Sea Urchins as Lab Animals for Reproductive and Developmental Biology

Amro Hamdoun, Catherine S Schrankel, Katherine T Nesbit, and Jose A Espinoza, University of California San Diego, San Diego, CA, United States

Early History of the Sea Urchin Embryo as a Lab Animal

The sea urchin has a rich history as a model organism, dating back to the mid 1800s (**Table 1**; reviewed in Ernst, 2011). Given the ease of gamete collection and embryo observation, the sea urchin became an important experimental model for 19th century scientists exploring the fundamental questions of development: What is the role of the nucleus in development (as sperm and egg each appear to have one)? What information exchange occurs at the union of egg and sperm? How does this enable the fertilized egg to blossom (or "differentiate") into an adult organism that exhibits the same body plan, cell types and organs as the parents? Early studies in sea urchins overturned long-held theories of preformation – that a miniature individual is already encapsulated in totem within a sperm (the "homunculus") – and demonstrated that the individual is formed by the fusion of nuclear material from both sperm and egg.

A Single Sperm for Every Egg

The sea urchin was used to study fertilization as early as the 1840s, by Derbs and colleagues (reviewed in Briggs and Wessel, 2006). In the later 1800s, using sea star and sea urchin eggs, Hermann Fol and Oscar Hertwig were the first scientists to observe that a single sperm penetrates the egg membrane during fertilization (Hertwig, 1877). Fol also observed that an envelope immediately forms around the egg, presumably to prevent additional sperm from fusing (known as "polyspermy") (Fol, 1877). Fol and Hertwig also documented that the nucleus of a successfully penetrated sperm will fuse with the nucleus of the egg.

Table 1 Milestones and major discoveries in sea urchin embryology ~1875–2000s

Fusion of sperm and egg nuclei at fertilization; fusion of a single sperm with the egg at fertilization	Hertwig (1877), Fol (1877)
Demonstration of phagocytosis and foundation of the cellular theory of immunology (Nobel Prize 1908, Elias Metchnikoff)	Metchnikoff (1893)
Chromosomes in nucleus are determinants for development; individual chromosomes possess different qualities	Boveri (1902)
DNA is found in chromosomes and RNA is found in cytoplasm of all cells; first correlation of RNA and protein synthesis	Brachet (1945)
Isolation of the mitotic apparatus during cleavage	Mazia and Dan (1952)
Discovery of long-lived maternal mRNAs and polyribosomes	Gross and Cousineau (1963), Monroy and Tyler (1963)
First characterization of hybridization kinetics of single and double-stranded DNA; gene-battery model of eukaryotic transcription proposed	Britten and Kohne (1968), Britten and Davidson (1969)
Cloning of the first eukaryotic gene (histone)	Kedes *et al.* (1975b)
Na+/H+ exchanger identified; first description of amino acid transporter activation at fertilization; first isolation of intact cortical granules	Vacquier (1975), Epel (1975)
First demonstration of the electrical block to polyspermy in an animal egg	Jaffee (1976)
Isolation of bindin, the first protein shown to bind sperm to eggs	Vacquier and Moy (1977)
Demonstration of the first cyclin (2001 Nobel Prize to Tim Hunt)	Evans *et al.* (1983)
First demonstration of chemotaxis of sperm to an egg-derived peptide (resact)	Ward *et al.* (1985)
Discovery of the Ca^{2+} messenger, cyclic ADP ribose	Lee *et al.* (1989)
Cloning of the first membrane receptor guanylyl cyclase, involved in sperm binding	Chinkers *et al.* (1989)
Accumulation of beta-catenin specifies the animal/vegetal axis of sea urchins	Wikramanayake *et al.* (1998); Logan *et al.* (1998)
Sea urchin endomesoderm GRN: the first and most extensive analysis of the specification of a germ layer lineage in any organism	Davidson *et al.* (2002)
Isolation of the bindin receptor EBR1 from sea urchin eggs	Kamei and Glabe (2003)
S. purpuratus genome sequenced: Expansion of innate immunity, defensome, and select TF gene families	Sodergren *et al.* (2006); special issue of Developmental Biology (Davidson *et al.*, 2006)
Identification of the primordial germ cell lineage	Juliano *et al.* (2006, 2010)
Demonstration of the activation of xenobiotic transporters at fertilization, and the xenobiotic metabolism pathways of the embryos; first complete description of embryonic and larval immune system of the purple sea urchin	Hamdoun *et al.* (2004), Shipp and Hamdoun (2012), Ho *et al.* (2016)
First demonstration of CRISPR/Cas9 editing in the sea urchin embryo	Lin and Su (2016)
Deuterostome origins of the Spemann organizer	Lapraz *et al.* (2015)

