Topic Introduction

The Use of *Xenopus* for Cell Biology Applications

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Problems of cell biology and the molecular controls underpinning them have been studied in the remarkably versatile *Xenopus* systems for many years. This versatility is showcased in several accompanying protocols, which are introduced here. One protocol demonstrates how the *Xenopus* embryonic ectoderm can be used to study the effects of mechanical cell deformation; another illustrates how the developing eye can be used as a platform for determining cell-cycle length. Two protocols show how extracts from *Xenopus* embryos can be exploited to characterize the behavior of specific intracellular proteins—specifically, to determine protein phosphorylation status and the ability to bind to chromatin. Finally, because specific antibodies to *Xenopus* proteins are pivotal reagents for cell biology and biochemistry applications, four protocols describing how to generate, purify, and assay the specificity of antibodies raised against *Xenopus* proteins are included in hopes of stimulating the expansion of these critical resources across the *Xenopus* community.

BACKGROUND

Xenopus has provided a remarkably versatile array of biological systems for the study of molecular controls, through biochemistry and cell biology all the way to investigation of the intricate interactions of cells and tissues in the developing and metamorphosing frog. Indeed, it is its exceptional versatility, and the ability to use it to study processes across such a wide range of biological scales, that has resulted in studies in *Xenopus* providing the underpinning of a surprisingly large amount of what we understand about other biological systems including humans. Moreover, we continue to develop new uses for the frog egg and embryo to study interesting biology, and different facets of this system can be readily adapted to shed further light on the biochemistry of developmental control. To understand how gene function feeds through to the behavior of tissues and organs, we must look to integrate our organism-level studies with an understanding of behavior of individual cells and the molecular controls that underpin this. Indeed, with the ever-expanding use of *Xenopus* in fields such as biomechanics and detailed molecular mechanisms, it is important to disseminate these new methodologies for others to use and adapt to address new questions.

The accompanying protocols present cell-based approaches that can be used to shed light on aspects of biology as diverse as cell-cycle dynamics and cell-shape response through to protein modifications and behavior. Moreover, methods are described for the selection of antigens, as well as the generation and purification of antibodies to *Xenopus* proteins that are the essential tool in a huge range of applications in modern biology.

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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.top105528

XENOPUS EMBRYOS AND TISSUES AS MODELS FOR CELL DYNAMICS

Effects of mechanical force on cell behavior are emerging as an important influence on processes including division, cell fate, and differentiation (Wyatt et al. 2016), although direct analysis of stretching and compressive forces on cells in living vertebrates can be challenging. The resilience of the blastula embryo animal pole ectoderm, the animal cap, and its ability to be cultured *ex vivo* in simple media combine with ready accessibility to make this tissue an excellent system for such studies (Stooke-Vaughan et al. 2017). In Protocol: **Applying Tensile and Compressive Force to *Xenopus* Animal Cap Tissue**, Goddard et al. (2019) take a classic cell biological approach to show how the effects of tensile force on cell behavior can be assayed in *Xenopus* animal cap ectodermal cells using confocal microscopy. The ready ability to apply very controlled forces to animal cap cells, and immunostaining of these tissues under stress, allows measurements to be taken using advanced microscopy under different conditions. Illustrating nicely the versatility of this approach, mathematical modeling has been combined with the ability to quantitate cell stresses and changes in cell shape to elegantly show that shape and stress have distinct effects on the cueing and orientation of cell division (Nestor-Bergmann et al. 2019).

