Sea Urchins as a Model System for Studying Embryonic Development

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Introduction

Developmental biology is the scientific discipline primarily concerned with embryogenesis, the process by which a single cell (the fertilized egg) gives rise to a multicellular organism. Since the emergence of this field as an experimental science in the late 19th century, biologists have relied on a relatively small number of model organisms, each possessing certain distinctive and useful characteristics, to study the cellular, molecular, and genetic mechanisms of embryogenesis. Sea urchins were one of the first animals to be used for this purpose. As the field of developmental biology has evolved over the decades, the experimental approaches that have been used to study sea urchin development have evolved in parallel, and this model system remains extremely useful today. This article provides a brief overview of (1) the salient characteristics of the sea urchin as a model system for developmental studies; (2) the historical contributions of sea urchins to this field; and (3) prominent, current areas of research. Aspects of each of these topics have been discussed in greater detail elsewhere (Ernst, 1997; 2011; Ettensohn et al., 2004; McClay 2011).

Phylogeny

Sea urchins, together with their close relatives the heart urchins and sand dollars, comprise Class Echinoidea within Phylum Echinodermata. There are several hundred extant species of sea urchins but only a handful are widely used for developmental studies. Sea urchins and all other echinoderms are invertebrate deuterostomes and, together with the hemichordates, form a sister group to the chordates. The embryogenesis of sea urchins is of considerable interest from an evolutionary perspective as it may illuminate features of the developmental program of the last common ancestor of modern deuterostomes. Our current view of the evolutionary history of sea urchins is based upon an extensive fossil record that extends back to the late Ordovician (>450 million years ago), a record that owes its robustness to the fossilizing properties of the calcified endoskeleton present in all sea urchins (Kroh and Smith, 2010).

Reproduction

Sea urchins are found in all oceans of the world, in habitats ranging from intertidal to depths of more than 5000 m (**Fig. 1**). Most species live in relatively shallow tropical or temperate waters. They are dioecious, but the males and females of most species cannot be distinguished based on external morphology. Although sexual reproduction is the norm, the larvae of several echinoid species are capable of asexual reproduction via splitting, or "cloning" (Eaves and Palmer, 2003; Vaughn and Strathmann, 2008). Sea urchins have a seasonal reproductive cycle which usually includes a single spawning season each year. There can be considerable variation in this seasonal reproductive cycle among different populations of the same species due to different local environmental conditions and even variation among individuals within a single population (Williamson and Steinberg, 2002).

Most sea urchins reproduce by broadcast spawning. The most common and ancestral mode of reproduction is indirect (planktotrophic) development, during which the fertilized egg is transformed into a swimming, feeding larva known as a pluteus larva, or echinopluteus. This embryonic phase of development is quite short (1–4 days, depending on the species and temperature). The pluteus larva is bilaterally symmetrical and bears almost no resemblance to an adult sea urchin. After a period of feeding and growth that can last several weeks, the rudiment of the adult body forms within the larva (Smith et al., 2008a). A rapid metamorphosis follows during which most larval tissues are discarded, and a radially symmetrical, juvenile sea urchin emerges from the remnants of the larval body (Burke, 1989). Direct (lecithotrophic) development, a mode of development that bypasses the feeding larval stage, has arisen recently and independently in multiple sea urchin clades (Strathmann, 1985; Wray and Raff,

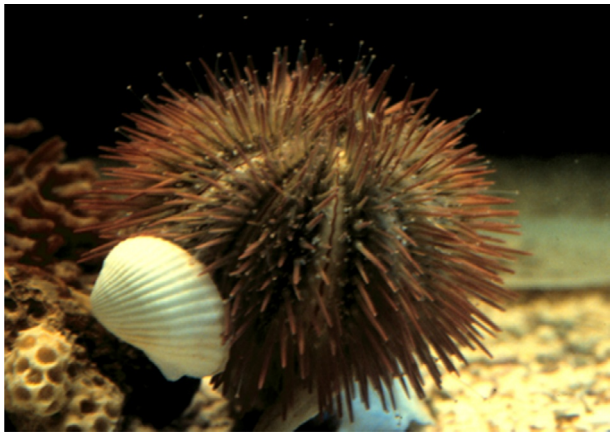

Fig. 1 Adult sea urchin (*Lytechinus variegatus*).

1991; Jeffery et al., 2003). Females of direct developing sea urchins produce fewer, larger eggs than their indirect developing counterparts, and many brood their young.