The Nuclear Basis of Inheritance

Fol and Hertwig's work on pronuclear fusion illuminated that the zygote contains nuclear substance derived from both parents. Biologist Theodor Boveri subsequently demonstrated that nuclei were necessary for proper development of the fertilized egg. Boveri also characterized that a normal set of chromosomes in each cell is required for early development (Boveri, 1902). By watching chromosomes separate during mitosis of urchin zygotes, Boveri was the first person to make the giant conceptual leap to state that Mendel's factors of inheritance must be on the chromosomes. Around the turn of the 20th century, Hans Driesch also used sea urchin embryos to define the equipotent potential of early quadrants of the embryo ("blastomeres"). He showed that one blastomere isolated from a 2 or 4-cell stage sea urchin embryo can develop into a smaller, yet complete larva. These experiments were refined by Sven Horstadius in the 1930s who clarified the role of localized determinates in the egg (molecules that define future body axes) by splitting or stretching individual blastomeres or embryos along different axes and assessing developmental outcomes (reviewed in Ernst, 2011).

Sea Urchins Used in the Lab

Major Species Used and Their Experimental Advantages

Sea urchins are ancient marine invertebrates of Class Echinodea within the phylum Echinodermata. There are approximately 900 extant species of sea urchins distributed throughout the world's coastal oceans. Of these a handful have been used as lab animals, primarily *Strongylocentrotus purpuratus, Lytechinus variegatus, Arbacia punctulata, Hemicentrotus pulcherrimus* and *Paracentrotus lividus* (**Table 2**) which are native to the Pacific, Atlantic and Mediterranean Oceans. Several of these species have fully sequenced and publically available genomes (www.echinobase.org). Two other species, including *Lytechinus pictus* and *Temnopleurus hardwicki* are also useful as lab models, due their relatively small adult size and shorter generation times.

Table 2 Summary of species information and breeding season for select sea urchin lab species

Species name	*Common name*	*Habitat range and temperature*	*Breeding season*[a]	*Egg diameter*	*Key developmental stages*[a] *(time after insemination)*	
Strongylocentrotus purpuratus	Purple urchin	Alaska-Baja California; 12–17°C	November–March	80 μm	Blastula	16 h
					Gastrulation	30 h
					Pluteus larva	4 Days
					Metamorphosis	40–80 Days
Lytechinus variegatus	Green urchin	North Carolina-Bahamas; 20–25°C	May–September	120 μm	Blastula	6 h
					Gastrulation	11 h
					Pluteus larva	20–24 h
					Metamorphosis	30–40 Days
Lytechinus pictus	Painted urchin	Southern California; 18–22°C	May–September	120 μm	Blastula	6 h
					Gastrulation	24 h
					Pluteus larva	48 h
					Metamorphosis	30–40 Days
Temnopleurus species (*T. toreumaticus* and *T. reevesii*)	Japanese urchin	Western Pacific; Japan and China Seas; 22–26°C	July–January	100 μm	Blastula	8–10 h
					Gastrulation	11–12 h
					Pluteus larva	28–30 h
					Metamorphosis	30–33 Days
Paracentrotus lividus	Mediterranean urchin	Mediterranean Sea-Eastern Atlantic; 18–25°C	May–September	75 μm	Blastula	4 h
					Gastrulation	5 h
					Pluteus larva	24 h
					Metamorphosis	20–30 Days

[a]Reproductive cycles and time to metamorphosis may fluctuate, based on local environmental conditions; the earliest pluteus larval stage (2–4 arm) is indicated.

