Int. J. Dev. Biol. 47: 153-160 (2003)

Using *Xenopus* as a model system for an undergraduate laboratory course in vertebrate development at the University of Bordeaux, France

MICHELLE OLIVE[1,4], PIERRE THIEBAUD[1,4], MARC LANDRY[2,4], MICHEL DUVERT[1,3,4], ALAIN VERNA[3,4], WILFRID BARILLOT[1,4] and NADINE THEZE[1,4,*]

[1]INSERM U441, Pessac, France, [2]INSERM EPI 9914, Bordeaux France, [3]SERCOMI and [4]UFR SDV, University Victor Segalen Bordeaux 2, Bordeaux, France

ABSTRACT The goal of this laboratory course is to introduce vertebrate developmental biology to undergraduate students, emphasizing both classical and contemporary aspects of this field. During the course, the students combine the use of living *Xenopus laevis* material with active tutorial participation, with the aim of illustrating how the fertilized egg can generate the diversity of cell types and complexity of pattern seen only a few days later in the embryo. Special emphasis is given to the observation and manipulation of living material. The laboratory course includes a comprehensive analysis of both oogenesis and early development and is divided into two overlapping parts that combine tutorial and practical approaches. The first part is devoted to oogenesis; oocytes are sorted out, allowed to mature *in vitro* and observed in histological section. In the second part, students perform an *in vitro* fertilization of *Xenopus* eggs and a mesoderm and neural induction assay of animal cap explants. Successful induction of the explants is confirmed by morphological, histological and molecular analyses. Finally, the students observe and comment on selected slides to illustrate the organization of the body plan of the amphibian embryo at an early stage of organogenesis.

KEY WORDS: *oogenesis, embryogenesis, body plan organization, histology, Xenopus laevis*

Background Information

Scholarly Interests of the Authors

We have been working for several years on muscle differentiation, focusing on the structure, expression and regulation of tropomyosin and myosin genes in *Xenopus laevis*. We have been involved more recently in a project based on smooth muscle cell differentiation during development. We are trying to understand the molecular and cellular mechanisms that underlie the reversible switch between undifferentiated mesodermal cells and smooth muscle cells. Another aspect of our work is to compare smooth muscle and cardiac phenotypes with emphasis on the identification of factors that can control marker genes in each cell type. Our approaches combine molecular techniques (cloning and analysis of specific markers) with developmental approaches (animal cap assay, overexpression of proteins in embryo, transgenesis, etc.).

Representative Publications

GAILLARD, C., THEZE, N., HARDY, S., ALLO, M.R., FERRASSON, E. and THIEBAUD, P. (1998). α-Tropomyosin gene expression in *Xenopus laevis* : Differential promoter usage and controlled expression by myogenic factors. *Dev. Genes Evol.* 207: 435-445.

GAILLARD, C., LERIVRAY, H., THEZE, N., COOPER, B., LEPETIT, D., MOHUN, T. and THIEBAUD, P. (1999). Differential expression of two skeletal muscle β-tropomyosin mRNAs during *Xenopus laevis* development. *Int. J. Dev. Biol.* 43: 175-178.

THEZE, N., HARDY, S., WILSON, R., ALLO, M.R., MOHUN, T. and THIEBAUD, P. (1995). The *MLC1f/3f* gene is an early marker of somitic muscle differentiation in *Xenopus laevis* embryo. *Dev. Biol.* 171: 352-362.

General Teaching Philosophy

Teachers need always meet the eager demands of students for practical courses that extend the theoretical concepts that they have been taught. Thus, it is important to show the experimental basis of theoretical approaches. Practical courses are intended not only to develop laboratory skills, but also to cause students to question natural phenomena and give them matters on which to think further. The general philosophy of the practical course described here is based on this idea. We wanted by means of this course to shift students from an inert listening status to active participation. Developmental biology is never so fascinating as when one is observing living material and exploring its entire dimension through experimental embryology. By working with living material and embryos, the students realize that modern

***Address correspondence to:** Dr. Nadine Thézé. INSERM U441, avenue Haut Lévêque, 33600 Pessac, France. Fax: +33-5-5636-8979. e-mail: nadine.theze@bordeaux.inserm.fr

