Topic Introduction

A Brief History of *Xenopus* in Biology

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Xenopus is one of the premier model systems to study cell and developmental biology in vivo in vertebrates. Here we briefly review how this South African frog came to be favored by a large community of scientists after the explosive growth of molecular biology and examine some of the original discoveries arising from this sturdy frog. Experimental embryology started in *Rana* but developed in newt embryos for historical reasons. A long lineage of mentorship, starting with Theodor Boveri, Hans Spemann, Fritz Baltzer, Ernst Hadorn, and Michail Fischberg, used newt embryos. In Oxford, Fischberg made the transition to *Xenopus laevis* because it was widely available for human pregnancy tests and laid eggs year-round, and he fortuitously isolated a one-nucleolus mutant. This mutant allowed nuclear transfer experiments showing that genetic information is not lost during cell differentiation and the demonstration that the nucleolus is the locus of transcription of the large ribosomal RNAs. With the advent of DNA cloning, the great equalizer among all fields of biology, microinjected *Xenopus* oocytes became an indispensable tool, providing the first living-cell mRNA translation, polymerase II and III transcription, and coupled transcription–translation systems in eukaryotes. *Xenopus* embryos provide abundant material to study the earliest signaling events during vertebrate development and have been subjected to saturating molecular screens in the genomic era. Many novel principles of development and cell biology owe their origins to this remarkably resilient frog.

INTRODUCTION

We owe a debt of gratitude to Hazel Sive for pioneering the Cold Spring Harbor *Xenopus* laboratory courses. The detailed protocols developed there—and compiled in *Early Development of Xenopus laevis* by Hazel Sive, Robert Grainger, and Richard Harland published by Cold Spring Harbor Press 20 years ago—have guided the *Xenopus* experimental work that has exploded in so many laboratories. The resilient frog is considered the old martyr of science. We can trace fundamental discoveries made with the tenacious *Rana* to Luigi Galvani, who in 1791 discovered electricity using isolated frog leg muscles. A few years later, Alessandro Volta used the same assay to invent the electric battery. Here we relate how a frog from remote South Africa became a powerful model system in biology in the course of a few decades.

EMBRYOLOGY

The beginning of experimental embryology can be traced to work with *Rana*. Wilhelm Roux killed one of the two first two embryonic cells in 1883. In 1895, Thomas H. Morgan further showed that if the dead cell is removed, the surviving cell could self-organize into a tadpole of half the normal size.

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From the *Xenopus* collection, edited by Hazel L. Sive.

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Cite this introduction as *Cold Spring Harb Protoc*; doi:10.1101/pdb.top107615

When biologists realized that development could be interrogated experimentally, research quickly shifted to newt (salamander) embryos, which had advantages for transplantation studies and a long history. This saga starts with Theodor Boveri, the father of European cell biology. He mentored Hans Spemann, who trained Fritz Baltzer, who in turn mentored Ernst Hadorn in Switzerland. A student of Hadorn's, Michail Fischberg, moved to Oxford, where instead of newts he started work on the frog *Xenopus*, and mentored J.B.G., who in turn mentored many, including E.M.D.R. and Ron Laskey, who mentored Richard Harland. Much of the current excitement in the *Xenopus* developmental biology field can be traced back to this lineage culminating in Fischberg and his transition to a frog from distant lands.

Xenopus laevis had been previously introduced in the United Kingdom by Lancelot Hogben, who had been teaching in Cape Town, South Africa, for the purposes of pregnancy tests. *Xenopus* females injected into the dorsal lymph sac with human urine containing human chorionic gonadotrophin respond by laying eggs the next day. This proved much more convenient than earlier pregnancy tests using rabbits. Gradually, improved *Xenopus* husbandry allowed the establishment of multiple *Xenopus* colonies throughout Europe and the United States. At the end of WWII, Pieter Nieuwkoop in Holland pioneered embryological studies in *Xenopus laevis*. In the wild, it lives under the paradisiacal blue skies of South Africa, yet it will lay eggs year-round after rains form puddles. This provided a fundamental experimental advantage over *Rana* and newts, which have short springtime breeding seasons.