Sources: Reproduced from Strathmann, R.R., 1987. Larval feeding. In: Giese, A.C., Pearse, J.S., Pearse, V.B. (Eds.), Reproduction of Marine Invertebrates. Volume IX: General Aspects: Seeking Unity in Diversity. Pacific Grove, CA: Blackwell Scientific Publications, Palo Alto/Boxwood Press, pp. 465–550 and Pearse, J., Cameron, R.A., 1991. Echinodermata: Echinoidea. In: Giese, A.C., Pearse, J.S., Pearse, V.B. (Eds.), Reproduction of Marine Invertebrates. vol. VI: Echinoderms and Lophophorates. Pacific Grove, CA: Boxwood Press, pp. 514–662.

The purple sea urchin, *S. purpuratus*, was the first echinoderm species to have its genome sequenced, and is the most widely used in the lab. The genome of *S. purpuratus* is approximately 814 million bases in length and encodes 23,300 genes (Sodergren *et al.*, 2006). As basal deuterostomes, the branch of evolution leading to vertebrates, sea urchins share many genes with humans. Complete developmental transcriptomes from multiple stages of *S. purpruatus* development are publically available at www.echinobase.org. Roughly one third of urchin genes encode proteins that are found in humans. These include many genes that are responsible for human genetic diseases. Remarkably, sea urchins also have a diverse repertoire of genes involved in hearing and vision, the latter genes functioning in photoreception of the adult tube feet. They also exhibit a significantly expanded innate immune gene repertoire compared to vertebrates.

The defining feature of sea urchins as lab animals is their extreme fecundity. Ripe animals can be easily induced to spawn by gentle shaking, injection of potassium chloride (KCl) into the coelomic cavity, or by mild electric shock. In the case of potassium chloride, the potassium ions cause muscle contractions that expel gametes. During spawning, eggs or sperm are released through five gonopores located at the top (aboral) surface of the animal, encircling the anus. A gravid female purple urchin yields 5–10 mL of packed eggs, while males produce approximately 0.5–1 mL of "dry" (i.e., prior to dilution of seawater) semen. "Dry" *S. purpuratus* semen is consistently 4×10^{10} cells per mL. Eggs with jelly coats removed are 2.8×10^{6} eggs per mL. Egg sizes vary from 70–150 μm in diameter depending on species (**Table 2**). Eggs can be maintained for several days in sea water containing antibiotic(s) such as ampicillin, sulfamethoxazole and/or trimethoprim to prevent microbial growth. Concentrated sea urchin sperm collected directly from the surface of the animal are immotile until diluted into sea water. If collected directly from the males, sperm can be stored for several days at 4°C.

Adult Anatomy and Anatomical Structure of Gonads

Sea urchins typically range in size from 6 to 12 cm. The largest species can reach up to 36 cm in diameter. Adult sea urchins have radial symmetry: the body is roughly spherical and is composed of five equally sized parts radiating out from a central axis. The inner shell, called a test, is made up of interlocking plates of calcium carbonate material and is covered by a thin skin and rows of spines (the classification "Echinoderm" derives from the Greek words for "spiny skin"). Spines vary in size and diameter according to species. An internal water vasculature system pumps water through the spines and tube feet, which are flexible tube-like structures that terminate with miniature suction cups. The internal organs are enclosed within the test, and the nervous system consists of a small nerve net encircling the mouth, from which five nerves enervate the rest of the animal. The mouth is found on the bottom, or oral face, of the animal. The sea urchin "jaw" consists of five bony teeth and tongue-like protrusion, collectively known as Aristotle's lantern.

Sea urchins are benthic creatures and eat plant and animal matter, largely preferring kelp, algae, and sponges in their rocky habitats, as well as decaying matter that settle down from the water column. Sea urchins have a simple digestive tract encircled within the test. The anus is located at the aboral surface of the animal. The body cavity has an open circulatory system and is filled with coelomocytes. Coelomocytes perform essential clotting, scavenging and phagocytosis functions. Passive gas exchange in the coelomic fluid occurs through gill-like structures located around the mouth.

The gonads also reside within the body cavity and comprise a much as 30% of the body mass. The gonad of the sea urchin is the major reproductive and nutrient storage organ for the adult. The reproductive cycle is unique to each species (see **Table 2**), and is closely linked to photoperiod, water temperature, and food availability (reviewed in Pearse and Cameron, 1991). The gonad is made up of reproductive cells (oogonium, oocyte and ovum stages for females; spermatogonium, spermatocyte, spermatid and spermatozoon for males) as well as storage cells, also known as nutritive phagocytes (NPs). Various proteins, carbohydrates and lipids that are needed for gametogenesis and emergency metabolism are stored within the NPs. The percentage of NPs vs reproductive cells contributes significantly to the size and quality of the gonad, and varies throughout the reproductive cycle.