0214-6282/2003/$25.00

Printed in Spain
www.ijdb.ehu.es

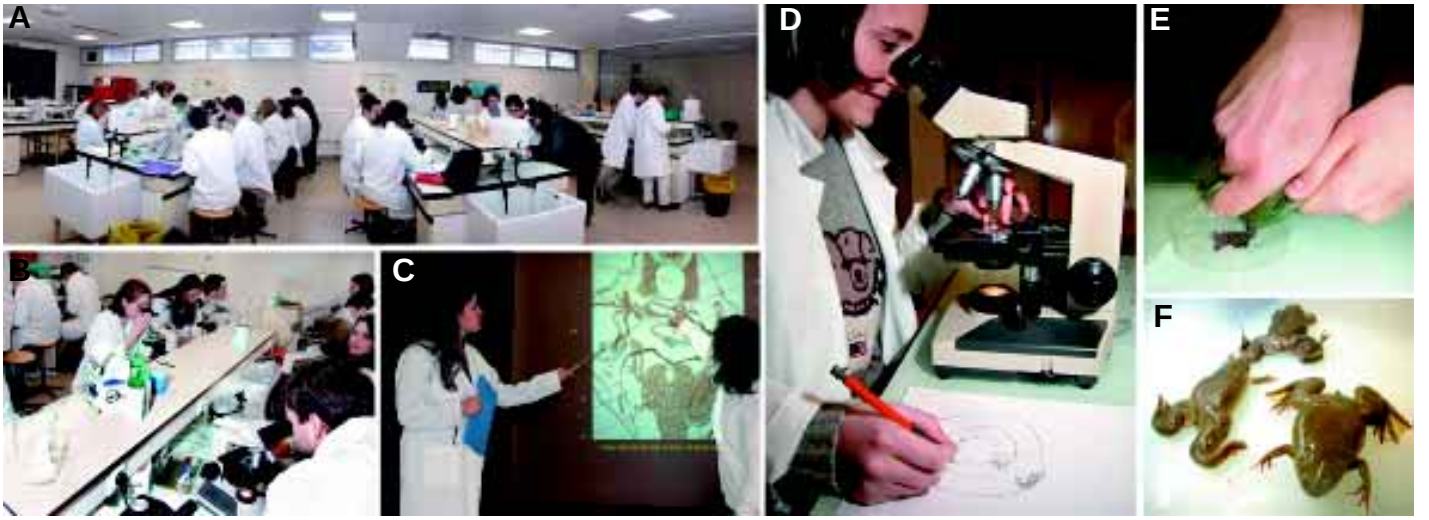

Fig. 1. Laboratory showing students participating in various activities. (A) *General view of the classroom with students.* **(B)** *Students working at the bench.* **(C)** *Students presenting slides of histological sections.* **(D)** *A student drawing while observing at the microscope.* **(E)** *A student collecting eggs for* in vitro *fertilization.* **(F)** Xenopus laevis *females.*

molecular tools are useless unless accompanied by observations and descriptions of natural phenomena, and the expertise of experimental embryology. They also come to understand how important were the contributions of the early embryologists. An amphibian model such as *Xenopus laevis* is suitable for such an endeavor. With this course, we aim to help students realize that this model makes it possible, through various approaches, to ask questions about fundamental processes of development in areas ranging from biochemistry and cellular and molecular biology to histology and physiology. Finally, this model system can be used to give students a good grasp of how theoretical principles can be subjected to experimental analysis.

We have at present a wealth of knowledge about *Xenopus laevis.* This amphibian offers us a remarkable opportunity to decipher the multiple processes that are involved in the building of a chordate or, more specifically, a vertebrate body plan. In less than two days, the structureless egg is converted into an embryo showing the basic vertebrate plan with the placements of future organs already determined. The student, through observation and manipulation, is urged to reflect on the numerous intra- and intercellular and tissular events operating during oogenesis and embryogenesis that gradually form the basic body plan of the embryo. These events include maternal determinants, cell-cycle molecules, cell-cell interactions and morphogenetic movements.

Introduction

This practical course is designed for the junior (third year) undergraduate level and is part of a unit called “Cell Biology and Development,” which represents 1/5 of the degree “Licence de Biologie Cellulaire et Physiologie.” The Cell Biology and Development unit comprises 100 h divided into lecture (48 h), tutorial (22 h) and laboratory (30 h), which includes 15 h for this practical course on development. To earn credit for this unit (9 ects), students must receive a grade of 10/20 or higher. The practical course is tightly coordinated with the lecture content and the tutorials.

Before taking the unit, students must have completed various courses in biology, including cell biology, genetics, molecular biology, descriptive embryology, histology, and physiology. As sophomore students (DEUG 2 level), they complete practical courses that introduce to them the histology of adult tissues and histochemistry techniques. They also complete a course on the descriptive embryology of invertebrates, prochordates, fish, and mammals relying mainly on observations of microscope slides. This Cell Biology and Development unit gives the students the necessary basis for a unit that they will take the following year, as senior undergraduates, called “Developmental Genetics,” which emphasizes gene function during the development of organisms.

The practical course of the unit Cell Biology and Development meets for two days in a specialized room equipped for the various observations and manipulations. Stereomicroscope projections and other images stored on the teacher’s computer can be viewed by means of a video camera and projector. The instructional technology used during the course includes Microsoft PowerPoint presentations, overhead projector transparencies, slides, and movies, as well as the chalkboard and Internet resources). Approximately 100 students are enrolled in the unit each year, and they are split into four groups for the practical courses. Three faculty members supervise the running of the course.

Course Structure and Content

The two-day course is tightly scheduled, especially the first day. During that day, lectures given by the teacher are interspersed with observation and manipulation of living material by the students. The time between the different manipulations of living material and the collection of data, often several hours, is used for other planned activities.

To make the organization of the practical course easy to understand, we will describe the schedule followed by the students and indicate by different symbols their activities at each step of the course:

- Listening to the teacher present the course topics and philosophy and describe the material available for student use
- Observation of living material and/or histological slides
- Practical manipulations

Day 1 - Morning

First, the teacher introduces the general topics of the course and its schedule. A detailed syllabus and course synopsis is given to each student. It includes a comprehensive description of the material and the methods the students will use, together with documents on oogenesis and embryo morphology and normal developmental tables of *Xenopus laevis.* The amphibian most widely used today in developmental research is the African clawed frog, *Xenopus laevis*. It is commercially available and easily maintained and produces a large number (1000-2000) of eggs in response to hormone stimulation. Its embryos develop rapidly and synchronously (hatching in 37 h at room temperature), and its eggs and early embryos are large enough in size for surgical manipulations. Embryos and isolated embryonic tissues can also be easily cultured for a minimum of several weeks. Fertilized eggs can be obtained throughout the year by using *in vitro* fertilization. Amphibian embryos provide excellent material for understanding the establishment of the vertebrate body plan during early development.

In the classroom (Fig. 1A), a pair of students is assigned to each bench, and the teacher shows them the various materials and solutions at their disposal.

Each pair has available the following equipment: dissecting microscope (LEICA, 10-50x magnification), light microscope (Olympus, 10, 40 and 100x magnification), stainless-steel forceps Dumont 5 (Moria, Paris), needles (25G × 5/8"), Gilson Pipetman (P1000, P200 and P20), 1% agarose-coated dishes, plastic beakers, tubes, Pasteur pipettes, needles and gloves, as well as equipment for mini-agarose gel electrophoresis analysis of RT-PCR products.

