**Cell and Organismal Biology**

***Early Amphibian Development-Practical 6***

Group A – March 7th, 10.15am-1.15pm, Biolab 2

Group B – March 8th, 10.15am-1.15pm, Biolab 2

**Purpose of practical**

To examine the structure of developing amphibian embryos

You will need a lab coat and pencils. You will be working in groups of four students.

**Assessment**

This is an assessed practical and counts for 2% of the overall module mark. The assessment will by a test on the VLE, which will be available from March 9th until 4.30 PM on March18th. If you are unable to attend the practical or carry out the assessment you may apply for mitigating circumstances via the normal route. In the absence of valid mitigating circumstances non-attendance or non-completion of the assessment will result in a zero mark.

**Introduction and Background**

The African clawed *Xenopus* frogs have been used in labs for many years. In the 1940s the females were used as part of a pregnancy test. In recent decades they have become a standard model for studying signalling processes in early development. They have a number of advantages as a model for studying development.

**Advantages**

* The adults are very easy to keep in a laboratory environment.
* The female can be induced with hormone injection to produce eggs all year round.
* The female can lay many eggs in a spawning.
* The eggs and embryos are large enough to allow discriminating micromanipulation, including ‘cut-and-paste’ experiments and microinjection.
* The embryos develop outside of the mother.
* The early stages of development are very rapid.
* The embryos can be grown at a wide range of temperatures (12OC to 25OC)
* Many developmentally important genes have been identified using *Xenopus* embryos.
* The *Xenopus* genome has been sequenced.



 ***Adult female Xenopus* *The life cycle of Xenopus***



***Developmental stages of Xenopus***

**The egg**

Characterized by a pigmented animal hemisphere and an unpigmented vegetal (not vegetable!) hemisphere.

**Cleavage stage**

Following fertilization the embryo undergoes a rapid set of cell divisions eventually giving rise to a hollow ball of cells known as the blastula. The cavity in the blastula is known at the blastocoel.

**Gastrula stage**

The specification of the three primary germ layers (ectoderm, mesoderm and endoderm) takes place in late blastula and early gastrula stages. The gastrula stage is characterized by complex cell movements which result in the endoderm and mesoderm moving inside the embryo and the ectoderm covering the outside of the embryo. Cells move inside the embryo via a structure known as the blastopore lip.

**Neurula stage**

During the neurula stage the dorsal ectoderm (neural plate) rolls up and forms the hollow dorsal nervous system that is present in all animals with backbones.

**Tailbud stage**

At the end of neurula stages the dorsal-ventral (back to front) and anterior-posterior axes (head to tail) of the embryo. The rudiments of all the major organs are present. The tailbud stage is characterized by differentiation of the cells in the major organs and elongation of the anterior-posterior axis of the embryo.

**Tadpole stage**

The major organ systems are all present and differentiated. The tadpole continues life as a feeding, free living organism.

***Question 1***

Why is it an advantage for developmental studies that the embryos develop outside of the mother?

***Question 2***

Why is it an advantage that the *Xenopus* genome has been sequenced?

**Task 1 Examination of cell cleavage in early development**

*Xenopus* eggs are fertilized in vitro using a suspension of sperm. After fertilization the thick jelly coat surrounding the zygotes is removed using a chemical treatment that breaks the disulphide bonds in the jelly protein. Removal of the jelly coat enables the embryos to be manipulated more easily. At room temperature the first cell division takes place after 1.5 to 2 hours following fertilization and culture. *Xenopus* embryos are cultured in simple salt solutions and can survive for many days without the need for food and nutrients.

***Question 3***

How can amphibian embryos survive for long periods without nutrients in the culture medium?

Eggs will have been fertilized 1 hour before the start of the practical.

* You will be provided with a small number of fertilized eggs by the demonstrators.
* Examine the fertilized eggs under the stereomicroscope. It is suggested that you look at the developing embryos every 10 minutes or so throughout the practical lesson.
* Record the time when each cell division begins
* In the space provided below draw pictures of the embryos after the first, second and third cell division.