In Oxford, Fischberg had the wisdom to breed a spontaneously arising mutant that had one nucleolus per diploid cell instead of the normal two. This invaluable genetic marker allowed his graduate student J.B.G. to reinterpret the nuclear transplantation studies started in *Rana* by Robert Briggs and Thomas King. This led to the demonstration that the nuclei of differentiated cells did not lose genetic material during development, as they were able to develop into fertile frogs (Fig. 1). Interest in *Xenopus* exploded when Donald Brown in the United States, with J.B.G., showed that zero-nucleolus tadpoles did not synthesize the large ribosomal RNAs. Igor Dawid, a colleague of Brown's, used *Xenopus* interspecies hybrids to show that mitochondria contain DNA, which is always inherited from the mother and not from the sperm. Ron Laskey, a graduate student of J.B.G.'s, showed that components accumulated in the oocyte initiated DNA replication in the egg.

With the advent of molecular cloning, the great equalizer between the different areas of biology, research in *Xenopus* was set for extraordinary growth from the 1970s onward.

FIGURE 1. A clone of *Xenopus laevis* frogs derived by nuclear transplantation of nuclei originating from a single embryo. The donor nuclei were transplanted into the eggs of a wild-type pigmented female (shown) and marked by both the one-nucleolus mutation and an albino mutation; all members of the clone are male. (Reproduced with permission from Gurdon JB. 2013. *Development* 140: 2449–2456, © Company of Biologists.)

THE *XENOPUS* OOCYTE AS A LIVING TEST TUBE

The abdomen of an adult *Xenopus* female is filled with thousands of large oocytes of 1.2 mm in diameter. When removed from the mother, oocytes can be cultured for several weeks in a simple saline solution developed by Lester Barth for neural induction studies in salamanders. Whereas the fertilized egg immediately replicates nuclei, the oocyte remains metabolically active but unchanged in simple culture conditions for a few weeks. Microinjected oocytes provided many breakthroughs in the analysis gene expression in vertebrates.

Microinjected oocytes provided the first translation system for eukaryotic mRNAs. Globin mRNA, purified from immature red blood cells, a gift from Jean Brachet and Hubert Chantrenne (Belgium), was shown by J.B.G. and Charles Lane to be efficiently translated into globin protein when injected into *Xenopus* oocyte. A decade later, the use of microinjected synthetic mRNAs transcribed in vitro, introduced by Douglas Melton, opened many doors.

Microinjection of DNA into the large oocyte nucleus, called the germinal vesicle, provided the first transcription system for eukaryotic RNA polymerases II and III. With the advent of DNA cloning, E.M.D.R. showed that *Xenopus* oocytes offered the first coupled transcription–translation system for the expression of cloned genes. To this day, the stability of transcription complexes is being studied by successive microinjections of DNA.

Proteins translated in oocytes, if they contain the proper signal sequences, can be either secreted or localized to the plasma membrane. The *Xenopus* oocyte provided the premier system to study the properties of cloned receptors and ion channels in electrophysiology. Nucleocytoplasmic transport of proteins and small RNAs microinjected into the cytoplasm, starting with iodinated histones, was shown. Further, gene expression of somatic nuclei from differentiated cells injected into the oocyte were reprogrammed to resemble that of embryos and stem cells.

The simple, but revolutionary, idea that underlies the fertile experimental heritage of the *Xenopus* oocyte is that if one introduces a single purified molecule at a time, the living test tube of the cell will take care of the rest.

EGG CELL-FREE EXTRACTS

Xenopus eggs can be broken by centrifugation to generate concentrated cytoplasmic preparations for the study of many cell biological processes. These extracts were initially developed by Yoshio Masui to study the assembly of the nuclear envelope around DNA. These cell-free extracts have been essential for studies of the cell cycle, mitotic spindle formation, microtubule assembly, and chromatin formation. Modern proteomic techniques have defined the composition of these extracts in detail and, because protein complexes can be depleted or added back, *Xenopus* extracts provide an essential postgenomic biochemical assay system.