The solutions used during the course are made by the teacher and kept at the appropriate temperature: cysteine (Sigma C7755), 2% solution prepared freshly in 1xMBS (88 mM NaCl, 1 mM KCl, 1 mM MgSO4, 5 mM HEPES, pH 7.8, 2.5 mM NaHCO3, 0.7 mM CaCl2, stored at 4°C); human bFGF (Life Technologies, 13256-029), 100 ng/µl in PBS, stored at -20°C; human activin A (R&D Systems 338-AC-005), 20 ng/µl solution in PBS, stored at -20°C; caffeine (Sigma C8960), 10 mM in water, stored at - 20°C; BSA Fraction V (Sigma A7906), 10% solution in water, stored at -20°C; progesterone (Sigma P0130), 10 µg/ml in ethanol, stored at 4°C; gentamicin (Life Technologies 15750-037), 50 mg/ml stored at room temperature; collagenase (Sigma C9891), 15 mg/10 ml in 1xMBS solution made freshly; RNA extraction kit (AMBION); and products for RT-PCR analysis.

In Vitro *Fertilization*

The teacher presents a short introduction on *in vitro* fertilization that highlights the ease with which it is possible to obtain a great number of eggs that develop synchronously and rapidly so that the interesting stages of axis formation and tissue differentiation are accessible in a relatively short time. While the first axis (animal-vegetal) of the embryo is prefigured in the oocyte, the second axis is determined at fertilization; the side opposite the sperm entry point is the future dorsal side of the embryo. The contact of sperm with the egg leads to the egg's activation. After a transient contraction wave of the cortex, the cortical granules discharge their glycoprotein content into the perivitelline space by exocytosis. The egg can then reorient itself in the perivitelline fluid. Because of the high content of yolk platelets in the vegetal hemisphere, the animal pole points upwards. The students' objective is to analyze descriptively the early phases of development and to obtain embryos for the animal cap assay.

The teachers previously obtained *Xenopus laevis* females and males from CNRS husbandry in Montpellier (France) and allowed them to recover for 2 weeks prior to being used for experimentation. Three females (Fig. 1F) and one male are used for each laboratory course. Most of the manipulations to obtain eggs and sperm for *in vitro* fertilization are described in the handbook *Early Development of Xenopus laevis* by Sive *et al.*, 2000. The stimulation of ovulation and testes preparation are performed by the teacher. Briefly, the females are primed with 50 units of human chorionic gonadotropin (hCG, Chorulon) the week before the course. Ovulation is induced by injection of 500 units of hCG in the dorsal lymph sac of the female on the evening before the course. The frogs are kept at 20°C overnight in a small water tank, and they shed eggs 10 to 12 h after stimulation.

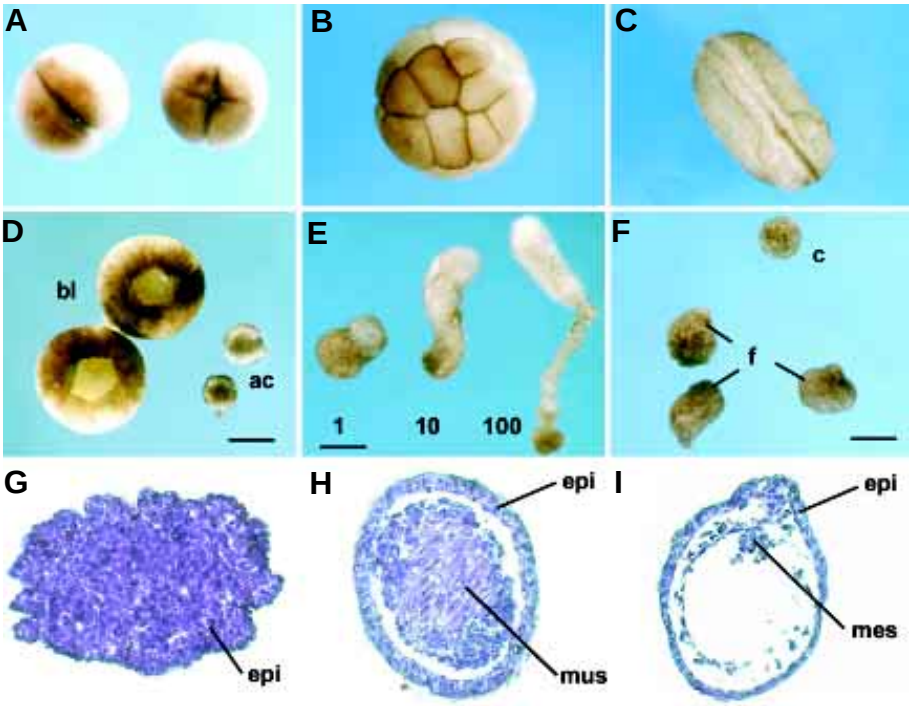

Fig. 2. Early embryos and animal cap explants obtained by students. (A) *2-cell and 4-cell embryos viewed from the animal pole.* **(B)** *16-cell embryo.* **(C)** Neurula-*stage embryo viewed from the dorsal side.* **(D)** *Animal cap (ac) explants dissected out from* blastula *embryos (bl).* **(E)** *Animal caps treated with 1 ng (1), 10 ng (10) or 100 ng (100) of activin.* **(F)** *bFGF-treated (f) and control (c) animal caps.* **(G)** *Histological section of an animal cap control.* **(H)** *Histological section of an activin-treated animal cap.* **(I)** *Histological section of a bFGF-treated animal cap. epi, epidermis; mus, muscle; mes, mesothelium. Bar (D-F): 500 µm.*