Cell size and shape can influence cell division and these links can be probed experimentally, but even fundamental questions such as defining the length of a cell cycle at different stages of embryogenesis can be hard to undertake in many organisms. However, because of the ready accessibility of its embryos, *Xenopus* has long been the model of choice to study how patterns of cell division change during embryonic development (Saka and Smith 2001; Vernon and Philpott 2003; Thuret et al. 2015). Although precise measurement of the length of phases of the cell cycle as well as the overall rate of cell division in the changing environment of the developing embryo is complex, it is probably most readily achieved using the special features we find in *Xenopus*. In Protocol: **In Vivo Assessment of Neural Precursor Cell Cycle Kinetics in the Amphibian Retina**, Locker and Perron (2019) show how cell staining and labeling for markers of different cell-cycle phases can again be combined with powerful mathematical modeling approaches to determine how cell-cycle time changes during retinal development. This methodology takes advantage of the accessibility of the *Xenopus* eye and the compartmentalization of proliferative and differentiation zones that is a key feature of *Xenopus* retina (Perron and Harris 2000), although the method should be readily adaptable to other tissues.

Xenopus egg and embryo extracts provide complex mixtures of proteins that can recapitulate a wide range of cell biological and macromolecular behaviors. (This is also explored in Introduction: **The Use of Cell-Free *Xenopus* Extracts to Investigate Cytoplasmic Events** [Gibeaux and Heald 2018].) As well as providing accessible systems to study coordination of macromolecular events such as mitotic spindle generation and nuclear fission, extracts from eggs and embryos can also be used to provide complex protein mixtures to probe protein behavior and modification *in vivo*. In Protocol: **Analysis of Phosphorylation Status of Ectopically Expressed Proteins in Early *Xenopus* Embryos**, Hardwick and Philpott (2019a) give a very simple method to assay the phosphorylation status of ectopically expressed proteins in *Xenopus* embryos, an approach agnostic to the kinases that are bringing about post-translational modifications. Embryonic cytoplasm provides a rich environment of such enzymes, particularly cyclin-dependent kinases, that may act together to bring about extensive phosphorylation of individual proteins, as is seen in the case of multisite phosphorylation of bHLH transcription factors in early embryos (Ali et al. 2011, 2014). The first steps in this protocol also give a more general, simple, and robust method for extracting proteins expressed in embryos for subsequent analysis by western blotting (see also the related antibody testing protocol introduced below).

Transcription factors and other chromatin-bound proteins can also be isolated from the DNA of developing embryos using Protocol: **Analysis of Chromatin Binding of Ectopically Expressed Pro-**

ANALYZING PROTEIN PHOSPHORYLATION AND CHROMATIN BINDING IN *XENOPUS* EMBRYOS

teins in Early *Xenopus* Embryos (Hardwick and Philpott 2019b), contributed by Laura Hardwick and Anna Philpott. Isolating proteins from developmentally staged embryos in this way would allow changes in DNA binding of individual factors across developmental time to be analyzed. In some cases, chromatin binding may be accompanied by post-translational modification. Combining the use of the chromatin isolation protocol with the protocol designed to analyze protein phosphorylation status was used to show that DNA-bound proneural transcription factors are phosphorylated on multiple sites after expression in *Xenopus* (Hardwick et al. 2019; Hardwick and Philpott 2019c). This type of analysis has formed the bedrock of further studies to uncover the molecular regulation by phosphorylation of this class of proteins (Ali et al. 2011, 2014; Azzarelli et al. 2017). These studies give a good example of how different facets of the use of *Xenopus* as a model system can be used together to reveal how biochemical regulation affects developmental function of control proteins.

GENERATING ANTIBODIES TO *XENOPUS* PROTEINS: CRITICAL ANALYTICAL TOOLS

Cell biology often requires the visualization of subcellular structures usually by specific detection of their protein components. Indeed, the use of antibodies to detect specific proteins underpins most studies of cell morphology and function. Specific antibodies are also vital tools in a wide range of studies of biochemistry, molecular biology, and embryology. However, the *Xenopus* community has lagged considerably behind many other model organism user groups in the generation of antibodies that recognize *Xenopus* proteins. The previously lower priority given to developing specific antibodies to frog proteins is partly due to the fact that classical uses of *Xenopus* systems such as gross morphological, embryological, and fate studies rely less on a mechanistic understanding of individual proteins than systems in which a more reductionist approach has been more prominent.