Characteristics as a Model System for Developmental Studies

Several factors contribute to the value of sea urchins as a developmental model:

1. *Large numbers of synchronously developing embryos can be obtained quickly and easily*. Gravid adults produce enormous numbers of gametes that are easy to collect in the laboratory. A variety of simple methods are used to induce gravid animals to shed their gametes, but injection of 0.5 M KCl into the body cavity is the most commonly used method (Foltz et al., 2004) (**Fig. 2**). A single female can provide many millions of oocytes and a single male many billions of sperm. Both types of gametes are fully competent to carry out fertilization without additional maturation. Fertilization and embryonic development occur externally, both in nature and in the laboratory. Development is initiated synchronously simply by mixing sperm and eggs.

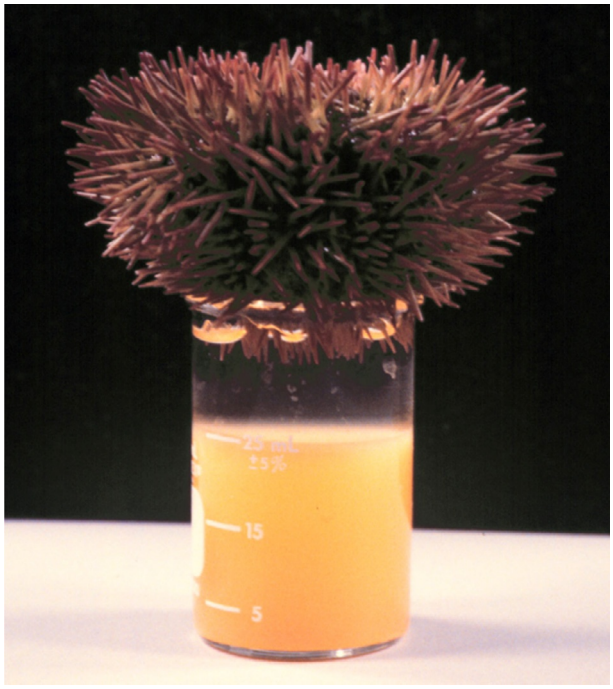

Fig. 2 Adult, female sea urchin spawning mature oocytes in the laboratory. This animal has released approximately 20 million oocytes.

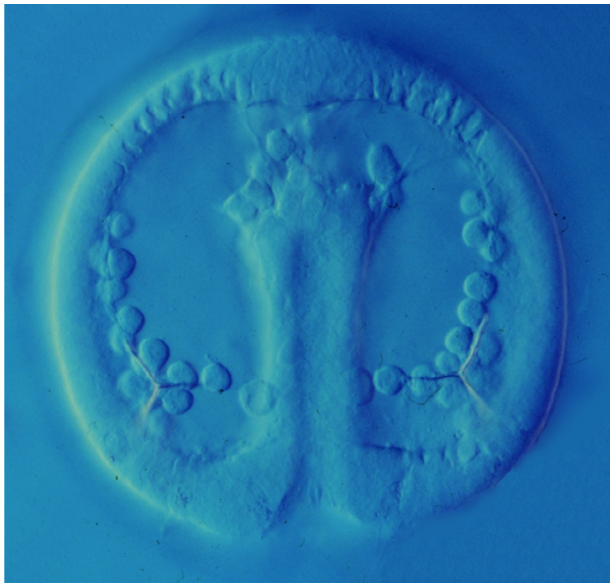

Fig. 3 Live sea urchin embryo at the late gastrula stage, viewed by light microscopy (differential interference contrast optics). The embryo is spherical and approximately 150 μm in diameter. Its optical transparency and simple structure makes possible the direct visualization of individual cells, even those deep inside the embryo.