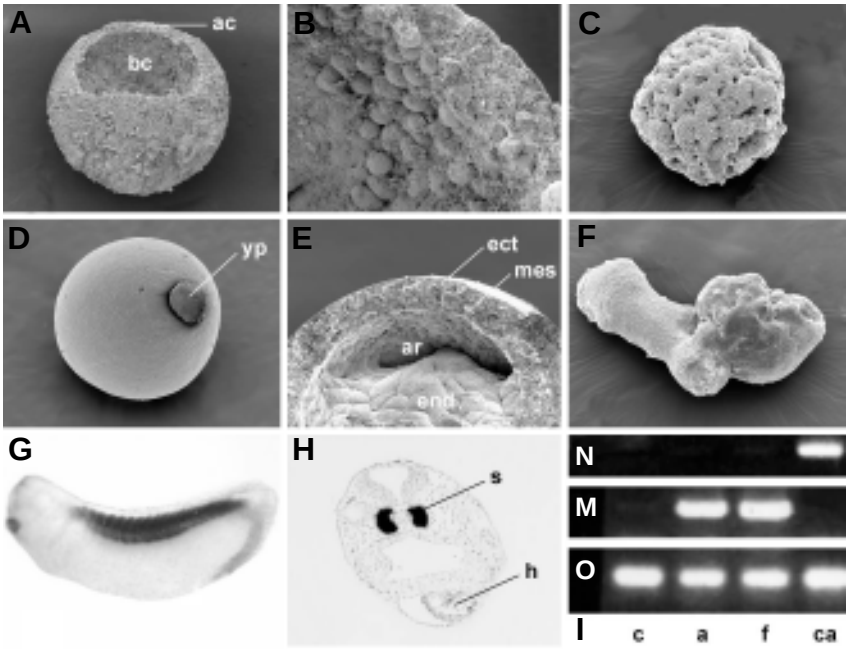

Fig. 3. Embryo morphology, muscle gene expression and animal cap induction analysis. *Scanning microscopy photographs (A-F).* **(A)** *Transverse section through the* blastula *showing blastocoel cavity (bc) and ectodermic animal cap (ac).* **(B)** *Blastocoel roof of (A) at higher magnification.* **(C)** *Control animal cap.* **(D)** *Late* gastrula *showing yolk plug (yp).* **(E)** *Transverse section through the gastrula showing archenteron (ar), ectoderm (ect), mesoderm (mes) and endoderm (end) cells.* **(F)** *Activin-treated animal cap.* **(G)** *Whole-mount* in situ *hybridization of tadpole embryo made with an* MLC1f/3f *mRNA antisense digoxygenin probe.* **(H)** In situ *hybridization, transverse section of tadpole embryo, with an* MLC1f/3f *mRNA* S^{35} *antisense probe. Somites (s) are strongly labeled but not the heart (h).* **(I)** *RT-PCR analysis of animal cap explants treated with activin (a), bFGF (f) or caffeine (ca), or untreated (c). The amplified fragments correspond to* N-CAM **(N)**, MLC1f/3f **(M)** *or* ODC **(O)** *mRNAs.*

Testes are isolated from a male frog, after sacrificing it by submerging it in 0.05% benzocaine, and kept in 1xMBS at 4°C until fertilization.

🛠 Just before collecting eggs, the students cut each testis in half and macerate a bit of a cut half with forceps. The students learn how to perform manual egg collection by using a gentle but firm touch. Eggs are collected in a dry plastic 90-mm dish (Fig. 1E) for no longer than 1 min once every hour for the first 2 or 3 h of shedding. With a pair of forceps, a piece of testis is gently rubbed over the eggs, and after 5 min, the dish is filled with 0.1xMBS. *In vitro* fertilization ensures the synchronous development of the embryos.

👁 Students observe the first sign of fertilization (contraction of the pigmented animal hemisphere) within a few minutes, and less than 30 min later they can see the eggs rotate, in response to gravity, after the swelling of the perivitelline space. Students let the embryos develop for about 1.5 h and then observe their first cleavage with the dissecting microscope. They are able to observe the early embryonic development (Fig. 2 A-C) and compare their observations with scanning electron microscopy photographs of embryos previously prepared by the teachers (Fig. 3 A,B and D,E) and with the normal table of development (Nieuwkoop and Faber, 1967).

During the first 6 h phase of cleavage, the *blastula* emerges as a multicellular morphological entity. The students dissect out the animal caps of the *blastula*-stage embryos (Fig. 2D). *In vitro* fertilization performed later in the day enables the students to observe the *gastrula* and *neurula* stages of the embryo the next day. These stages illustrate the generation of the basic vertebrate body plan (Fig. 2C).

Oocyte Maturation

👂 While they are waiting for the embryos to develop, the students attend a short lecture on oogenesis in the amphibian. The oocyte stores a huge amount of material, such as the nucleoprotein, cytoskeletal proteins, cell-surface proteins and enzymes. The mature *Xenopus* oocyte presents a primary animal-vegetal axis, which formed during oogenesis and is externally visible as a dark pigmented animal pole and a white vegetal pole. Internally, the accumulation of yolk platelets in the vegetal hemisphere leads to the dislocation of the nucleus into the animal hemisphere. Conversely, there is an accumulation of ribonucleoparticles according to a vegetal-animal gradient. The animal-vegetal axis of the oocyte corresponds to the future anteroposterior axis of the embryo. Among the mRNAs accumulated during oogenesis are some, which code for molecules such as growth factors or secreted signalization factors, that have a spatially restricted localization and constitute cytoplasmic determinants that can specify the cell fate of embryonic cells. Fully grown *Xenopus* oocytes are arrested in their development at the late G2 phase of meiosis I and must progress to the second meiotic metaphase before fertilization is possible. Further oocyte maturation is triggered by progesterone and may be viewed as a complex series of events by which a cell makes its transition from the G2 phase to the M phase of the cell cycle.

The teachers prepare ovaries the day before the course from a mature female anesthetized by submergence for 10-15 min in 2% phenoxyethanol. A small incision through the skin and muscle layer of the abdomen is made, and a piece of ovary is removed and placed in 1xMBS and kept at 16°C. Oocytes for experimental use can easily be removed from the ovarian tissue either by dissection or by collagenase treatment. The collagenase treatment is performed by incubating ovaries for 30-60 min in 0.15% (w/v) collagenase in 1xMBS with no calcium. Oocytes are then extensively washed in 1xMBS + calcium. The remaining follicle cells surrounding the oocytes are then manually removed with a pair of forceps. These oocytes are used for both *in vitro* maturation and germinal vesicle extraction.

🛠 Students select 10 healthy stage-VI defolliculated oocytes and incubate half of them in 1xMBS with 10 μg/ml of progesterone while leaving the other half (control) in 1xMBS. The timing of maturation depends on oocyte preparation and may vary between females. In our conditions, we routinely observe maturation within 4-6 h.

