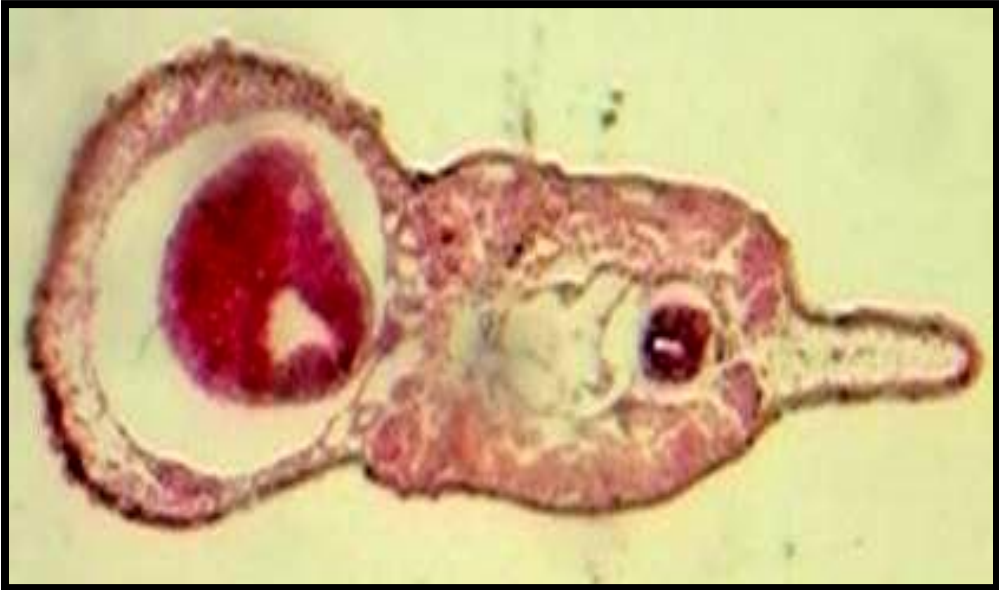


Level of the glottis



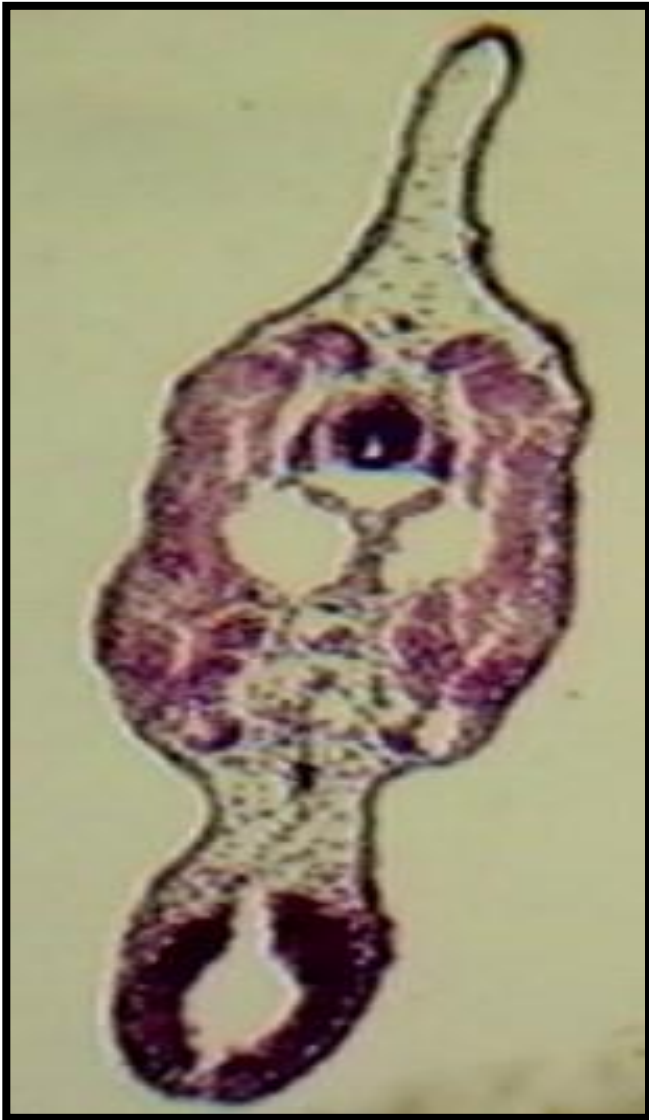
Level of the lung bud

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Level of the mesonephric duct

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Level of the proctodeum