***Question 4***

What is the length of the cell cycle in the early embryo?

***Question 5***

Does the volume of individual cells grow during early cleavage stages of amphibian development?

**Task 2 Examination of fixed specimens from different stages of development**

A number of fixed specimens of embryos from different stages of development will be provided in an agarose coated Petri dish.

* Draw and attempt to identify the developmental stages provided in the space provided below.

**Task 3 Examination of cleavage stage embryos**

***Question 6***

How many cells are present in your cleavage stage embryo?

***Question 7***

How many rounds of cell division were necessary to give rise to this number of cells?

**Task 4 Examination of late blastula stage embryo**

***Question 8***

Are the cells bigger in the animal hemisphere or vegetal hemisphere?

* Under a microscope, use the blade provided to cut open the embryo along the animal to vegetal axis.
* In the space below draw what you see and label the blastocoel cavity and cells of the animal and vegetal hemispheres.

**Task 5 Examination of gastrula stage embryos**

* Draw a vegetal view of the gastrula stage embryos.
* Label the structure where cells are moving into the interior of the embryo (blastopore lip).
* With the blade provided bisect the embryo all the animal to vegetal axis.

***Question 9***

How does the internal structure of the gastrula differ from that of the blastula stage embryo?

**Task 6 Examination of neurula stage embryos**

* Attempt to identify the developing nervous system in the neurula stage embryos and label this on your drawing of the neurula stage embryo.

**Task 7 Examination of tailbud stage embryos**

During tailbud stages tissue differentiation begins. The structure of the trunk and tail is dominated by repeated, arrow shaped structures called somites. Somites are present in all vertebrate embryos and give rise to the axial skeleton (ribs and vertebrae) and all skeletal muscle of the adult.

* Identify the somites on you picture(s) of tailbud embryos.
* Label the anterior and posterior ends of the tailbud embryo.
* Under the microscope, use the scalpel to cut a transverse section through the mid-trunk of the tailbud embryo (see below). Using the needle flip the trunk section on end and observe.
* Attempt to identify the structures shown in the diagram below.



 ***Transverse section through a tailbud stage embryo***

**Task 8 Examination of a tadpole stage embryo**

* Attempt to identify the following structures in the tadpole
1. The mouth
2. The anus
3. The gut
4. The lens
5. The somites
6. Pigment cells

**Task 9 Examination of gene expression patterns in fixed specimens**

In situ hybridization is a sensitive technique for determining where and when a gene is active during development. The technique allows an investigator to detect the presence of a given messenger RNA (mRNA) within the cells of the embryo. The presence of the mRNA is visualized by a coloured precipitate. A number of in situ hybridization specimens will be provided in for you to examine.

***Brachyury***

The *Brachyury* gene codes for a T-box transcription factor and its expression is activated by signalling by TGF-beta family proteins such as nodal.

***MyoD***

The *MyoD* gene codes for a basic helix loop helix transcription factor which regulates the expression of genes coding for proteins such as actin and myosin.

* Draw pictures of the stages provided in the space provided over the page.

***Question 10***

Which of the germ layer is expresses *brachyury* in gastrula and early neurula stage embryos?

***Question 11***

What does this technique tell you about the localisation of the brachyury protein within the developing embryo?

***Question 12***

Where is the *MyoD* gene expressed in the developing embryo?

***Question 13***

What cell-type in the adult do you predict is derived from *MyoD* expressing cells in the embryo ?

**Task 10 Examination of display items**

Please take some time to look at the adult *Xenopus* on display. There will also be a video video demonstration of a living late stage tadpole and some twinned tadpoles.

* Look for the beating heart and circulatory system of the tadpole.

**Question 14**

What famous embryological experiment results in the formation of a twinned embryo similar to the ones on display?