Topic Introduction

The *Xenopus* Oocyte: A Single-Cell Model for Studying Ca^{2+} Signaling

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In the four decades since the *Xenopus* oocyte was first demonstrated to have the capacity to translate exogenous mRNAs, this system has been exploited for many different experimental purposes. Typically, the oocyte is used either as a “biological test tube” for heterologous expression of proteins without any particular cell biological insight or, alternatively, it is used for applications where cell biology is paramount, such as investigations of the cellular adaptations that power early development. In this article, we discuss the utility of the *Xenopus* oocyte for studying Ca^{2+} signaling in both these contexts.

BACKGROUND

The oocyte is the cellular precursor for the development of specialized cells. Oocytes from the South African clawed frog *Xenopus laevis* provide the most commonly used oocyte model for studying the properties, organization, and cellular roles of Ca^{2+} -permeable channels and transporters. For experimentalists, the *Xenopus* oocyte brings together in a single cell the capacity to investigate protein function and cell biological processes on a colossal scale and across a range spanning from single molecules to entire genomes (Miledi et al. 1983; Sonnleitner et al. 2002; Demuro and Parker 2006; Halley-Stott et al. 2010). Key mechanistic principles of Ca^{2+} signaling have been demonstrated in this model—for example, identification of receptors and channels that mediate Ca^{2+} signals and characterization of how these proteins work (Lubbert et al. 1987; Mak and Foskett 1994; Beene et al. 2003; Goldin 2006), observation of unique spatiotemporal profiles of agonist-triggered Ca^{2+} signals (Lechleiter et al. 1991a,b), mechanistic dissection of Ca^{2+} oscillations (Camacho and Lechleiter 1993; Girard and Clapham 1993), demonstration of the functional coupling of endoplasmic reticulum (ER) and mitochondrial Ca^{2+} stores (Jouaville et al. 1995), and resolution of how cellular Ca^{2+} signals arise through orchestration of localized Ca^{2+} -release events (Parker and Yao 1991; Parker et al. 1996). Emerging *Xenopus* resources for the two species commonly used in the laboratory (*X. laevis* and *X. tropicalis*, the latter being a smaller and more quickly developing diploid frog) span genomic and bioinformatic data (Bowes et al. 2010), and reagent repositories (Pearl et al. 2012), as well as methods for high-throughput functional genetic assays and transgenesis (Harland and Grainger 2011). These toolsets underscore the broad utility of *Xenopus* as an easily maintained and readily manipulated tetrapod model relevant to human development and disease.

In the broadest view, two applications bring experimentalists to the *Xenopus* oocyte. The first application is as an incubator for gene expression divorced from cell biology—a biological test tube

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(Gurdon et al. 1971; Barnard et al. 1982). Classically, this encompasses gain-of-function analyses to resolve the properties of heterologously expressed gene products (e.g., by recording the electrophysiological signatures of cell-surface proteins [Stuhmer and Parekh 1995; Goldin 2006] or by identifying genes themselves using expression cloning [Lubbert et al. 1987; Markovich 2008]). The second application is to study the cell biology of the *Xenopus* oocyte in its own right. The oocyte is a remarkable cell that is uniquely tailored to complete maturation, fertilization, and early embryogenesis following a massive accumulation of resources during oogenesis. The trajectory of a cell that remodels from growth without division (pre-fertilization) to division without growth (early embryogenesis) affords remarkable opportunities to study the cellular specializations that enable such behavior. Consequently, the impact of studies using *Xenopus* oocytes/eggs and their extracts on many aspects of cell biology has been extensive (Brown 2004). Of course, these dual applications of the *Xenopus* system are interdependent—both derive from the endogenous synthetic capacity of the oocyte (hijacked by injection of foreign genes) and the fact that the resultant expression is supported on such a gigantic scale.

ADVANTAGES OF THE *XENOPUS* OOCYTE AS A LIVING TEST TUBE

There are several reasons why the *Xenopus* oocyte is a facile system for heterologous expression.

