Topic Introduction

Using the *Xenopus* Oocyte Toolbox

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The *Xenopus* oocyte is a unique model system, allowing both the study of complex biological processes within a cellular context through expression of exogenous mRNAs and proteins, and the study of the cell, molecular, and developmental biology of the oocyte itself. During oogenesis, *Xenopus* oocytes grow dramatically in size, with a mature oocyte having a diameter of ~ 1.3 mm, and become highly polarized, localizing many mRNAs and proteins. Thus, the mature oocyte is a repository of maternal mRNAs and proteins that will direct early embryogenesis prior to zygotic genome transcription. Importantly, the *Xenopus* oocyte also has the capacity to translate exogenous microinjected RNAs, which has enabled breakthroughs in a wide range of areas including cell biology, developmental biology, molecular biology, and physiology. This introduction outlines how *Xenopus* oocytes can be used to study a variety of important biological questions.

BACKGROUND

The *Xenopus* oocyte has been called a “living test tube” (de Robertis et al. 1977) and has proven to be an invaluable system for investigating a myriad of biological questions. Research using *Xenopus* oocytes has revolutionized the fields of cell and molecular biology, resulting in many “firsts” including, but not limited to, the identification of the nucleolus as the site of ribosomal RNA transcription (Brown and Gurdon 1964), the isolation of a eukaryotic gene (Birnstiel et al. 1968; Brown and Weber 1968), in situ hybridization (Gall and Pardue 1969), in vivo expression of exogenous mRNA (Gurdon et al. 1971), transcription of a cloned eukaryotic gene (Brown and Gurdon 1977), sequencing of a eukaryotic gene (Fedoroff and Brown 1978), and isolation of a eukaryotic transcription factor (Engelke et al. 1980). The large size of the *Xenopus* oocyte and the availability of large quantities of oocytes continues to facilitate microinjection approaches to study cellular functions of exogenous proteins, as well as biochemical approaches to study fundamental cellular processes.

OOGENESIS

As depicted in Figure 1, oogenesis in *Xenopus* is divided into six stages (I–VI) based on size and morphology (Dumont 1972). Stage I oocytes are transparent, lacking yolk, and display the first hallmark of polarity along the animal-vegetal (AV) axis: The Balbiani Body, or mitochondrial cloud (Heasman et al. 1984), is present on the vegetal side of the nucleus or germinal vesicle (GV). Oocytes

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FIGURE 1. Depiction of the six stages (st.; I–VI, from left to right) of oogenesis in *Xenopus*. The diameters of each oocyte, stages of vitellogenesis (or yolk accumulation), and the animal-vegetal (AV) axis are shown. The nuclei (or germinal vesicles [GVs]) are indicated by circles, and the Balbiani Body in the stage I oocyte is depicted in gray on the vegetal side of the GV, which is in the center. In the stage IV oocyte, the GV is displaced toward the animal hemisphere, where accumulating pigment is indicated by brown shading. The GV is further displaced toward the animal hemisphere in stage V and VI oocytes, where accumulated pigment is indicated by dark brown shading and increased yolk accumulation is indicated by yellow shading.

grow dramatically during oogenesis—from 50 μm at the beginning of stage I to 1.3 mm by the end of stage VI—with much of this growth due to the accumulation of yolk during vitellogenesis, which refers to the uptake of the yolk precursor protein vitellogenin (Wallace and Dumont 1968). Vitellogenesis begins in stage II oocytes and continues through stage V of oogenesis (Dumont 1972). By the end of oogenesis (stage VI) the vegetal yolk mass comprises 70% of total oocyte yolk protein (Danilchik and Gerhart 1987) and 80% of total oocyte protein (Wiley and Wallace 1981). Oocyte pigmentation begins in stage III, with polarization of the pigment first evident in stage IV and increasing through stage VI, such that the animal hemisphere is darkly pigmented in fully grown oocytes (Dumont 1972).

In Protocol: **Isolation of *Xenopus* Oocytes** (Newman et al. 2018), procedures for the isolation of various stages of *Xenopus* oocytes are detailed, and each of the other accompanying protocols uses distinct stages of oogenesis. Protocol: **Whole-Mount In Situ Hybridization of *Xenopus* Oocytes** (Bauermeister and Pieler 2018), Protocol: **Fluorescence In Situ Hybridization of Cryosectioned *Xenopus* Oocytes** (Neil and Mowry 2018), and Protocol: **Whole-Mount Immunofluorescence for Visualizing Endogenous Protein and Injected RNA in *Xenopus* Oocytes** (Jeschonek and Mowry 2018), use stage II–IV oocytes. Protocol: **Isolation and Analysis of *Xenopus* Germinal Vesicles** (Morgan 2018) uses stage IV–V oocytes, and Protocol: **Heterologous Protein Expression in the *Xenopus* Oocyte** (Marchant 2018), Protocol: **Patch-Clamp and Perfusion Techniques to Study Ion Channels Expressed in *Xenopus* Oocytes** (Zhang and Cui 2018), and Protocol: **Oocyte Host-Transfer and Maternal mRNA Depletion Experiments in *Xenopus*** (Houston 2018) use stage VI oocytes. Protocol: **Microinjection of *Xenopus* Oocytes** (Aguero et al. 2018) describes procedures for microinjecting DNA or RNA molecules into stage VI *Xenopus* oocytes.