👁 Students check the oocytes that have been treated by progesterone from time to time in order to detect the first indication of

maturation, i.e., the appearance of a white spot at the animal pole of the oocyte (Fig. 4D). This white spot corresponds to the displacement of the pigments of the animal pole after dissolution of the oocyte nucleus (also called the germinal vesicle). They have reproducibly found that 50% of progesterone-treated oocytes show a white spot in 4-6 h.

🛠 👁 The students observe the ovaries under the dissection microscope and can visualize the major morphological events of oogenesis. They describe the overall structure of the ovaries: bags of highly vascularized connective tissue with branched structures (Fig. 4A). The ovarian tissue is dissected, and the students sort out the oocytes and order them according to the conventional criteria established by Dumont (1972), who described six stages (I-VI) of oocyte development (Fig. 4C). Small unpigmented oocytes are in early stages of oogenesis (Fig. 4B), while pigmented ones are in late oogenesis stages (Fig. 4D). Special attention is paid to stage-VI oocytes, which show a distinctive pigment pattern with a dark brown animal hemisphere and a white vegetal hemisphere, emphasizing the polarity of the oocyte that originates during oogenesis (Fig. 4D).

🛠 👁 In the immature stage-VI oocyte, the germinal vesicle is always located toward the animal pole, whereas the large yolk platelets are concentrated mainly toward the vegetal pole. The students dissect out the germinal vesicle (GV) by using a fine needle and forceps. While grasping the oocyte with forceps, they puncture the animal pole with the needle and gently press the oocyte to expel the nucleus (Fig. 4E). They are able to observe the size of the nucleus, which is about 10^5 larger than that of a normal somatic tissue cell, and its location close to the animal pole.

Oogenesis

👂 The teacher comments on histological sections projected onto a screen. The goal of these observations is to illustrate via cytology and cytochemistry some of the major features of oocytes and their constituents together with a more precise presentation of the internal structure: lampbrush chromosomes and the mitochondrial aggregate known as the Balbiani ring, visible during the previtellogenic stage; abundant nucleoli; lipid deposition in yolk platelets during late oogenesis; and cortical granules in the peripheral cytoplasm. The size of the germinal vesicle and the lobed appearance of its nuclear envelope can be clearly seen.

The teachers have previously prepared a set of histological sections: Ovaries are fixed in Bouin's fluid solution, embedded, sectioned and stained according to different protocols (trichrome, Unna blue, periodic acid Schiff, and alcian blue) to characterize cytochemically significant molecules and organelles.

👁 The students use the light microscope to make observations (Fig. 1 B,D) of the histological sections, and they must make annotated drawings of the slides. A composite of the slides given to the students is shown in Fig. 5. Students may note, for instance, the modifications to the nuclear envelope and the appearance of extra nucleoli and lampbrush chromosomes in the nucleoplasm (Fig. 5 A-C). They may also observe cortical deposition of pigments and cytoplasmic yolk platelets (Fig. 5 D-F).

Day 1 - Afternoon

Animal Cap Assay

👂 The students listen to a short introduction on the animal cap assay and its usefulness for the study of embryonic induction processes. The basic body plan of the amphibian embryo arises through a sequence of inductive interactions; mesoderm is induced first, followed by neural induction (Gurdon, 1987). The mesoderm arises from cells at the equator of the *blastula* as a consequence of signals produced by vegetal cells during the cleavage phase. This signal was demonstrated by Nieuwkoop, who cultured explants of animal pole, marginal zone, or vegetal pole cells from the *blastula*, alone or in combination. When animal pole cells are cultured alone they differentiate into an atypical type of epidermis, whereas vegetal pole cells become poorly differentiated endoderm. Combinations of animal and vegetal pole cells, however, differentiate into a variety of mesodermal cell types. The animal cap assay constitutes a simple way to test for mesoderm inducers. In this assay, explants of the animal pole are cultured in a well-defined medium containing soluble factors to be tested. Several peptide growth factors belonging to the fibroblast growth factor (FGF) and the transforming growth factor (TGF) families have been identified as strong candidates for the natural *in vivo* mesoderm inducing factor.

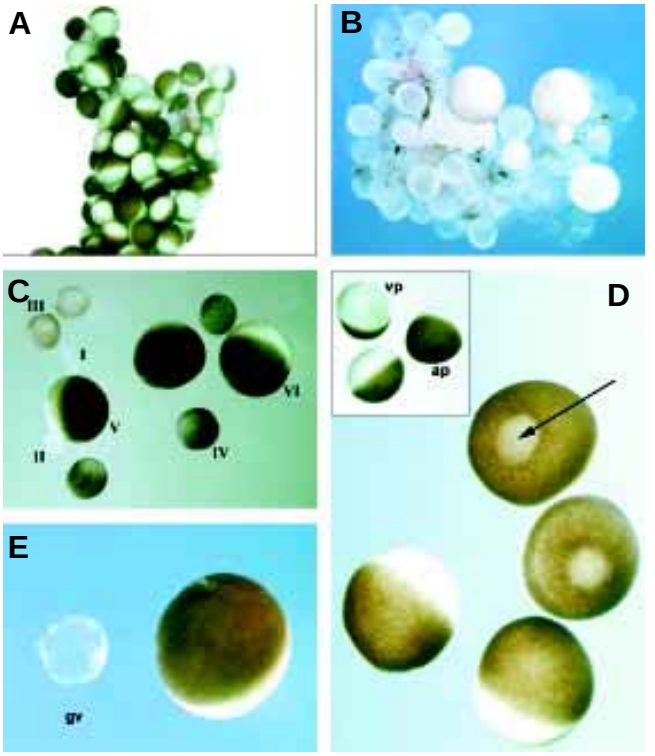

Fig. 4. Observation of *Xenopus laevis* ovaries and oocytes. (A) View of an ovary. **(B)** *Group of early stage (I-III) oocytes.* **(C)** *Stage-I to stage-VI oocytes sorted by students.* **(D)** *Progesterone-treated oocytes showing a typical white spot (arrow) at the animal pole, indicative of maturation. Inset shows animal (ap) and vegetal (vp) pole views of stage-VI oocytes, and an equatorial view.* **(E)** *Germinal vesicle (gv) extracted from a stage-VI oocyte, 1.2 mm in diameter.*