- Oocytes are easy to obtain. Frog colonies are inexpensive to maintain and husbandry is straightforward. Surgical extraction protocols are simple and oocytes are abundant (a mature female frog has >20,000 oocytes). Alternatively, oocytes and ovaries can be directly sourced from commercial vendors (e.g., EcoCyte Bioscience and Nasco) or resource centers (Pearl et al. 2012).
- Fully grown *Xenopus* oocytes are robust. Following the completion of oogenesis, stage VI oocytes are completely synchronized by arrest at the end of the G₂ phase of meiosis I and can remain in that state in vivo (possibly for years) until hormonal maturation results in the completion of meiosis and ovulation. As a colossal, autonomous “organ system” (Soreq and Seidman 1992), the oocyte is capable of being maintained in vitro for extended periods of time (typically <2 wk post-injection [Stuhmer and Parekh 1995]).
- The large size of oocytes used for expression studies (~1.0–1.3 mm in diameter, stage V and VI [Dumont 1972]) allows for manual microinjection methods. Constructs can be introduced by injection of cDNA into the germinal vesicle (a target ~0.4 mm in diameter) or by cytoplasmic injection of RNA (or rarely cDNA [Geib et al. 2001]). Multiple species can be injected simultaneously, which is important for (i) Ca²⁺ channel proteins that function as complexes where the relative levels of mRNA for different subunits can be titrated to optimize functional expression, and (ii) co-injection of mRNA with modified tRNAs for unnatural amino acid labeling (Beene et al. 2003). Proteins, vesicles, and whole organelles have also been reconstituted for functional assays (Le Caherec et al. 1996; Sheu and Sharma 1999; Palma et al. 2003; Au et al. 2010; Halley-Stott et al. 2010). All these microinjection protocols can be accomplished without elaborate equipment.
- The oocyte is a promiscuous yet faithful expression system. Ca²⁺ channels/transporters from a broad swathe of eukaryotic organisms (Table 1) as well as animal virus proteins (e.g., viroporins [Antoine et al. 2007]) can be functionally expressed. Prokaryotic channels have also been expressed, although examples are less numerous (Bocquet et al. 2007; Choi et al. 2010; Maksaev and Haswell 2011), possibly reflecting divergent environmental requirements for channel function relative to their native systems. The use of the oocyte as an ex situ system for protein analysis is especially important for organisms where in situ recordings would be challenging.
- Functional assays are easy to perform. Again, the large size and spare translational capacity (Moar et al. 1971) of the oocyte facilitate observation and integration of responses. The oocyte provides a huge canvas for imaging—with a total plasma membrane area of ~20 mm², membrane domains

TABLE 1. Broad species specificity for functional protein expression in *Xenopus* oocytes

Model organism	Species	Expressed construct	References
Plant (dicot)	<i>Arabidopsis</i>	Ca ²⁺ -regulated channel (glutamate receptor)	Roy et al. 2008
Plant (monocot)	<i>Oryza</i>	Ca ²⁺ permeable channel (HKT)	Lan et al. 2010
Green algae	<i>Chlamydomonas</i>	Light-activated Ca ²⁺ channel (ChR2)	Nagel et al. 2003
Alveolate	<i>Plasmodium</i>	Ca ²⁺ ATPase (PfATP6)	Eckstein-Ludwig et al. 2003
Amoeba	<i>Dictyostelium</i>	P ₂ X receptors (dP ₂ X)	Ludlow et al. 2009
Euglenozoa	<i>Trypanosomes</i>	K ⁺ transporter (TbHKT1)	Mosimann et al. 2010
Yeast	<i>Saccharomyces</i>	Outward rectifier K ⁺ channel (YORK)	Lesage et al. 1996
Sponge	<i>Amphimedon</i>	Inward rectifier K ⁺ channel (AmqKir)	Tompkins-Macdonald et al. 2009
Mollusc	<i>Loligo</i>	Voltage-activated Ca ²⁺ channel (Ca _v 2)	Kimura and Kubo 2002
Worm	<i>C. elegans</i>	Ca ²⁺ permeable channel (MEC4[d])	Bianchi et al. 2004
Insects	<i>Blatella, Drosophila</i>	Voltage-activated Ca ²⁺ channel (DSC1, BSC1)	Zhou et al. 2004; Zhang et al. 2011
Flatworm	<i>Schistosoma</i>	Ligand-gated Ca ²⁺ channel (P ₂ X)	Agboh et al. 2004
Cnidarian (jellyfish)	<i>Cyanea</i>	Voltage-activated Ca ²⁺ channel (CyCa ₁)	Jeziorski et al. 1998
Echinoderm (sea urchin)	<i>Strongylocentrotus purpuratus</i>	Intracellular Ca ²⁺ Channel (TPC)	Brailoiu et al. 2009
Chordate (tunicate)	<i>Halocynthia</i>	Voltage-activated Ca ²⁺ channel (TuCa1)	Izumi-Nakaseko et al. 2003

Where possible, these examples illustrate Ca²⁺-permeable channels or transporters. Because of limited space, only single examples are shown for each model.