2. *Embryonic development is rapid.* In many warm-water species, only 1–2 days are required for the fertilized egg to develop into the pluteus larva. One practical consequence of this rapid development is that the time between experimental manipulations and analysis of their developmental effects is very short.
3. *The embryo has a simple structure.* At the late blastula stage, the embryo is a simple, hollow ball composed of about 800 cells surrounding a central cavity (the blastocoel). The number of cells approximately doubles by the end of gastrulation but does not exceed 2000–3000 cells during pre-feeding development. The embryo and pluteus larva have relatively simple morphologies and only 15–20 distinct cell types arise during embryogenesis.
4. *The embryo is highly transparent*. The embryos of many species of sea urchins are almost as clear as glass (**Fig. 3**). This transparency, coupled with the anatomical simplicity of the embryo, facilitates in vivo light microscopic observations of development. Thus, biologists have constructed a very detailed picture of sea urchin embryogenesis at the cellular level and can easily and quickly assess the developmental effects of experimental perturbations.
5. *Small molecules can be used to perturb development*. Sea urchin embryos readily take up many small molecules, including chemical inhibitors and biosynthetic precursors from the surrounding seawater. In the case of chemical inhibitors, this allows precise temporal control over molecular perturbations.
6. *Diverse tools are available for analyzing and manipulating gene expression*. Sea urchins are well suited to all modern approaches for analyzing gene expression, including whole mount immunostaining and in situ hybridization to detect specific proteins and mRNAs, respectively, quantitative polymerase chain reaction (QPCR) to measure RNA levels, and high-throughput methods of gene expression profiling such as Nanostring analysis and RNAseq. Fertilized eggs can be injected with a wide variety of reagents, including DNA, RNA, antisense oligonucleotides, and fluorescent cell markers. Injected mRNAs may encode wild-type or mutant (e.g., dominant negative) proteins. Currently, the standard approach for blocking the function of specific genes is to interfere with the translation or splicing of the target RNA by injecting morpholino antisense oligonucleotides (Angerer and Angerer, 2004). More recently, CRISPR/Cas9-mediated gene editing has been used to efficiently block the function of specific genes during development (Lin and Su, 2016; Oulhen and Wessel, 2016a). Molecular manipulations can be targeted to specific cells by injecting molecules into blastomeres, by using photoactivatable reagents that can be selectively activated in specific cells by light, or by transplanting cells in order to create chimeric embryos that contain a mixture of normal and modified cells. Transgenic embryos are produced by microinjecting linearized plasmids or bacterial artificial chromosomes (BACs) into fertilized eggs, which results in the random incorporation of transgenes into the genomic DNA of a subset of cells in the early embryo in a mosaic fashion (McMahon et al., 1985). This approach has been used in combination with reporter genes to dissect cis-regulatory elements that control the temporal and spatial patterns of transcription of many developmental genes (Smith, 2008).
7. *Extensive genomic resources are available*. As described below (Current Work, Genomics), annotated genome assemblies of various levels of completeness are available for several sea urchin species and constitute an immensely valuable resource for research. Also available are temporal expression profiles for all genes expressed during development, the spatial expression patterns of most regulatory (transcription factor-encoding) genes, and many other genomic resources.

Limitations: Like all model organisms, sea urchins have certain limitations. The most important of these is that the generation time of sea urchins (i.e., the time from egg to egg) is relatively long, lasting several months even in rapidly developing, warm-water species. In addition, adult animals require a relatively large amount of laboratory space for their maintenance compared with some other developmental models. For these reasons, studies that require multiple rounds of mating are very rarely carried out in the laboratory. Conventional forward genetic screens have not been practical and there are no stable transgenic strains of sea urchins (although, as noted above, methods for transient transgenesis are well developed). There are no inbred strains; therefore, biologists work with natural populations of animals, which exhibit some degree of genetic polymorphism. There are also no immortalized cell lines derived from sea urchin tissues, although primary cultures of embryonic cells are simple to establish. Lastly, the limited reproductive season of these animals is a drawback. In practice, this can be mitigated by obtaining animals from different locations over the course of the year (thereby exploiting local variations in reproductive cycle within a species) or by working with multiple species that have different reproductive seasons. Adults obtained in gravid condition can be maintained in the laboratory for several weeks without a decline in fertility.