Reproductive Cycles

Despite the differences in spawning seasons for individual sea urchin species (**Table 2**), sea urchin reproductive cycles can be divided broadly into four stages (reviewed in Walker *et al.*, 2007; Pearse and Cameron, 1991). Stage I occurs immediately post-spawning (egg or sperm release) and lasts for about 3 months. Reproductive cells remain sparse at the beginning of Stage I, as NPs phagocytose unused egg and sperm precursors. The number of NP cells increases during this stage, yet reproductive cells eventually reemerge around the periphery of the gonads by the end of Stage I. In Stage II, which lasts 3–4 months, NP renewal continues and reproductive cells increase in number and size at the gonad periphery. By Stage III, the reproductive cells migrate into the gonad and continue to proliferate. This stage can last several months depending on the species, and the ratio of reproductive cells to NPs increases markedly during this time. At stage IV, gonads are packed full of differentiated gametes: eggs or sperm, whereas NP numbers are reduced or absent. Spawning occurs towards the end of Stage IV, in which most or all of the gametes are released. In the lab, this can be stimulated by 0.5 M KCl injection or by gentle shaking.

Fertilization and Early Cleavages of the Sea Urchin Embryo

Fertilization of Sea Urchin Eggs

The sea urchin is an excellent organism for live demonstration of fertilization and early development in lab or classroom settings (see Relevant Websites section). *In vitro* fertilization is easily conducted by mixing diluted eggs and sperm of the same species in sea

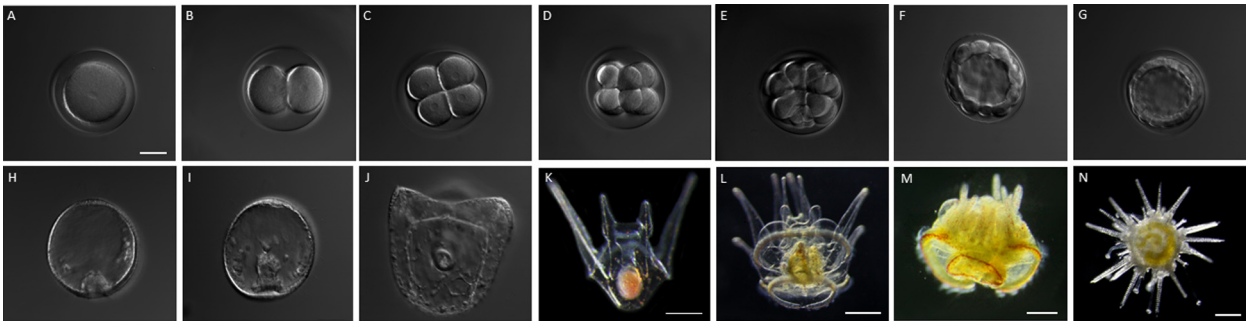

Fig. 1 Larval development of the sea urchin, *Lytechinus pictus*. Sea urchins were raised at room temperature. (A) Newly fertilized zygote; (B) 2-cell stage, 1.5 h post fertilization (hpf); (C) 4-cell stage, 2.5 hpf; (D) 8-cell composite image of two focal planes within an embryo, 3.5 hpf; (E) 16-cell stage, 4hpf; (F) 60-cell stage, 5hpf; (G) Early blastula before hatching from the fertilization envelope, 6.5 hpf; (H) Early gastrula, 24hpf; (I) Mid-stage gastrula, 26 hpf; (J) Prism larvae, 43.5hpf; (K) 4-arm pluteus larvae; (L) 8-arm stage; in a competent larvae, the rudiment that becomes the juvenile body begins to form around the region of the gut (yellow mass in the center of the embryo); in (M) the rudiment grows and the juvenile emerges from the larval body during metamorphosis; (N) Newly settled juvenile urchins are approximately 1 mm in diameter and form mouth parts to begin feeding on benthic diatoms and biofilms after 3 days. Later, their diet incorporates kelp and other macroalgae. Settlement of the juvenile takes approximately 4–5 weeks from fertilization. Scale bar = 50 μm for (A)–(J). Scale bar = 0.25 mm for panels (K)–(M), and 0.5 mm for panel (N).

water. Given that sea urchin spawn externally, mechanisms exist to ensure species fidelity of the sperm and egg interaction. Sea urchin sperm contain a molecule known as bindin that is released during the acrosome reaction, and binds to vitelline layer of conspecific eggs. Evolution of this molecule is essential for the reproductive isolation of different species of sea urchin (Vacquier and Moy, 1977).

