Preface

Sea urchins and other marine invertebrates have contributed fundamentally to our knowledge of cell, molecular, and developmental biology. These contributions, ranging from the discovery of cyclins in the sea urchin, to cellular immunity in the starfish, to transmission of nerve cell signals in the squid giant axon, arise from the incredible diversity of these organisms, the ease with which samples can be prepared, and the availability of sufficient numbers of samples. With sea urchins, for example, you can relatively rapidly obtain large quantities of eggs all of which are arrested at the same stage of the cell cycle. This natural synchronization of these cells has proven useful for understanding the regulation and control of the cell cycle.

One of the most famous contributions of marine invertebrates to cell and molecular biology is the discovery of cyclins [1]. Over several summers at the Marine Biology Laboratory in Woods Hole, MA, Tim Hunt and colleagues demonstrated a protein band that disappeared in fertilized or activated sea urchin eggs, while the other proteins continued to be synthesized [2]. Further analysis showed that this protein band was continuously synthesized before being destroyed about 10 min before each cell division. Following this initial discovery, a similar behavior was discovered in cycling clam eggs and, as we now know, is universally responsible for regulating the cell cycle [3].

These interesting organisms have also contributed basic knowledge that leads directly to discoveries in human physiology with direct relevance to human health. For example, Ilya Mechnikoff and Paul Ehrlich shared the Nobel Prize in Physiology and Medicine in 1908 for their discoveries in immunity. In his Nobel lecture, Mechnikoff describes his work on comparative embryology as being crucial for the ultimate discovery of phagocytosis [4]. Enabling his discovery was the fact that many of the "lower animals" (his words) are transparent and thus allow visualization of cells within the living organism. He observed cells within the body cavity of bipinnaria larvae from the starfish and it occurred to him that these might be like blood cells from vertebrate organisms and that they may play a role in the individual's defense against microorganisms. He tested his idea by inserting a thorn (different sources say a rose thorn or a thorn from a tangerine tree) into the larva and observing that it was surrounded by mobile cells the following morning. In later studies, he observed similar behavior in "water fleas" (*Daphnia*) infected with microbes and then transferred his theories to humans and the infection-fighting role played by phagocytes [4, 5].

With the development of genome projects for many different phyla, the potential for these organisms to contribute more to our understanding of basic cell and molecular processes increases. This Method in Molecular Biology volume addresses the use of sea urchins and other marine invertebrates in the cell and molecular biology laboratory. It covers all aspects of the process beginning with caring for the animals, obtaining gametes, and producing embryos for a variety of organisms in the first chapters. We move on to describing

methods for imaging and other useful experimental tools for cell and developmental biology research. The final portion of the book will present a variety of molecular biological methods and strategies for utilizing the sea urchin genome.

Chapters 1–5 present methods for culturing and caring for a variety of marine invertebrate organisms. Hopefully, these chapters will inspire you to try a new model system in your own research program. In Chapter 1, Richard Strathmann describes techniques for the culture of marine invertebrates. These methods are streamlined and adaptable to the larvae of diverse animals. Many labs would like to give these fantastic animals a try and this chapter is just what you are looking for to get started. The provided methods are sufficient for taking your cultures from fertilization, through embryogenesis and up to metamorphosis. Chapter 2 is a very special chapter, written by Charles Lambert just before his passing in 2011. With great thanks to Gretchen Lambert, his chapter was completed and edited for inclusion in this volume. This chapter focuses on obtaining gametes, performing in vitro fertilization, and culturing embryos from the three different orders of ascidians. Because of their special place as basal chordates, understanding maturation, fertilization, and early development in this important group of animals has been very informative. The Drs. Lambert worked on these intriguing animals throughout their productive careers and have inspired many scientists along the way. Chapter 3 by Anthony Pires describes the culture of the widespread limpet *Crepidula fornicata* from spawning to metamorphosis. This mollusc has emerged as a very useful model system for the study of embryonic and larval life histories because of the development of useful tools including a well-resolved fate map, development of loss- and gain-of-function strategies, and an emerging genomics resource [6]. The culture of the mysterious placozoan *Trichoplax adhaerens* is described by Andreas Heyland and colleagues in Chapter 4. These basal metazoans have interested scientists since their discovery in 1883 because they were difficult to classify, appearing perhaps as cnidarians or sponges [7]. They have also generated recent interest since the publication of their 98 million base pair genome because they show great promise for understanding the evolution of the basic animal body plan [8]. A method for culturing bryozoan larvae and colonies is presented by Michael Temkin in Chapter 5. The Bryozoa are a phylum of diverse aquatic invertebrates that are found in fresh and salt water. There are over 4,000 living species known! Bryozoans are found in the fossil record, but their relationship to other organisms is not certain, making them an interesting group to study for evolutionary reasons.

