

BIOLOGY 340
Comparative Embryology

Laboratory Exercise 2
Laboratory Examination of an Amphibian:
The Frog

Introduction

You are about to begin an examination of one of the classic animals in comparative embryology. The aquatic nature and ease of breeding in frogs have made them a favorite of developmental biologists for over a century. In this laboratory, you will not be examining live specimens; rather, you will be examining fixed specimens. This is obviously at some level artificial, as it allows the instructor to present only ideal sections for examination. However, this is an excellent introduction to the early stages of a vertebrate before you go through the examination of serial slices of reptiles/birds and (latter) mammals in subsequent sections of the course.

As before, when starting this laboratory, be sure to review with your laboratory partners (and the laboratory instructor if necessary) proper care and use of compound microscopes. As always, be sure to keep scopes and objectives clean, and never, never, never focus down into a slide. Much pain and suffering awaits the student from whose station the instructor hears the telltale “crack” of a slide.

Materials

A small box containing eighteen prepared slides of frogs ranging from tissue samples of the testes and ovaries of adults, to eggs, to free-swimming 10mm larvae is available for examination. Each group of students should take one of these along with a microscope for use with the slide.

Important!!!

DO NOT move slides from one slide set to another. Each set is a group of coordinated slides. It is imperative that you do not mix slides between their original boxes.

Hints

The common tendency is to crank the illumination way up and open the microscope’s diaphragm entirely. Remember that these are very small organisms, easily washed out of view from too much illumination. Start on low to medium levels of illumination to find structures in the embryo slices. Additionally, this can sometimes give a slightly greater depth of field for viewing.

However, as these are histological slices, you will not be presented with the same three-dimensional difficulty as with the sea urchins. Thus, you will be much more likely to see

everything in a particular view of an organism in one focal plane. Nonetheless, you may have to focus up and down minimally with the fine focus to see all structures. This reinforces one of the great exercises of comparative biology – the understanding of three-dimensional organisms from a series of two-dimensional images.

Examination of Frog Embryos

Note that the preserved slides include a mix of all different stages illustrated in your laboratory manual. You will have to scan around the area of the slide, as well as up and down through differing focal planes to see all that your slide has to offer. Do not hesitate to view those of your fellow students if they find a particularly well-defined example.

Unsegmented Egg

See figure 6.26A in your laboratory manual for an example. Frog eggs at this stage are normally surrounded by a thin **VITTELINE MEMBRANE** and a series of **JELLY MEMBRANES**. The methods of sectioning the embryos of this stage do not preserve the jelly membranes. However, the thin red line you can see at the edge of the egg is the demarcation of the vitelline membrane.

Early Cleavage Stages

See figure 6.26B-D in your laboratory manual for examples. In slides labeled early cleavage stage, you should be able to see between two and six-to-eight cells. Note that cleavage is complete between individual cells. Despite the **MESOLECITHAL** nature of frog eggs, they still go through **HOLOBLASTIC CLEAVAGE**. Because of the method of mounting the slice of the egg on the slide, they are often distorted, and stretched from one side to another.

Later Cleavage Stages

See figure 6.26E-G in your laboratory manual for examples. In slides labeled later cleavage stage, you can usually see a slice of an embryo demonstrating between six to ten cells. This means that the entire embryo has many more than that in the entirety of its sphere. Sometimes embryos in these slides are verging on the blastula stage and thus demonstrate the beginnings of the internal fluid-filled blastocoel. Note whether any of the cells look significantly larger than any of the others. This would be an indication that you can see some cells of the **VEGETAL HEMISPHERE** as opposed to cells of the **ANIMAL HEMISPHERE**.

Exercise 340-2-1: In the space provided below, draw representative examples of embryos of the 8-16 cell stage.

Blastula Stage Embryos

Take out the slide in your set marked “Frog blastula typical sagittal section”. Only one embryo will be on each slide, so it should be fairly easy to find. Although the **BLASTOCOELE** will be clearly visible in your section, note that it is asymmetric in its placement. Unlike the condition in sea urchins and amphioxus where the blastula is surrounded by a single layer of cells, the walls of the amphibian blastula are multicellular and the blastocoele is eccentrically placed. Cells toward the vegetal pole of the embryo are markedly larger than those toward the animal pole. As the process of gastrulation will begin with blastopore formation in the endoderm of the vegetal pole, it will necessarily proceed relatively more slowly than the condition in sea urchins or amphioxus. Figures 6.26H-I and 6.27B-D will aid in your examination.