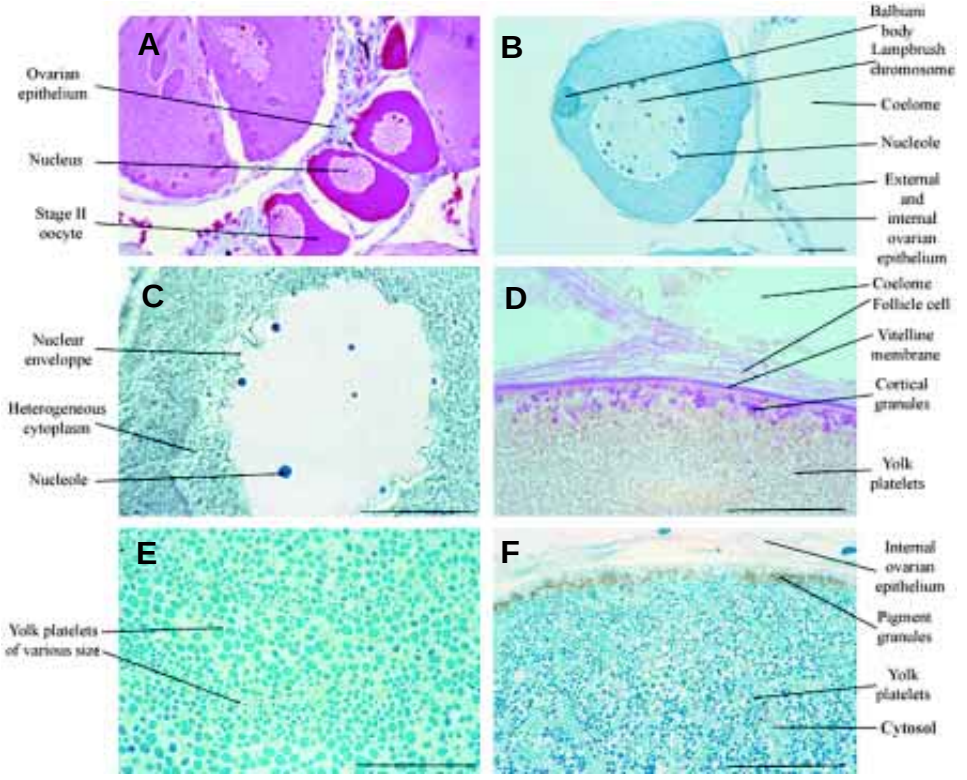

Fig. 5. Histological, cytological and histochemical observations of the principal features of *Xenopus laevis* oogenesis. *Stained, paraffin-embedded* Xenopus laevis *ovary sections.* **(A)** *Trichrome staining showing oocytes at various stages.* **(B,C)** *Unna blue staining showing previtellogenic stage-II oocyte.* **(D)** *P.A.S. staining of a vitellogenic oocyte.* **(E,F)** *Unna blue staining of vitellogenic oocyte. Bars: 100 μm in A-D and F; 25 μm in E.*

An early response to mesoderm induction is a change in shape of the explant, involving elongation and constriction, that mimics gastrulation movements. Induction can also be analyzed at the molecular level by the detection of mRNA of structural muscle genes (for example, myosin or actin), because muscle is the most abundant mesoderm-derived tissue and the most abundant cell type to arise from animal-vegetal cell combinations.

The second major induction that operates in the embryo is neural induction, where the newly formed mesoderm induces ectoderm to differentiate into neural tissue. The animal cap assay can be used to test the neural inductive capacities of various substances. For instance, caffeine treatment of cells, which triggers Ca+ second messenger release, can cause cells to follow a neural pathway. Neural cell differentiation can be monitored by the appearance of N-CAM mRNA, a molecular marker indicative of neural tissue differentiation.

The goal for students is to use an animal cap explant assay to test the inductive capacities of activin and bFGF, two soluble molecules that belong respectively to the TGF and FGF families of growth factors. They also test the effect of caffeine on neural induction. They score mesoderm and neural induction both at morphological and molecular levels, the latter through RT-PCR analysis of muscle or neural gene mRNA. They also make observations of histological sections of animal caps, both induced and not induced by growth factors.

The embryos that have developed from the *in vitro* fertilization performed in the morning reach the *blastula* stage early in the afternoon and are ready for the animal cap assay. The embryo is surrounded by gelatinous membranes (jelly coat) and a tight-fitting vitelline membrane. Any operation on the embryo requires, as a first step, the removal of these membranes. Before the students start preparing the animal caps, the teacher explains to them how to remove the vitelline membrane and excise the animal cap. The explanation is accompanied by a demonstration performed under a dissection microscope on which a camera is mounted and shown on a screen (Fig. 1C). The operation is carried out in 60-mm plastic Petri dishes with their bottoms lined with 1% agarose (dissolved in 1X MBS) to prevent the embryonic tissue from adhering to the plastic surface. The vitelline membrane, which lies close to the egg surface, can be manually removed with a pair of forceps. The embryo is then placed with the animal pole facing up, and the animal cap is dissected out with the forceps. The cap is placed in the dish with the inner surface facing up to preserve its integrity.

🛠 The students dejelly embryos for 5 min in a 2% cysteine solution, pH 8.0, and then carefully rinse them 5 times in a 1-L beaker with tap water before transferring them to a clean dish in 0.1x MBS. Afterwards, they can begin with the dissection. There is variability in the number and healthiness of the embryos, but every student usually dissects out at least 10 caps. Once the students have enough caps to make 4 distinct pools, the caps are transferred to a multiwell plate. Each well has an agarose bed containing either neutral buffer (control) or buffer with either activin, bFGF, or caffeine and 50 μg/ml gentamicin and 0.1% BSA. Each student group uses a different concentration of growth factors so that they can compare among themselves the effect of varying the concentration. If necessary, the teacher helps the students at every stage of the preparation of the animal cap: removal of the vitelline membrane, cap excision, and transfer to the multiwell plate. The caps are left overnight at room temperature and analyzed the second day.