A Brief Overview of Embryonic Development in Indirect Developing Sea Urchins

Developmental studies have focused primarily on species that exhibit planktotrophy, as this is the most common mode of development within the clade and is particularly easy to study in the laboratory. The eggs of indirect developing species are isolecithal (i.e., they have sparse, evenly distributed yolk) and typically 100–200 μm in diameter. They have completed meiosis and are polarized along at least one major maternal axis, the animal–vegetal (A–V) axis. Cleavage is holoblastic (i.e., cleavage furrows pass completely through the egg). A stereotypical pattern of cleavage produces a 16-cell embryo composed of three tiers of cells arranged along the A–V axis; the mesomeres, macromeres, and micromeres. Continued mitotic cell division produces a spherical blastula consisting of 500–800 cells organized as a monolayered epithelium surrounding the blastocoel. Gastrulation begins 8–10 h after fertilization in warm-water species and results in the internalization of the vegetal region of the blastula, which consists of the presumptive mesoderm and endoderm. Sea urchins are deuterostomes; they gastrulate through a blastopore which becomes the anus, while the mouth forms later as a secondary opening between the ectoderm and the archenteron. Gastrulation includes several modes of cell movement, including ingression (epithelial–mesenchymal transition), epithelial invagination, and oriented cell rearrangement. During postgastrula development, the definitive organs of the pluteus larva are elaborated, including a calcitic endoskeleton, a tri-partite intestinal tract, musculature, a simple nervous system, a ciliary band (which is used for locomotion and feeding), connective tissue, and coelomic pouches.

Gene transcription begins shortly after fertilization and the rate of RNA synthesis/nucleus is greatest during early cleavage (Davidson, 1986). Early embryonic gene expression territories arise as a result of the partitioning of asymmetrically distributed maternal factors into distinct cell lineages during cleavage. These initial territories are refined and further partitioned by a suite of intercellular signals (Angerer and Angerer, 2003). Several embryonic cell types express terminal differentiation genes even prior to the onset of gastrulation. Additional cell–cell interactions continue during gastrulation and postgastrula development, leading to the emergence of the 15–20 cell types characteristic of the pluteus larva. Despite the early territory-specific gene expression evident in the sea urchin, the developmental programs of several cell lineages remain remarkably plastic, even late in embryogenesis (Sharma and Ettensohn, 2011; Cheng et al., 2014).

Historical Contributions of Sea Urchins to Developmental Biology

1. *Early contributions to embryology and genetics*: Early studies on the gametes and embryos of sea urchins led to many fundamental advances in the fields of embryology and genetics. The earliest papers describing the in vitro fertilization and development of sea urchin embryos were published separately by Dubosse, Derbes and von Baer in 1847 (see Briggs and Wessel, 2006). Early work with sea urchins revealed the central role of chromosomes and the nucleus in heredity and development (Hertwig, 1876), the nonequivalence of chromosomes (Boveri, 1907), and the asymmetric distribution of factors in the egg cytoplasm (Selenka, 1883; Boveri, 1901). The blastomere isolation experiments of Driesch were among the very first in the field of experimental embryology and led to the discovery of regulative development (Driesch, 1892). Several decades later, the famous cell isolation and transplantation experiments of Hörstadius provided evidence of inductive interactions among embryonic cells and helped establish the concept of developmental gradients (Hörstadius, 1939).
2. *The advent of the modern era*: During the middle decades of the 20th century, work with sea urchin embryos led to important advances in the new field of molecular biology, including contributions to the emergence of the central dogma (reviewed by Ernst, 2011). Work by R. Britten, E. Davidson, and others made sea urchins a preeminent model for the quantitative analysis of gene expression during early development (Davidson, 1968). At the same time, studies using sea urchin gametes and cleavage stage embryos made fundamental contributions to our understanding of fertilization (Epel, 1967; Steinhardt et al., 1971; Steinhardt and Epel, 1974; Johnson and Epel, 1976; Vacquier and Moy, 1977; Zucker and Steinhardt, 1979) and cell division

(Rappaport, 1961, 1967; Evans et al., 1983). Time-lapse microscopy of live sea urchin embryos led to a new appreciation of the dynamic cell shape changes and cell movements that underlie embryonic development (Gustafson and Wolpert, 1967).