Exercise 340-2-2: In the space provided below, draw the blastula stage embryo of a frog.

The Gastrula

Using the slides labeled “Frog crescent blastopore section” and “Frog yolk plug typical sagittal section”, examine the early **GASTRULA** stage. Figure 7.19C-I and 7.20B-F will aid in your examination.

The large yolky cells of the prospective endoderm prevent the large-scale invagination that we saw in sea urchins and amphioxus. Instead, the blastopore of the frog makes its first appearance as a shallow indentation. (As in amphioxus, the blastopore does appear in the region of the prospective endoderm.)

Note the materials that lie on the surface of the blastula that are destined to move inwards through the blastopore: prechordal mesoderm (which will give rise to parts of the braincase), notochordal mesoderm, lateral mesoderm, and endoderm. Epidermal ectoderm will remain outside to give rise to skin structures.

The distribution of endoderm viewed in sagittal section reveals a large **YOLK PLUG** plugging the blastopore. Identify the yolk plug in the blastopore, and at each edge of it find the **DORSAL LIP OF THE BLASTOPORE** and the **VENTRAL LIP OF THE BLASTOPORE**. At this stage, mesodermal materials move in at the dorsal lip and ventral lip. The space opening to the dorsal lip of the blastopore is the **ARCHENTERON**. Note, as the archenteron expands in volume the remaining volume of the blastocoel decreases. Remember, this is a sagittal view. In other words, it is a midline section that runs from head to tail. Thus, it is running the length of the developing gut tube.

Exercise 340-2-3: In the space provided below, draw a diagrammatic illustration of the early gastrula showing the yolk plug and developing gut. With the aid of Figure 7.19 of your lab manual, color code the endodermal, mesodermal, and ectodermal components of the embryo. The endoderm will give rise to the lining of the gut. Ectodermal derivatives include the skin (epidermal ectoderm) and the neural tube (neural ectoderm). Mesoderm will give rise to the serially segmental somites as well as the lining of the coelom.

Early Development of the Neural Plate

Using the slide labeled “Frog neural plate typical cross section”, you will now turn to a transverse section of the frog embryo. This slide will give you a simple introduction to the transverse section. Using figure 8.16, identify the space of the archenteron, the yolk-laden endodermal cells ventral to the archenteron, and the surface ectoderm.

Next the surface ectoderm of the dorsal midline will begin to thicken as the **NEURAL PLATE**. To see this more clearly, use the slide labeled “Frog Neural Fold typical cross-section”. This slide will include at least three or four different slices of an embryo that show the neural thickening much more dramatically. Use figures 8.15 and 8.16 of your lab manual to aid in the identification of the **NEURAL FOLD**. The neural fold is a result of differential growth and upward movement of the **NEURAL ECTODERM**.

Just ventral to the developing neural fold, the **NOTOCHORD** has now become clearly definable. It appears as a solid circle. Consider what this means in three dimensions. A series of solid circles stacked together indicate that the notochord is in fact a rod in three dimensions. If you have difficulty conceptualizing this, see figure 8.21. The notochord is flexible from side to side, but (as in amphioxus and all other chordates) prevents telescoping of the embryo.

Just lateral to the notochord on each side, you should now be able to distinguish a block of mesodermal tissue that then tapers more distally to surround the upper part of the gut tube. The blocks on either side of the notochord are **SOMITES**, whereas the mesoderm that tapers off more ventrally and distally is the **LATERAL PLATE MESODERM**. Figure 3.7 will help you to see the different, discrete mesodermal structures. As you scan back and forth from one slice to another, you may notice that the somites change in size, and sometimes don't appear visible at all. This is because they are **SEGMENTAL STRUCTURES**. That is serially repeating structures. As serially repeating structures, you may occasionally be between somites in certain sections. Contrast this with the notochord or the neural fold. These are both continuous, or **TRANS-SEGMENTAL STRUCTURES**.