Day 2 - Morning

Animal Cap Analysis

👁 The students examine, under the dissection microscope, the morphological modifications, if any, to the caps. They usually find that activin treatment of the caps induces the explants to elongate more or less while bFGF treatment has a mild effect, producing pear-shaped explants (Fig. 2 E,F). Caffeine-treated explants show no morphological modifications. The control explants maintained in a neutral buffer remain round and resemble a wrinkled ciliated sphere (Fig. 2F). The students compare their observations with scanning microscopy photographs of caps cultured by the teachers (Fig. 3 C,F).

👁 To interpret the results of the animal cap studies, students observe histological sections of the explants that were previously prepared by the teachers. Figure 2 G-I shows examples of sections of explants. The control cap is composed of epidermal cells (Fig. 2G), whereas muscle cells can be identified in the activin-treated cap, and the cells of the bFGF-treated cap have differentiated into typical mesothelium cells (Fig. 2 H,I).

🛠 👁 To analyze changes in the mRNA content of the caps at the molecular level, total RNA is extracted from 5-10 pooled caps of each treatment group and RT-PCR is performed with different primers. Amplified DNA fragments are analyzed by electrophoresis on an agarose gel (Fig. 3I). Because muscle development is the most conspicuous criterion for mesoderm induction, primers specific to the gene *MLC1f/3f*, which is skeletal muscle-specific, are used (Thézé *et al.,* 1995). A PCR analysis is also performed with neural *N-CAM*-specific primers and with ubiquitous primers for *ODC*. *MLC1f/3f* mRNA is specifically detected in activin- and bFGF-treated caps, but *N-CAM* mRNA is detected only in caffeine-treated caps.

Gastrulation

👁 👂 The students watch a 15-min videotape on the early development of the amphibian *Triturus alpestris,* narrated by the teacher, who explains that gastrulation occurs during the early embryogenesis of vertebrates, immediately following the *blastula* stage. More broadly defined, gastrulation is the phase of development during which the three primary germ layers are established: the ectoderm, the endoderm and the mesoderm. Gastrulation involves a series of morphogenetic movements leading to the formation of the vertebrate body plan. Following this short presentation, the students annotate diagrams of various sagittal embryo sections that illustrate the succession of events occurring during gastrulation.

Day 2 - Afternoon

Body Plan Organization

👂 The teacher explains how the body plan of the embryo is sequentially built. By the end of neurulation, the axes of polarities (antero-posterior, dorso-ventral, left-right) have been definitively established, and tissues and organs can now develop in their appropriate locations during the organogenesis phase. The morphological analysis that the students made during day 1 of the course must be completed by a histological characterization of the organs that appear during late developmental stages. The aim is to characterize and observe the relative positions of future organs in relation to the polarities of the embryo. Students may also realize that the stages of organogenesis have phylogenetic implications. They see the notochord wrapped by cartilaginous outlines that prefigure adult bone structures and the axial skeleton. They see the pronephros and then the kidneys, the rhombencephalon (the predecessor of the adult posterior brain) and larval organs such as gill arches that will be replaced in the adult by the lung.

It is also important to be able to analyze the spatio-temporal expression of tissue-specific marker genes. The teacher therefore introduces *in situ* hybridization and antibody-staining techniques. The student must learn to recognize, by using selected histological sections, the four types of tissues and to understand their embryological origins. The nature of the tissues is confirmed by *in situ* hybridization with specific probes.

First, however, students make an oral presentation during which they must comment, in an interactive fashion, on histological sections projected on a screen (Fig. 1C). These slides consist of transversal and sagittal sections through the head and trunk of a tadpole. The Socratic method, in which emphasis is placed on questioning and where a response leads to a new question, is used. The students must identity structures and support their identifications.