The field of cell biology developed in response to advances in technology, particularly along with advances in microscopy. The light microscope allowed direct visualization of cells and some subcellular structure, which continued to advance following the introduction of electron microscopy that allowed for visualization of subcellular detail and even to the level of individual proteins. In Chapters 6–9, methods are described for acquiring and analyzing visual information in cell and molecular biology. George von Dassow details methods for processing of images from confocal microscopes in Chapter 6. This is a very practical and useful chapter written in an engaging style. Among many other things, readers will learn how to process 3D images, how to use the free ImageJ software, and how to deal with background noise. In Chapter 7, George Shinn details methods for imaging chaetognatha internal anatomy by light and transmission electron microscopy. The detailed methods described are applicable to most any marine invertebrate organism. There is a particularly extensive and useful **Note** section in this chapter, which will allow the novice to avoid the common pitfalls encountered when learning how to process embedded and sectioned specimens for light and electron microscopy. John Buckland-Nicks gives an excellent description of scanning electron microscopy in Chapter 8 with a focus on visualization of marine

invertebrate gametes. The chapter describes some background theory before giving detailed steps that could be replicated for any organism. Particular attention is paid to the delicate nature of gametes, particularly oocytes. Again, the extensive use of the **Notes** section provides the reader with sufficient advice for successfully adopting these methods in their own laboratory. In Chapter 9, Robert Burke and colleagues give a detailed description of several techniques useful for imaging neural developing in sea urchins. They consider many topics that will be of interest to anyone using light microscopy to study cell and molecular biology (which is probably all cell and molecular biologists). Some items covered include a comparison between the use of paraformaldehyde and methanol as fixatives. What is compatible with in situ hybridization techniques? Can this be combined with immunolabeling of a specific protein antigen. Even if you've done these methods before, you will learn something new in this chapter.

Chapter 10 by Anthony Morgan and Antony Galione describes the preparation of sea urchin egg homogenates that preserve biological function. These preparations have been invaluable for the study of signal transduction in these cells, particularly the regulation of calcium release in response to a variety of agonists. The homogenates allow for visualization of both calcium release from internal stores and the study of the uptake of calcium as the signal is completed. Using homogenates also removes the necessity of microinjection for introducing molecules or proteins into the system. However, microinjection remains a useful method for studying cell biology in these systems, as detailed in Chapter 11 by Alex McDougall, Karen Wing-man Lee, and Remi Dumollard. They describe methods for the microinjection of mRNA expressing fluorescently tagged proteins and for following the expression of these proteins from the egg to the tadpole in the ascidian. As they mention, this method could be combined with a variety of gene knockdown methods to give a very comprehensive picture of specific developmental processes. While described for the ascidian, these methods will also certainly be applicable and useful and most any system that allows for microinjection (basically every egg)! Methods for isolating specific cell types from the sea urchin are described by Celina Juliano, S. Zachary Swartz, and Gary Wessel in Chapter 12. The sea urchin model system has been extraordinarily useful for understanding gene expression in the early embryo and during cell specification. This chapter details methods for isolating these different types of cells, based upon gene expression patterns, which will allow for downstream analysis of specific cell lineages that would not otherwise be possible. This lab thinks outside the box, so you don't know what ideas may be generated by reading their protocol. Chapter 13 by Samantha Cihal and David Carroll describes a method for labeling cell surface proteins in living starfish oocytes and methods for the further analysis of these proteins and, perhaps, their interaction with intracellular signaling molecules. It is hoped that this procedure would be generally useful to most cell types.