In the lab, when mature gametes are used at appropriate concentrations, successful fertilization routinely exceeds 95% of all eggs. Embryos develop through gastrulation within 2–3 days of fertilization, requiring nothing more than temperature-controlled sea water (**Fig. 1**). The feeding larval stage of the sea urchin, termed the pluteus larva, begins several days after fertilization and lasts from several weeks to months depending on species (**Table 2**). The rudiment, or juvenile sea urchin, develops indirectly within this larva and sheds the larval body upon settlement onto the substratum. The larvae and newly settled urchin juveniles feed on phytoplankton including microalgae and diatoms such as *Rhodomonas* and *Nitzschia*. Juveniles become gravid between 6 months and 2 years post-settlement, depending on the species.

Egg Activation and Surface Reorganization

Eggs of sea urchins are mature at release, having completed meiosis and arrested at the G1 phase of mitosis. Eggs are quiescent at spawning, but undergo a massive increase in metabolic activity at fertilization (reviewed in Pearse and Cameron, 1991). The initial signal for activation is a wave of calcium ions that sweeps across the egg, from the point of sperm fusion. Calcium release from intracellular stores is mediated by canonical second messengers, including the molecule cyclic ADP ribose (cADPR) which was discovered in sea urchins (Lee *et al.*, 1989). These initial signaling events lead to a rapid change in membrane voltage from −70 to +20 mV, which acts as a fast block to fusion of other sperm with the egg (Jaffee, 1976). This is termed the fast block to polyspermy.

Following fertilization, dramatic cell surface reorganization involving sequential waves of vesicles insertion and membrane retrieval transforms the surface of the egg from one adapted to bind and fuse sperm to one adapted to promote development (reviewed in Vacquier, 1981). A conspicuous consequence of this reorganization is elevation of the fertilization envelope (**Fig. 1(A)**), which occurs within minutes of fertilization and is mediated by the exocytic release of 16,000 (in *S. purpuratus*) cortical granules docked at the egg surface. This envelope is formed by elevation of a glycoprotein meshwork under the plasma membrane termed the egg vitelline layer as proteins are released from the cortical granules (Vacquier *et al.*, 1973). The resulting envelope is hardened by enzymes present in the cortical granules. This envelope acts as the slow block to polyspermy, and remains around the embryo until hatching thus likely also acting to protect against physical damage of the embryo.

The surface changes in the egg also include the activation of a several types of membrane transport activity including a Na^+/H^+ exchanger, amino acid transporters, and xenobiotic transporters. The exchanger alkalinizes and activates the egg, while amino acid transporters concentrate nutrients from sea water into the egg (Epel, 1975). A notable surface change is an 80–100 fold increase in multidrug transporters activity by insertion of vesicles containing a homolog of mammalian P-glycoprotein (P-gp), the protein responsible for chemotherapeutic resistance of several types of cancer (Hamdoun *et al.*, 2004). In early development, P-gp is responsible for effluxing environmental toxins and metabolic by-products from the embryo.

Early Cleavage Stages

An important feature of sea urchin embryonic development is the synchrony of development across embryos. As such, the early cleavage stages of sea urchins have been favorite models for the study of cell division. The mitotic apparatus was first isolated as

a structure in the sea urchin (Mazia and Dan, 1952), as were cyclins, which are cell cycle regulators that oscillate in abundance during the cell cycle and thereby regulate mitotic progression (Evans *et al.*, 1983). The duplication of the centrosome, which gives rise to the two poles of the mitotic apparatus and is the heart of the division apparatus of all eukaryotic cells, was first isolated from sea urchin zygotes (Thompson-Coffe *et al.*, 1996).