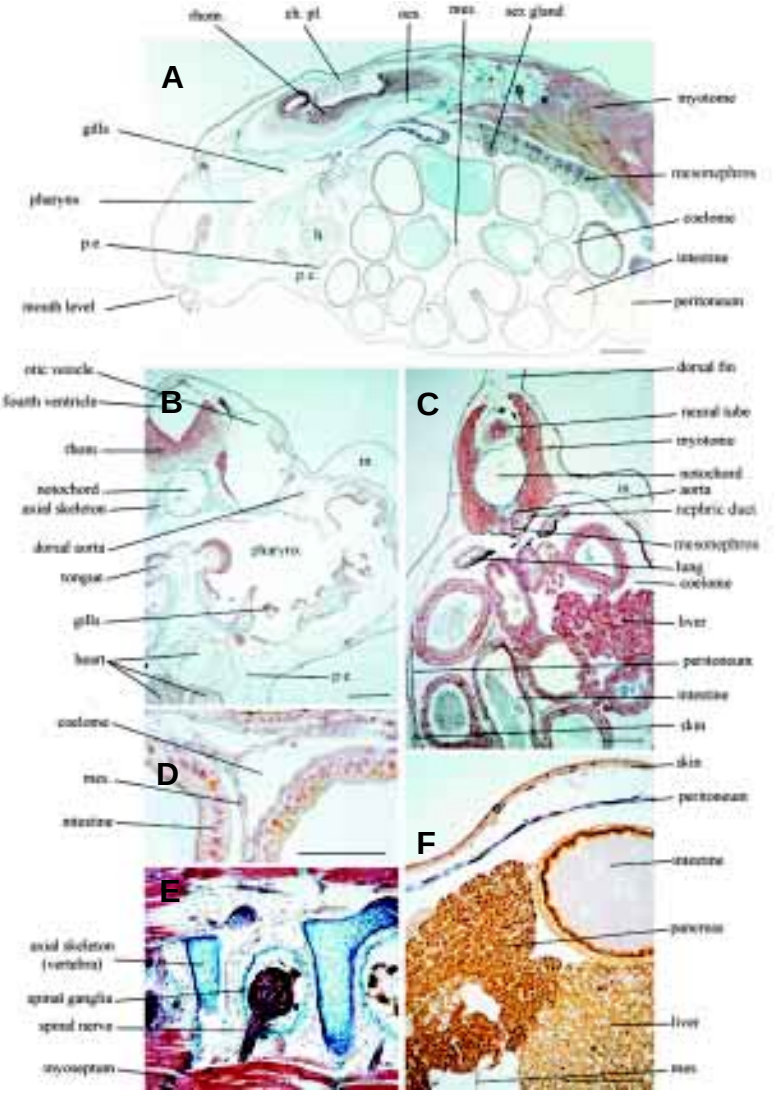

Fig. 6. Histological sections of embryos illustrating body plan organization. *Trichrome stainings of paraffin-embedded* Xenopus laevis *tailbud sections.* **(A)** *Sagittal section of the anterior third of an embryo. (B-D, F) Transverse sections.* **(B)** *is a section through the pharynx and gills and* **(C)** *is through the trunk.* **(D)** *is a detail of intestine and peritoneum.* **(E)** *Sagittal section illustrating metamerization.* **(F)** *is a detail of various viscera. ch.pl., choroid plexus; mes, mesentery; oes, esophagus; p.e., pericardial epithelium; rhom, rhombencephalon. Bars: 200 μm.*

- One of the histological sections observed under the microscope is assigned by the teacher to each group of students to be drawn and annotated with a complete legend. The histological slides of tadpole sections are similar to those that they have been observing on the screen. They are asked to recognize the central nervous system, notochord, somites, and gut, as well as more specialized organs such as heart, aorta, kidney (pronephros and mesonephros), liver, pancreas, muscles, and the membranes surrounding the different organs (peritoneum, pericardium) (Fig. 6 A-F). For each slide, they are asked to indicate the correct orientation of the body axis (dorsal, ventral, anterior, posterior).
- Students have several slides illustrating *in situ* hybridization of embryos with a somitic gene-specific probe (Fig. 3H). They may also observe, under the dissection microscope, a whole-mount *in situ* hybridization of an embryo performed by using the same gene as probe (Fig. 3G). This illustrates the spatial expression of different marker genes.

Examination

At the end of the laboratory course, the students write a report (worth 25% of the final mark) that is then evaluated by the teachers. The students' performance in this practical course is evaluated on the basis of the following 5 points:

- Successful completion, results, and discussion of the *in vitro* maturation of the stage-VI oocytes.
- Successful completion, results, and discussion of the *in vitro* fertilization and the animal cap assay.
- Drawings of microscope slides of oogenesis (one early and one late stage).
- Drawings of two transverse sections (head and trunk) and annotation of a diagram of a sagittal section of a tadpole.
- Annotation of a diagram illustrating the process of gastrulation (early vertebrate embryogenesis).

At the end of the year, a written examination containing both technical and theoretical questions requiring the interpretation of a scientific paper in the context of developmental biology completes the student evaluation. It accounts for 75% of the final mark for the unit. The objective is to integrate both lecture and laboratory courses.

Recommended Resources

Textbooks for Assigned Readings

COLLENOT, A. and SIGNORET, J. (2000). *L'organisme en développement. La construction de l'adulte.* Hermann, Paris.

DARRIBERE, T. (1997). *Biologie du développement. Le modèle amphibien.* Diderot Editeur, Arts et Sciences, Paris.

FRANQUINET, R. and FOUCRIER, J. (1998). *Atlas embryologie descriptive.* Dunod, Paris.

HAUSEN, P. and RIEBELL M. (1991). *The early development of Xenopus laevis. An atlas of histology.* Verlag der Zeitschrift für Naturforschung, Tübingen.

HOURDRY, J. (1998). *Biologie du développement. Morphogenèse animale, unité et diversité des métazoaires.* Ellipses, Paris.

LEMOIGNE (1997). *Biologie du développement,* 4th edn, Masson, Paris.

SIGNORET, J. and COLLENOT, A. (1991). *L'organisme en développement. Des gamètes à l'embryon.* Hermann, Paris.

Video Projection

LUTHER, W. and KUCZKA, H. Furchung und Gastrulation in *Triturus alpestris.* Service du Film de Recherche Scientifique.

Websites for Consulting

http://www.snv.jussieu.fr/bmedia/
http://www.ucalgary.ca/uofc/eduweb/virtualembryo/
http://worms.zoology.wisc.edu/embryology_main.html
http://zygote.swarthmore.edu/

References

DUMONT, J.N. (1972). Oogenesis in *Xenopus laevis* (Daudin). I. Stages of oocyte development in laboratory maintained animals. *J. Morphol.* 136: 153-179.

GURDON, J.B. (1987). Embryonic induction - Molecular prospects. *Development* 99: 285-306.

NIEUWKOOP, P.D. and FABER, J. (1967). *Normal table of Xenopus laevis* (Daudin), 2nd edn. North Holland, Amsterdam.

SIVE, H.L., GRAINGER, R.M. and HARLAND, R.M. (2000). *Early development of Xenopus laevis. A laboratory manual.* Cold Spring Harbor Laboratory Press, New York.

THEZE, N., HARDY, S., WILSON, R., ALLO, M.R., MOHUN, T. and THIEBAUD, P. (1995). The *MLC1f/3f* gene is an early marker of somitic differentiation in *Xenopus laevis* embryo. *Dev. Biol.* 171 : 352-362.

Acknowledgements

The practical courses are supported by the University Victor Segalen Bordeaux 2. We are indebted to Jean-Pierre Berthelé for assisting us at various levels in the preparation of the course. We thank Christiane Salat for the preparation of histological sections and Chrystelle Dulou for help in the preparation of buffers. We also thank Hughes Bretheau from the Department "Communication, Audivisuel, Multimedia" of the University Bordeaux 2 for his helpful assistance with photography. We thank Anne Thiébaud for carefully reading the manuscript.