GERM LAYER DIFFERENTIATION

A great advantage of the amphibian embryo is that the future tissue allocations can be targeted shortly after fertilization, when a less pigmented dorsal crescent forms as a result of a cortical rotation of the egg. Studies on dorsal–ventral cell lineages, and the effect of microinjected molecules on them, can be followed in great detail. The dorsal crescent becomes the Spemann organizer at gastrula, which is the source of dorsal–ventral differentiation signals (Fig. 2). Explants of animal cap cells at blastula differentiate into epidermis, but if they are treated with TGF- β growth factors such as activin, they will differentiate into mesoderm—and at higher doses into endoderm, as shown by Jim Smith and Makoto Asashima. Within the ectoderm, the inhibition of BMP and TGF- β signaling results in the induction of the central nervous system. Studies in *Xenopus* have greatly enhanced our understanding of

FIGURE 2. The Spemann organizer transplantation experiment in *Xenopus*. A small region of the dorsal blastopore lip transplanted into the ventral side of a gastrula host (*lower right*, albino transplanted tissue) results in a perfectly patterned second body axis. The Spemann organizer region has been subjected to saturating molecular screens that identified many novel secreted growth factor antagonists such as Chordin, Noggin, Frzb, Cerberus, and Dickkopf. (Reproduced with permission from De Robertis JB, 2006, *Nat Rev Mol Cell Biol* 7: 296–302, © Springer Nature).

morphogen gradients in development and provided the guiding principles for the recent protocols used to channel the histotypic differentiation of mammalian embryonic stem cells in culture.

REVERSE GENETICS IN *XENOPUS*

Synthetic mRNAs transcribed from cloned genes allowed many gain- and loss-of-function studies. Dominant-negative constructs can inhibit gene function, and overexpression can test for the function of novel genes. Functional screens to identify novel molecules were performed in microinjected embryos using pools of mRNAs transcribed from cDNA libraries, followed by sib selection. The *Xenopus* Spemann organizer proved a particularly rich fishing ground for novel molecules, many of which were, unexpectedly, found to be secreted inhibitors of growth factors, such as Noggin, Chordin, Frzb, Cerberus, and Dickkopf. Genes transcribed by a common signaling pathway could be identified as synexpression groups in large-scale in situ hybridization screens of embryos. A notable gene isolated in *Xenopus* was the first *Hox* gene, although in this case E.M.D.R. used probes previously identified by Walter Gehring in *Drosophila*. (Interestingly Gehring, like Fischberg, was a graduate student of Hadorn's, closing the circle in this lineage of developmental biologists.) A convenient loss-of-function system that depletes both maternal and zygotic mRNAs was provided by antisense morpholino oligonucleotides (MOs), first introduced by Janet Heasman, which have been extremely effective.

One limitation of *Xenopus laevis* is its long generation time of more than a year. This led to the introduction of *Xenopus tropicalis*, which reproduces in 4–6 mo, is suitable for transgenesis and site-directed mutagenesis, and has a diploid genome. *Xenopus laevis* is a subtetraploid frog that originated as a hybrid between two different species. The completion of the *Xenopus laevis* genomic sequence by Richard Harland and colleagues was a crucial landmark that provides an invaluable resource for gene targeting and understanding the role of gene loss and duplication during evolution. With the proliferation of candidate target genes from human genetics, microinjected *Xenopus* embryos provide an in vivo model to rapidly screen their relevance to disease in a vertebrate.

CONCLUSION

In a few decades, a vibrant scientific fellowship has developed among those who love experimenting with the embryos of this resilient frog with their own hands. This community benefits from the biannual *Xenopus* meetings, a superb Xenbase database and the National *Xenopus* Resource (NXR) supported by the National Institutes of Health (NIH), and last but not least the Cold Spring Harbor Laboratory courses on *Xenopus* development. The new book *Xenopus: A Laboratory Manual*, edited by Hazel Sive, provides biologists with an invaluable collection of protocols that ensure that *Xenopus* researchers will continue discoveries on the mysteries of vertebrate development well into the future.

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Cold Spring Harb Protoc; doi: 10.1101/pdb.top107615 originally published online March 30, 2021

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