The Hedgehog signaling pathway is an important regulator in many different developmental processes and in a wide variety of organisms. It represents a fundamental decision-making pathway. Methods to study the contribution of this pathway to development in the sea urchin by microinjection of morpholino oligos or with small molecule inhibitors are described in Chapter 14 by Jacob Warner and David McClay. Sea urchins have been useful model systems for the study of fertilization and also for the early developmental events that occur following fertilization. In Chapter 15, Jolanta Kisielewska and Michael Whitaker report a clever method for using fluorescence to monitor DNA replication in living cells, along with methods for introducing other fluorescently labeled proteins into these embryos.

Protein phosphorylation regulates the activity of many signaling molecules, in both a positive and negative fashion. As more is learned about developmental mechanisms, it is

becoming clear that the phosphorylation state of these signaling proteins is critical for their function. In Chapter 16, Jose Escalona and Stephen Stricker detail methods for an immunoblotting method to quantifiably analyze protein phosphorylation changes in nemertean oocytes during maturation. These methods can be easily adapted for use in other oocytes or cell types. One big advantage to the method described is that it is film based and, as such, will be useful in a wide variety of situations, including when working at marine laboratories.

In Chapter 17, Aditya Sethi, Robert Angerer, and Lynne Angerer detail methods for the analysis of gene regulatory networks during development in the sea urchin using multicolor fluorescent in situ hybridization (*FISH*) combined with immunohistochemistry. These techniques will allow spatial and temporal resolution of gene expression changes in whole embryos. Julio Harvey outlines a very practical application of a DNA hybridization assay in Chapter 18 to identify specific organisms from environmental water samples. This protocol, called the sandwich hybridization method, makes use of two different DNA probes—one to capture the target and the other for identification. This separation allows for an increased signal-to-noise ratio and it also allows for quantification in the described microarray format.

As mentioned above, the sea urchin has proven to be a perfect model system for the study of fertilization. In recent years, tremendous progress has been made in identifying components of the fertilization pathway and to understand the mechanism of the initial activation event. Chapter 19 by Michelle Roux and Kathy Foltz takes this analysis to the next level by describing methods for the analysis of either individual signaling molecules or global signaling pathways at fertilization in the sea urchin system. Because of the development of the sea urchin genome project (*see* Chapter 20 in this volume), modern genomic and proteomic methods can be applied to this fundamental biological problem. This chapter does a fantastic job introducing the promise and the potential problems that can be encountered when beginning such a research program. Chapter 20 details bioinformatic methods for efficient use of the sea urchin genome data. Andrew Cameron takes us through SpBase, the home of the Sea Urchin Genome Database. From this starting point, the proteins, genes, and genome data can be analyzed using a variety of publicly available software programs. The possibilities are tremendous and available for all to take advantage.

The super-versatile starfish oocyte system is utilized in Chapter 21 by Eiichi Okumura, Masatoshi Hara, and Takeo Kishimoto. They describe methods for the preparation of antibodies for use as specific inhibitors in the oocyte (and even in the nucleus). The protocol details each step of the way, from designing and producing the antigen, to immunizing the animals and purifying the antibodies by affinity chromatography to the microinjection process. This is a remarkably comprehensive chapter and will prove very useful for labs looking to design a project from start to finish to understand the function of a specific protein. In our final chapter, Tetsuo Kida, Shinjiro Matsuda, Atsushi Kuyama, and Tetsuo Toraya describe a method for identifying the 1-methyladenine receptor using photoaffinity labeling. The chapter also details their progress in the identification of a promising target for the long-sought-after 1-MA receptor.

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