To view the completion of neural tube formation, use the slide labeled “Frog neural tube typical cross-section”. In this section, the neural fold will have zipped up dorsally, and where like tissue has touched like tissue, will have fused to form the **DORSAL HOLLOW NERVE TUBE** (or more simply, **NEURAL TUBE**). Ventral to this you should still see the notochord, the lateral plate mesoderm flanking the gut tube, the yolk-rich endodermal lining of the gut tube, and the epidermal ectoderm. The epidermal ectoderm now covers the outer surface of the embryo now that the neural tube has closed and sunk ventrally into the embryo.

Exercise 340-2-4: In the space below, diagram the process of closure of the neural fold into the neural tube. Be sure to label other structures too, including the notochord, the lateral plate mesoderm, the endodermal lining of the gut tube, and the epidermal ectoderm.

Further Development of the Frog Embryo

Using the slide labeled “Frog 4 mm embryo typical cross section”, you will now investigate the development of the neural crest cells, further differentiation of the somites, the gut tube, and some elements of the head. The slide should include four to five slices from a single individual. Figures 8.23E-I will aid you in your identifications.

Immediately dorsal to the dorsal hollow nerve cord, right at the junction where it detached from the epidermal ectoderm, you should see loose, mesodermally arranged cells streaming down to either side. These are the NEURAL CREST CELLS (see figure 8.23D-F particularly).

As in earlier stages, as you scan back and forth from one slice to another, you may notice that the somites change in size, and sometimes don't appear visible at all. Recall that this is because they are segmental structures – serially repeating structures. As serially repeating structures, you may occasionally be between somites in certain sections.

If this set of slices includes a cut through the head region, you might be able to see the olfactory capsule of the developing nose or the retina and/or lens of the developing eye.

Sagittal Section of the 4mm Frog Embryo

Using the slide labeled “Frog 4mm embryo typical sagittal section” and Figures 8.21 and 8.22 of your laboratory manual, identify as many structures as possible. Be sure to try to distinguish between segmental and trans-segmental structures. Of the trans-segmental structures, attempt to identify – from dorsal to ventral – the dorsal hollow nerve cord, notochord, gut tube, and the yolk-laden cells lining the ventral margin of the gut tube.

Segmental structures will not be visible in the mid-sagittal section. However, in parasagittal sections, you should be able to see some of the repeating somites.

7 mm and Larger Embryos

Although your laboratory manual doesn't include diagrams of more advanced stages, your experience from previous parts of this exercise will allow you to identify at least some of the structures of 7 mm and 10 mm embryos.

Sagittal sections of the 7 mm embryo will demonstrate further development of the postcranial structures. You should still be able to find the dorsal hollow nerve cord, notochord, gut tube, somites, and neural crest tissue. In some fortuitous sections, you might be able to see a branch coming out of the dorsal hollow nerve tube laterally. This would be a segmental spinal nerve.

7 mm embryo slides usually include some sections through the head. The dorsal most tube remains the dorsal hollow nerve tube, though in the head it's known by its more common name for the cranial outpost of the tube – the **BRAIN**. The next big tube immediately ventral to the brain will be the **PHARYNX**. If you can spot a large condensation of cells just below the surface of the skin, it is most likely the **OTIC CAPSULE**. If you spot a condensation of cells formed into a disc and clasped by a cup of more medial cells, these are probably the **LENS** and **RETINA** of the eye respectively.

If you see a series of bits of tissue that appear to be external to the head proper itself, you are seeing a section through the larval **EXTERNAL GILLS**.

In sagittal section, you should be able to identify all of the structures you saw in the 4 mm sagittal embryo. Additionally, you should be able to see the thin membrane at the prospective mouth, or **STOMADEUM**, just before it breaks through.

In the case of 10 mm embryos, continue to practice finding slices through both segmental and trans-segmental structures when observing the transverse sections. Wherever possible, try to determine not only the structure you are observing, but also the embryological derivation in terms of germ layer. In sagittal sections, find all of the same (but now larger) structures you found in the 7 mm embryo. By this stage, you should see that the **MOUTH** has broken through. The large mass of dense, epithelial tissue visible ventral to the gut tube is the developing **HEART**.