USING *XENOPUS* OOCYTES TO STUDY POLARITY AND EARLY DEVELOPMENT

During oogenesis, *Xenopus* oocytes synthesize the large store of maternal mRNAs and proteins that will be necessary to program early embryogenesis until zygotic transcription begins at the mid-blastula transition (Newport and Kirschner 1982). The highly active transcription required for this results in lampbrush chromosomes, which are a topic of Protocol: **Isolation and Analysis of *Xenopus* Germinal Vesicles** (Morgan 2018). Additionally, during oogenesis, a subset of mRNAs are polarized along the AV axis, providing the basis for developmental polarity in the oocyte and patterning in the embryo (King et al. 2005; Medioni et al. 2012). *Xenopus* oocytes have been an important model system for studying the mechanisms driving mRNA transport and localization (Houston 2013), and several

accompanying protocols are relevant to this topic: Protocol: **Whole-Mount In Situ Hybridization of *Xenopus* Oocytes** (Bauermeister and Pieler 2018) presents whole-mount in situ hybridization as a technique for spatial analysis of RNA expression in oocytes. Protocol: **Whole-Mount Immunofluorescence for Visualizing Endogenous Protein and Injected RNA in *Xenopus* Oocytes** (Jeschonek and Mowry 2018) details techniques for analyzing spatial distribution of both endogenous proteins and microinjected RNAs. Protocol: **Fluorescence In Situ Hybridization of Cryosectioned *Xenopus* Oocytes** (Neil and Mowry 2018) describes a FISH approach, combined with cryosectioning, for high-resolution imaging of endogenous RNA distribution in oocytes. Analyzing the contributions of maternally inherited mRNAs to early embryogenesis is of critical importance in *Xenopus* and many systems. Protocol: **Oocyte Host-Transfer and Maternal mRNA Depletion Experiments in *Xenopus*** (Houston 2018) details procedures for using host-transfer techniques to assess effects on embryonic development after manipulating expression of maternal mRNAs in oocytes.

USING *XENOPUS* OOCYTES TO STUDY PHYSIOLOGY, CELL, AND MOLECULAR BIOLOGY

Xenopus oocytes have long been an invaluable tool for expression and analysis of heterologous proteins and, as noted above, inspired characterization as a “living test tube” (de Robertis et al. 1977). Proteins produced from mRNA microinjected into the cytoplasm (Gurdon et al. 1971) or DNA microinjected into the nucleus (Mertz and Gurdon 1977) are robustly expressed and appropriately processed and trafficked. The development of in vitro procedures for production of synthetic RNAs (Krieg and Melton 1984) further expanded the versatility and effectiveness of the oocyte as a system for analyzing the function of expressed proteins. Protocol: **Microinjection of *Xenopus* Oocytes** (Aguero et al. 2018) provides procedures for microinjection of *Xenopus* oocytes and Protocol: **Heterologous Protein Expression in the *Xenopus* Oocyte** (Marchant 2018) details procedures for nuclear injection of cDNA constructs for expression of heterologous proteins in oocytes. Both of these protocols can be adapted to study any gene of interest. The large size of the oocyte is of particular benefit to electrophysiological approaches to study channel proteins, such as acetylcholine receptors (Miledi et al. 1982) and many others (Dascal 1987). In Protocol: **Patch-Clamp and Perfusion Techniques to Study Ion Channels Expressed in *Xenopus* Oocytes**, Zhang and Cui (2018) describe the use of *Xenopus* oocytes to study heterologously expressed ion channels. The protocol can be applied to study a variety of ion channels and neurotransmitter receptors. Together, these protocols provide a toolbox for using *Xenopus* oocytes to carry out functional studies for an investigator’s protein of interest.

SUMMARY

For many decades, the *Xenopus* oocyte has proven to be an invaluable system for addressing fundamental biological questions. The protocols introduced here will assist investigators in continuing to use and study this remarkable cell.

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