Current Work

1. *Genomics*: A fundamental advance was made when the initial assembly and annotation of the genome of the purple sea urchin, *Strongylocentrotus purpuratus*, a species widely used for developmental studies, was published in 2006 (Sea Urchin Genome Sequencing Consortium, 2006). The *S. purpuratus* genome assembly and annotation have since been refined and many additional genomic resources have been generated for this species, including temporal gene expression profiles for all embryonically expressed genes, spatial expression (in situ hybridization) data for most regulatory genes and many other embryonically expressed genes, and genome-wide chromatin accessibility (ATAC-seq) data across development (Howard-Ashby et al., 2006; Materna et al., 2010; Tu et al., 2012, 2014; Cameron, 2014). With the advent of RNA-seq, a method for quantifying RNA levels on a genome-wide scale, gene expression data from the gametes and embryos of many sea urchins and other echinoderms are accumulating rapidly (Rafiq et al., 2014; Tu et al., 2014; Barsi et al., 2015; Gildor et al., 2016; Israel et al., 2016; Janies et al., 2016; Pérez-Portela et al., 2016). These studies provide a detailed picture of the programs of differential gene expression deployed in the sea urchin embryo. A genome web browser and database, originally called SpBase (Cameron et al., 2009) but renamed Echinobase, serves as a central repository for genomic information not only for *S. purpuratus* but also for a rapidly growing number of other echinoderms.
2. *Gene regulatory networks*: Work pioneered by E. Davidson's laboratory at Caltech has established the sea urchin as a preeminent experimental model for the elucidation of developmental gene regulatory networks (GRNs). These are dynamic networks of regulatory and signaling genes and specify combinatorial interactions among these genes as well as their inputs into downstream effectors. GRNs are proving to be powerful tools for analyzing the genetic control and evolution of development (Peter and Davidson, 2015). Current work using sea urchins is aimed at elucidating GRN architecture (Li et al., 2014; Rafiq et al., 2014; Andrikou et al., 2015) and evolution (see 4, below), the control of tissue morphogenesis by GRNs (Saunders and McClay, 2014; Martik and McClay, 2015; Ettensohn and Dey, 2017), and the regulation of GRNs by intercellular signaling pathways (Cui et al., 2014; Sun and Ettensohn 2014). GRN analysis has been enhanced by recent technical advances, including the multiplexing of reporter constructs (Nam and Davidson, 2012) and FACS-based isolation of specific early embryonic cell types (Barsi et al., 2015). Work on the regulatory genomics of sea urchins is spurring the application of similar approaches to other animal models (Van Nostrand and Kim, 2011; Satou and Imai, 2015).
3. *Embryonic patterning*: Recent studies on embryonic axis formation have revealed essential roles for conserved signaling pathways, most notably the TGFβ and Wnt signaling pathways, in patterning the embryo along the A–V, dorsal–ventral, and left–right axes (Duboc et al., 2004; 2005; Luo and Su, 2012; McIntyre et al., 2013; Range et al., 2013; Lapraz et al., 2015; Range and Wei, 2016). In most respects, these studies point to a striking evolutionary conservation of basic patterning mechanisms across deuterostomes, although sea urchin-specific inventions have also been revealed (Revilla-i-Domingo et al., 2007; Haillot et al., 2015). Embryonic precursors to the germ line have also been identified (Yajima and Wessel, 2011) and important features of their distinctive molecular program of specification have been elucidated (Swartz et al., 2014; Oulhen and Wessel, 2016b).
4. *Evolutionary developmental biology*: Work with sea urchins has stimulated the emergence of a new field, comparative regulatory genomics, which explores GRN evolution. In this context, several recent studies have illuminated comparative aspects of skeletal, muscle, and neural development (Angerer et al., 2011; Bishop et al., 2013; Andrikou et al., 2015; Cheatle Jarvela et al., 2016; Garner et al., 2016; Mao et al., 2016; Cary and Hinman, 2017). Other studies have examined the rewiring of GRNs during the major shift in developmental mode from indirect to direct development (Smith et al., 2008b; Israel et al., 2016) and aspects of the robustness and evolvability of GRNs (Garfield et al., 2013; Gildor and Ben-Tabou de-Leon, 2015; Ben-Tabou de-Leon, 2016).

Prospects

Many of the characteristics of sea urchins that first attracted developmental biologists to this model system more than a century and a half ago remain remarkably relevant today. An organism that can provide large numbers of synchronously developing embryos of any embryonic stage is particularly well suited to modern, high-throughput approaches for analyzing genome structure and gene expression. The optical transparency of the embryo continues to be vitally important as new light optical tools, including new light-based biosensors and automated approaches to image generation and analysis, are developed (Villoutreix et al., 2016). The external development of the embryo and its permeability to small molecules means that new generations of chemical inhibitors (e.g., Tran and Zheng, 2017) can be exploited. Powerful molecular approaches for manipulating gene function, including morpholinos and CRISPR/Cas9-mediated gene editing, have mitigated many of the limitations of sea urchins with respect to transmission genetics. Comparative/evolutionary studies on sea urchins and other echinoderms continue to benefit from a robust phylogeny and from the increasing availability of diverse species (and their embryos) across a wide range of evolutionary distances. For all these reasons, sea urchins continue to be a powerful experimental model for dissecting fundamental mechanisms of embryonic development.

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