The first three cleavages of sea urchins are synchronous and symmetric. By the fourth cell cycle, cells at the vegetal pole of the embryo undergo two critical asymmetric cleavage events. The fourth cleavage produces four cells at the vegetal pole termed micromeres that eventually contribute to the larval skeleton. At the fifth cleavage, the micromeres divide once more to form four daughter cells termed the small micromeres. These small micromeres express canonical molecular markers of the germline (*vasa*, *nanos* and *piwi*) and are the presumptive primordial germ cells (PGCs), the embryonic precursors to the germline (Juliano *et al.*, 2006, 2010). Ablation of these cells results in formation of adults that are morphologically normal but lack egg or sperm (Yajima and Wessel, 2012).

Segregation of the Primordial Germ Cell Lineage

The germline lineage contributes to gametogenesis (the development of egg and sperm) in adult organisms. The location and developmental stage of PGC specification differs among species. For many animals, PGCs are specified by an inherited mechanism, in which determinants within the oocyte get partitioned into cells that subsequently acquire the germline fate. In contrast, an inductive signaling mechanism generates the germline in other animals. Sea urchins have a distinct embryonic segregation of PGCs. They appear to utilize an inherited mechanism of germline formation, whereby germline genes present in the egg are selectively retained only in the PGCs, and degraded elsewhere in the embryo (reviewed in Wessel *et al.*, 2014).

Sea urchin embryos form four PGCs (also termed small micromeres), at the vegetal pole at the fifth cleavage. The cells divide only once more in early development to form eight cells. The eight PGCs are motile cells capable of migration shortly after their formation, although they remain relatively stationary on the developing gut tube during gastrulation (Campanale *et al.*, 2014). At the end of gastrulation these cells home to the left and right coelomic pouches in a characteristic 5-left/3-right pattern (Pehrson and Cohen, 1986) (**Fig. 2**). The cells on the left seed the germline of the juvenile, while those on the right pouch undergo apoptosis. The evolutionary significance of this asymmetric germ cell segregation, and subsequent programmed death of the right fated cells, remains unclear. An intriguing hypothesis is that this serves as some PGC quality control mechanism.

The motility mechanisms for left-right segregation to the coelomic pouches are not yet well understood. It is hypothesized that the extended filopodia sense migratory cues, in the form of a morphogen or signaling gradient. In other animals, germline chemoattractants are derived from rhodopsin, CXCR4, or phospholipid signaling pathways. In the sea urchin system, the exact signals that home the PGCs have yet to be determined, although several transcription factors that operate in the left coelomic pouches (Materna *et al.*, 2013) have been implicated upstream of the homing signal (Martik and McClay, 2015).

An important aspect of PGC biology in all animals is that specification and migration occurs during a vulnerable window of inductive intercellular signaling and (re)programming. This requires a system that protects the cell from mutagens, as these could directly harm the next generation. Unexpectedly the protective transporters that are activated at fertilization are selectively reduced in the sea urchin primordial germ cells (Campanale and Hamdoun, 2012), suggesting a potential trade-off between signaling and protection pathways. The significance of this down-regulation is unclear, however it could be required for the detection of developmental signaling molecules. This phenomenon has been exploited to load sea urchin primordial germ cells with fluorescent dyes and isolate them to purity using flow cytometry (Swartz *et al.*, 2014).