~100-fold larger than those attainable in mammalian cells can be imaged (Ottolia et al. 2007). The same holds for intracellular imaging, where large regions of the ER can be monitored for Ca²⁺ release activity. With regard to integration of electrophysiological responses, detectable currents as small as tens of nA can be recorded by two-electrode voltage clamp (i.e., a single ion channel opening [1 pA; $P_{\text{open}} = 0.1$] in every 40 μm^2 of membrane). Copy numbers of expressed proteins are large ($\sim 5 \times 10^8$ [Sigel 1990]), correlating with synthetic rates of ~100–200 molecules of protein per injected mRNA molecule per day (Gurdon et al. 1971; Halley-Stott et al. 2010). With sensitive endogenous Ca²⁺-activated chloride currents (Miledi and Parker 1984; Schroeder et al. 2008), store-operated Ca²⁺ entry (Yu et al. 2009), and exogenous reporters for Ca²⁺ signals, the impact of heterologously expressed proteins on Ca²⁺ signals can easily be assessed. Such advantages have been leveraged to the extremes as shown by protocols for high-throughput drug screening or ensemble imaging of single channel Ca²⁺ fluxes (Demuro and Parker 2006; Goldin 2006; Papke and Stokes 2010).

XENOPUS OOCYTES AS A REAL CELL: FUNCTIONAL ARCHITECTURE OF Ca²⁺ SIGNALS

Amphibians possess an oogonial stem cell population that generates new oocytes every year. The process of oocyte growth is long (~2 yr) and discontinuous, during which the microscopic oocyte increases many times in size (>10,000-fold) to become a full-grown stage VI oocyte. It is important to understand that during oogenesis, the cell is actively accumulating (lipid) and synthesizing (proteins, mRNAs) reserves of material to autonomously support early embryonic development. The large size of the oocyte results from receptor-mediated endocytosis of yolk protein precursors synthesized in the liver to provide nutrient stores for embryogenesis. The huge nucleus of the growing oocyte engages in protracted and intense RNA synthesis. This is illustrated by the existence of “lampbrush” chromosomes imparting transcriptional activity at rates 1000-fold greater than observed in *Xenopus* embryonic cells (~1.1 ng RNA/h [Anderson and Smith 1978; Anderson et al. 1982]). Collectively, this absorptive and synthetic activity results in a prolonged warehousing of resources to power early embryogenesis. Table 2 conveys the massive scale and enrichment of resources within a *Xenopus* oocyte compared to a generic mammalian cell—the oocyte nucleus alone is ~40,000 times the volume of a tissue culture cell!

The large dimensions and protein content of a full-grown oocyte facilitate a scale and scope of experimental assays that would prove exacting to execute in other cells (including oocytes from other

TABLE 2. Comparison of single cell resources of a *Xenopus* oocyte versus a mammalian cell

		<i>Xenopus</i> oocyte	Mammalian cell (HeLa)	Enrichment (fold)	References
Cell volume		~1 μ L	<1 pL	10^6	Fujioka et al. 2006; Sims and Allbritton 2007
Total protein		~125 μ g	~100–700 pg	10^5 – 10^6	Hallberg and Smith 1975
Total RNA		~4–5 μ g	~1–30 pg	10^4 – 10^6	Sindelka et al. 2010
Nucleus	Volume	~40 nL	~0.2 pL	10^5	Fujioka et al. 2006
	Nucleoporins	~50,000,000	~5000	10^4	Lenart and Ellenberg 2003
	Nucleoli	1000	~1	10^3	Brown and Dawid 1968
ER	Ribosome number	~ 1×10^{12}	~ 1×10^7	10^5	Wolf and Schlessinger 1977; Nielsen et al. 1982
Mitochondria	Number	10,000,000	100–1000s	10^5	Callen et al. 1980; Marinos 1985
	DNA	~4.5 ng	~45 fg/cell	10^5	Webb and Smith 1977; Legros et al. 2004
Plasma membrane	Area	~18–20 mm^2	~2000 μm^2	~ 10^4	Boulter et al. 2006; Sobczak et al. 2010
	Turnover	~750,000 $\mu\text{m}^2/\text{h}$	2000 $\mu\text{m}^2/\text{h}$	~ 10^2	Steinman et al. 1976; Zampighi et al. 1999

