
Preface

Fully grown (stage VI) *Xenopus laevis* oocytes are very large cells, each measuring more than 1 mm in diameter, with an enormous nucleus (also called germinal vesicles) of 300 μm across. These cells are very rich in RNA, each containing up to 4.3 μg total RNA including about 40 ng poly(A)-containing RNA (mRNA), and up to 4 μg of ribosome RNA. The protein content of each oocyte is equally impressive, totaling more than 500 μg . When data are available, the quantities of specific proteins are staggering. For example, each oocyte contains an estimated 50 to 70 ng (1.25–1.75 μM) of the catalytic subunit of protein kinase A (*I*). Several thousand stage VI oocytes can be readily obtained from a single female (costing about US \$25). These oocytes are also physiologically synchronous (arrested at the diplotene stage of the first meiotic prophase) and will remain so for a considerable duration of time (routinely up to a week) when simply placed in an isotonic saline solution with appropriate antibiotics. The lack of requirement for external nutrients or other factors also means easier and better control for experimental manipulations.

Oocytes are best known for their extremely high capacity of protein synthesis—200–400 ng proteins per day per oocyte. Therefore, it is not surprising, but remarkable nonetheless, that one can measure chick brain GABA receptor activities in oocytes injected with total chick brain mRNA, as pioneered by Ricardo Miledi (*see* Chapter 24 for another innovative technique by Professor Miledi). Equally impressive, but less well known, is the assembly of infectious virus particles in frog oocytes via co-injection of genomic viral nucleic acids and capsid mRNAs (*see* Chapter 26). Frog oocytes are perhaps the only commonly used experimental model in which single cell enzymology is not only possible, but routine. For the vast majority of modern day molecular biologists who are most familiar with Western blotting, imagine that you can routinely perform anti-MAP kinase immunoblotting (developed within seconds of the addition of ECL reagents) with as little as one-tenth of a single cell! Because of this unique advantage, Ferrell's group was able to demonstrate the ultrasensitive, positive feedback-regulated, all-and-none MAP activation in single cells (*2*).

For all its advantages, a “frog oocyte facility” is easily and inexpensively assembled. For a low-budget setup, all you need are a dissecting microscope with an external fiberoptic light source (\$3000), a microinjector (\$1500–3000) and a pipet puller (\$1000). Even more important, you do not need extensive training or practice to acquire the necessary techniques, including mRNA injection, because the oocytes are so large. I was never formally trained in a “frog lab.”

Xenopus Protocols: Cell Biology and Signal Transduction is not intended to duplicate or replace the comprehensive frog book edited by Brian K. Kay and H. Benjamin Peng (*3*). Rather, it is intended to complement the Kay/Peng book by focusing on the versatility of frog oocytes and egg extracts in cell biology and signal transduction. As such, no information is included for the even more popular uses of

X. laevis as a developmental model for studying vertebrate embryogenesis. Readers interested in embryogenesis are also referred to a more recent book (4).

Readers will find that different laboratories use slightly different protocols for the same purposes (isolation of oocytes by collagenase treatment and preparation of egg extracts, just to name a couple). I have decided to allow these repetitions as it is impossible to choose one over another. In addition, some modifications may be inherent to the specific biological processes that are being investigated.

I have for many years felt that this wonderful experimental system is underutilized. I hope that *Xenopus Protocols: Cell Biology and Signal Transduction* will serve to increase awareness among the scientific community of the many possibilities that this system can offer. I am particularly intrigued by the possibility that *Xenopus* oocytes will serve prominently as a cell-based model to study functional genomics and proteomics in the postgenomic era.

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References

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