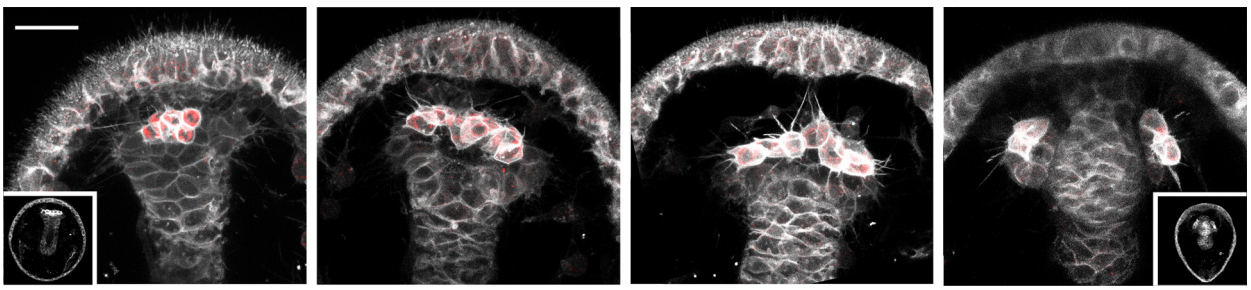

Fig. 2 Migration of sea urchin primordial germ cells (PGCs) in *S. purpuratus*. The 8 PGCs (red) are located in a cluster at the tip of the developing gut tube (the archenteron) at the end of gastrulation (left inset). The PGCs at this stage are rounded, and maintain membrane (grey) contact with neighboring PGCs. Once migration is initiated the PGCs adopt an amoeboid shape and have short filopodia. In the final stage of migration the PGCs produce long filopodia and segregate into coelomic pouches on the left and right side of the archenteron (right most panel, right inset). Scale bar = 20 μm.

Genetic and Cellular Control of Embryonic Development in Sea Urchins

Polarity and Establishment of the Major Body Axes

Both cellular and developmental manifestations of polarity are apparent in sea urchin eggs and early embryos. Purple sea urchin eggs have a polarized distribution of molecules that regulate axis formation along the animal-vegetal axis (A/V; i.e., top/bottom or future neural vs endomesoderm territories). In one species of urchin, *Paracentrotus lividus*, a reddish band of pigment granules is concentrated in the vegetal pole of embryo. In the purple sea urchin, the establishment of the A/V axis is initiated through β-catenin accumulation in the vegetal half of the egg (Wikramanayake *et al.*, 1998). Nuclearization of β-catenin and other maternal factors in the vegetal half of the developing embryo drives Delta/Notch signaling transduction. This initiates an endomesodermal program (future endoderm and mesoderm) in a ring of vegetal cells of the early embryo (Logan *et al.*, 1998; Davidson *et al.*, 2002). These include proteins in the *wnt* pathway, which are signaling pathways that turn on additional gene networks responsible for A/V axis formation. The secondary body axis, the oral-aboral (OA) axis (analogous to the dorsal-ventral axis), is established during cleavage stage by a maternal distribution of mitochondria (Coffman and Davidson, 2001). Downstream nodal signaling in the presumptive oral ectoderm further reinforces the OA axis (Lapraz *et al.*, 2015).

Cellular polarity, defined here as the segregation of membrane compartments to apical (outward facing) and basal (inward facing) compartments, is evident as early as the two-cell stage in sea urchins (Alford *et al.*, 2009). This polarity is also characterized by the segregation of thousands of membrane projections, known as microvilli, to the outward facing surfaces of the embryo. These microvilli are dynamic and grow and shrink with fertilization and the progression of the cell cycle (Schroeder, 1978). They appear to function in control of vesicle trafficking events and to increase the surface area of the embryo for absorption of nutrients from sea water. The individual cells of the embryo do not collectively form an epithelium until later in development when the blastocoel forms. At this stage (blastula) the embryo resembles a hollow ball of cells (**Fig. 1(F)**), with tight junctional complexes between cells and polarized distribution of membrane proteins to apical and basal surfaces.

Gene Regulatory Networks of Development

For scientists studying the flow of information from the nucleus, the sea urchin has long been an attractive model organism. Major advances in understanding or describing gene regulation of development, often for the first time, were pioneered in sea urchin embryos (reviewed in Ernst, 2011). Such discoveries include differential localization of DNA and RNA in the cell, maternal long-lived mRNAs present in the oocyte, functional separation of transcription and translation, and cytoplasmic polyadenylation of mRNAs (**Table 1** and references therein). Indeed, the first eukaryotic genes to be cloned were histone genes from the sea urchin (Kedes *et al.*, 1975).