Estimates are taken from HeLa cells (an example of an immortalized mammalian cell line) and *Xenopus* oocytes. Examples from other cell lines and *Xenopus* eggs are substituted where we were unable to locate relevant data in the literature.

species). A clear utility is for studying organelle properties and dynamics. The oocyte presents huge spatiotemporal canvases for imaging the native organization and function of Ca^{2+} channels and the properties of heterologously expressed constructs. The size of the oocyte nucleus facilitates studies of transport via single nuclear pore complexes (Peters 2006) and single-channel electrophysiology of intracellular Ca^{2+} channels (Mak and Foskett 1994). Although biochemical techniques are generally less sensitive, the abundance of resources allows for single-cell application of techniques usually applied at a population level. Examples include magnetic resonance spectroscopy and single-cell mass spectrometry for chemical imaging (Lee et al. 2006; Fletcher et al. 2007). A clear caveat is that these experimental opportunities occur in the context of a highly specialized cell—although the oocyte contains plentiful reserves of protein and RNA (the majority is yolk [$\sim 80\%$ of protein] and rRNA [95% of RNA]), content is not enriched proportionally to somatic cells (Brown and Dawid 1968; Chase and Dawid 1972) and organelle morphology, protein distribution, and trafficking events are uniquely tailored to oocyte cell biology (Brown and Dawid 1968; Wall and Meleka 1985; Cordes et al. 1995; Zampighi et al. 1999). Finally, the large size of the oocyte presents challenges, restricting kinetic analysis of currents recorded by conventional two-electrode voltage clamp and optical turbidity restricts imaging depth (Stuhmer and Parekh 1995; Marchant and Parker 2001b; Goldin 2006).

Nevertheless, the *Xenopus* oocyte has proved invaluable for scientists interested in Ca^{2+} signaling. Key advantages include:

- the predominant expression of a single intracellular Ca^{2+} channel (type 1 inositol 1,4,5-trisphosphate (InsP_3) receptor [Parys et al. 1992; Zhang et al. 2007])
- the existence of endogenous phosphoinositide-coupled receptors that release Ca^{2+} and endogenous electrophysiological readouts for Ca^{2+} release activity (Miledi and Parker 1984; Schroeder et al. 2008; Yu et al. 2009)
- the presence of multiple organelle types in the oocyte periphery, including the cortical ER band from which a variety of spatial and temporal patterns of Ca^{2+} release can be triggered and imaged
- the capacity to image InsP_3 receptor function and organization at high resolution (single channels in situ) and over protracted periods of time, for example, during the dramatic reorganizations of ER morphology that occur during oocyte maturation; such studies have shown that InsP_3 receptor sensitivity is regulated with high spatial acuity in both the oocyte and egg, even between contiguous ER regions (Marchant and Parker 2001a; Boulware and Marchant 2005, 2008)
- the utility of the oocyte as a polarized cell, relevant for studying the overall distribution of Ca^{2+} signaling components needed for developmental axis formation (Kume et al. 1997; Saneyoshi et al. 2002)

- finally, as discussed above, the ability to heterologously express proteins that impact Ca²⁺ homeostasis

Methods for utilizing *Xenopus* oocytes to study Ca²⁺ signaling are described in **Nuclear Microinjection to Assess How Heterologously Expressed Proteins Impact Ca²⁺ Signals in *Xenopus* Oocytes** (Lin-Moshier and Marchant 2013a) and **A Rapid Western Blotting Protocol for the *Xenopus* Oocyte** (Lin-Moshier and Marchant 2013b). Both protocols are also applicable for probing the functional architecture of Ca²⁺ channels over a longer time frame, for example, during oocyte maturation into a fertilizable egg.

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