Of course, in recent decades, the functions of different genes have been extensively studied in *Xenopus* embryos, but when gene expression is assayed, this is typically undertaken by using a mechanism that detects mRNA rather than by using antibodies to detect proteins. However, the detection of mRNA transcripts will only ever give part of the story. Protein half-lives can vary enormously, so direct extrapolation from the RNA level to the protein level is often not possible (Peshkin et al. 2015). Furthermore, post-translational modification can also affect protein half-life and activity (Hindley et al. 2012). However, the need for reliable antibody resources to a large range of *Xenopus* proteins has not gone unnoticed; for more than 10 years, a number of *Xenopus* Principal Investigator forums have highlighted improving antibody resources for use by the *Xenopus* community as a very high priority. Increasing the number of antibodies validated for activity against *Xenopus* proteins can be achieved either by generating antibodies to *Xenopus* epitopes and/or by screening antibodies raised to proteins from other species for cross-reactivity with *Xenopus*. Both approaches will be important avenues to exploit if we are to move forward toward the ultimate goal of having ready availability of validated antibodies to all *Xenopus* proteins under investigation by our community.

Although there are many widely available protocols to generate antigens for inoculation that are not specific to *Xenopus* proteins, Protocol: **Raising Antibodies for Use in *Xenopus*** (Piccinni and Guille 2020a), by Maya Piccinni and Matthew Guille, describes a simple method for the generation of *Xenopus* proteins in HEK cells for immunization and gives tips on how to select an appropriate antigenic region. The method they describe has an advantage over the use of bacterially expressed proteins for host immunization because some post-translational modifications only found in eukaryotic cells may be present, and protein folding and stability is more likely to mimic that found in *Xenopus* cells. Further details are also given for antigen purification and immunization of hosts in this protocol.

Once immunization has been undertaken, it is important to separate the antibodies from extracted serum and to further purify against the selected antigen to enhance the specificity and reduce the background in subsequent antibody uses. Protocol: **Purifying Antibodies Raised against *Xenopus* Peptides** (Piccinni and Guille 2020b), also by Maya Piccinni and Matthew Guille, describes

one way to isolate IgG immunoglobulin from whole serum using a caprylic acid extraction protocol. This is followed by a method for affinity purification and desalting of specific antibodies using a peptide antigen column. After antigen-specific antibody purification, a robust method is required to determine the specificity and potency of the generated antibody against the antigen in question. In Protocol: **Confirming Antibody Specificity in *Xenopus***, Martin et al. (2020) describe a strategy that can be used to verify that the new antibody recognizes its target. This strategy involves knocking down or enhancing the expression of the target in embryos and then performing a western blotting analysis with the new antibody to evaluate its specificity for the target. In Protocol: **Assessing the Immune Response When Raising Antibodies for Use in *Xenopus***, Piccinni and Guille (2020c) describe a simple method to determine whether a strong and specific antibody response has been generated to a specific protein expressed in *Xenopus* embryos, as assayed by western blotting. Taken together, these four protocols give a roadmap for generating new antibody resources for *Xenopus* proteins, but there are many additional and well-established ways to generate antigens for immunization, purify antibodies, and test their affinity and specificity that are equally applicable across all species.

CONCLUSION

Overall, the protocols introduced here give a snapshot of the many versatile ways that *Xenopus* can be used to study a large range of cell biological questions, complementing other protocols in the collection that describe the use of *Xenopus* oocytes and eggs, embryos, and frogs. *Xenopus* is truly an organism of remarkable versatility, and we continue to make great strides in using the many facets of the *Xenopus* system to study problems in innovative ways, as well as continually developing new reagents like antibodies to make those studies possible.

ACKNOWLEDGMENTS

I thank Ron Laskey, Marc Kirschner, and John Gurdon for teaching me almost everything I know about frogs and the many past and present members of the Philpott laboratory for stimulating discussions. I am supported by Cancer Research UK Programme Grant A25636 and Wellcome Trust Investigator Award 212253/Z/18/Z.

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Cold Spring Harb Protoc; doi: 10.1101/pdb.top105528 originally published online March 29, 2021

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