Studies in sea urchin embryos have generated exceptionally detailed descriptions of embryonic gene regulation and function. Reports in the 1970s and 1980s marked the birth of linking gene regulation definitively to developmental differentiation. By the 1990s and 2000s a "systems biology" approach to development – involving the generation of gene regulatory network (GRN) models – was established using the sea urchin (Davidson *et al.*, 2002). GRNs model hierarchical cell fate decisions by describing the interactions between transcription factors (TFs) and the promoters of their target genes. These cascades in gene regulation result in several layers of differentiation, from the initial patterning of major axes and body part plans, to the establishment of terminal cell-type specific differentiation batteries (reviewed in Davidson,2010). The ability to selectively identify and perturb TF expression within the sea urchin embryo enabled the characterization of one of the most detailed gene expression analyses of any embryonic event described to date: endomesoderm specification (Davidson *et al.*, 2002). GRNs for other major developmental processes in the sea urchin have been characterized as well, including epithelial to mesenchymal transitions (EMT), biomineralization, coelomic pouch development and PGC homing, and muscle and immunocyte specification.

An active area of research in the sea urchin is devoted to linking the information encoded in the GRN to cellular events occurring during development. One example is EMT, a complex mix of changes in cell adhesion and migration that accompanies a cell's transition from an epithelial to mesenchymal phenotype. These events have been well-characterized at the GRN level in sea urchin primary mesenchyme (Saunders and McClay, 2014). In this context, distinct regulatory sub-circuits that involve at least thirteen different TFs work in parallel to initiate various EMT processes. These include remodeling of the basement membrane, apical constriction, epithelial de-adhesion, directed motility, loss of apical-basal polarity, and acquisition of mesenchymal adhesion and polarity.

Protection of the Sea Urchin Embryo From Pathogens and Environmental Stressors

Sea urchin embryos and larvae develop in the marine environment without maternal protection from environmental stressors. An integral aspect of development is protection of the germline and developing embryo from external mutagens or pathogens. Immune competence during development was first characterized in Echinoderm embryos in the late 1800s. Comparative embryologist Élie Metchnikoff observed wound healing and phagocytic behaviors in mesenchymal cells of sea star embryos. He documented the functional parallels between the Echinoderm phagocytes and vertebrate macrophages (Metchnikoff, 1893). In 1908, he was awarded the Nobel Prize in Physiology or Medicine for defining the "universality of phagocytosis", a fundamental contribution to the field of

cellular immunology. Nearly a hundred years later, molecular biology has allowed for the characterization of immunocytes in the sea urchin larvae (and analogous adult coelomocytes) at the genetic level. The sequencing and assembly of the *S. purpuratus* genome identified an expanded innate gene repertoire (over 10-fold of that found in vertebrates), and illuminated the shared regulatory heritage of sea urchin and vertebrate immune systems (Hibino *et al.*, 2006; Rast *et al.*, 2006). These genes and TFs have now been mapped across adult and larval immune cell types and in responses to various pathogens (reviewed in Buckley and Rast, 2017).

Sea urchin embryos also express several hundred genes associated with xenobiotic metabolism (or the "chemical defensome") in the first several days of development (Goldstone *et al.*, 2006). These include anti-oxidants, heat shock proteins, xenobiotic transporters and transcription factors that sense and respond to small molecules. The best studied of these defense pathways is the xenobiotic transporter system, including members of the ATP Binding Cassette (ABC) transporter and Solute Carrier (SLC) family. These transporters translocate a wide range of hydrophobic small molecules across membranes including xenobiotics and even signaling molecules. Sea urchin embryos have homologs of several human transporters responsible for chemotherapeutic resistance of cancer cells including ABCB1 (aka P-gp), ABCC1 (aka MRP1) and ABCG2 (aka BCRP). These genes are selectively regulated throughout embryonic and larval development, for either broad protective mechanisms, or for discrete functions in developmental signaling or morphogenesis (Shipp and Hamdoun, 2012).

Genome Manipulation and the Future of Sea Urchin Research

The unique biology and experimental advantages of the sea urchin mean that this animal will likely continue to serve an important role in developmental and reproductive biology research. Recent technological advances in gene editing are opening the door to the possibility of stable genetic manipulation as seen in fruit flies, mice and zebrafish. The ability to efficiently deliver molecules into the sea urchin embryo by microinjection has made it relatively easily to apply CRISPR-CAS gene editing systems to knockout genes in this embryo (Lin and Su, 2016). It is anticipated that these technologies will soon enable researchers to not only describe reproduction and development in this animal, but also to manipulate the genome across generations. This will prove valuable for the modeling of human disease pathways in the sea urchin, or for the development of new technologies to understand how the environment influences development.

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