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17 Echinoderms

Focus on the Sea Urchin Model in Cellular and Developmental Biology

Florian Pontheaux, Fernando Roch, Julia Morales and Patrick Cormier

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17.1 HISTORICAL CONTRIBUTIONS OF SEA URCHIN GAMETES AND EMBRYOS

Sea urchins, and in particular their gametes, have been an important experimental model since the end of the 19th century and throughout the 20th century (reviewed in Monroy 1986; Briggs and Wessel 2006; Pederson 2006; Hamdoun et al. 2018). From Aristotle's description of sea urchins' feeding apparatus (350 BCE) to the genome sequencing of *Strongylocentrotus purpuratus* in the 21st century, echinoderms on many occasions have been the involuntary protagonists of the history of science (Sodergren et al. 2006; Pederson 2006). Indeed, as we will discuss in detail in the following sections, sea urchins have played a paramount role in the fields of embryology and cell biology (Pederson 2006; Briggs and Wessel 2006).

17.1.1 HOW DID THE OPTICAL TRANSPARENCY OF SEA URCHIN EGGS FOSTER SIGNIFICANT ADVANCES IN THE UNDERSTANDING OF FERTILIZATION?

As early as 1840, Derbès, like Dufossé or von Baër, was probably seduced by the transparency of the sea urchin egg, which makes these animals an excellent experimental model system for the study of fertilization (Derbès 1847).

Sea urchins are gonochoric, and their gametes can be easily obtained in large quantities: a single female can produce millions of eggs, and males release an even greater quantity of functional gametes. In addition, both eggs and sperm are immediately competent to accomplish fertilization without any complementary maturation. Consequently, the simple mixing of sperm and eggs initiates fertilization and development, which take place externally. Using this material, Derbès was able to produce accurate descriptions of fertilization, holoblastic radial cleavages and larval development. The size of the sea urchin eggs ($\approx 100 \mu\text{m}$ diameter, see Table 17.1) and the optical characteristics of their oligolecithal cytoplasm make them a valuable system for manipulation, microinjection and observation under optical microscopy (Angione et al. 2015; Stepicheva and Song 2014). Derbès was the first scientist to hypothesize the existence of a transparent layer surrounding the unfertilized eggs: the egg-jelly. However, he did not properly grasp the importance of this protective coat, as he suggested that it was dispensable for fertilization (Briggs and Wessel 2006). We now know that this glycoprotein meshwork has several functions, which include attracting and activating the sperm and providing a carbohydrate-based mechanism to allow species-specific recognition (Vilela-silva et al. 2008). In fact, jelly layers

secrete chemo-attractants that drive sperm swimming, and more than 100 sperm-activating peptides have been identified in the egg-jelly of various sea urchin species (Darszon et al. 2005).

Two of these peptides are known as Resact, isolated from *Arbacia punctulata*, and Speract, purified from *Strongylocentrotus purpuratus*. They bind to their respective receptors that are placed on the sperm's outer membrane and trigger changes in sperm metabolism and motility by regulating its membrane potential (Darszon et al. 2005). Sperm swimming toward the egg is controlled by flagellar curvature modifications, which depend on oscillations in the intracellular Ca^{2+} concentration $[\text{Ca}^{2+}]_i$ (Böhmer et al. 2005). The egg-jelly is also responsible for the induction of the acrosome reaction of the sperm (Santella et al. 2012). In *S. purpuratus*, fucose-sulfate polymers are the jelly-coat specific components prompting acrosome reaction (SeGall and Lennarz 1979), a process that can be separated in consecutive phases (Vacquier 2012). First, the outer acrosomal membrane fuses with the plasma membrane of the sperm head, triggering actin polymerization. Then, the acrosomal vesicle releases its contents. Finally, the Bindin protein present on the acrosomal membrane is exposed to the egg surface. The acrosome reaction is essential for fertilization and ensures that it only occurs between gametes of homologous species. Fucose-sulfate polymers are central components of the egg-jelly, and their diversity seems to confer specificity to egg and sperm interactions (Pomin 2015).

The most remarkable and accurate observations of Derbès concern the establishment of the fertilization envelope. He documented the effects elicited by sperm on the egg for the first time, including the separation of the vitelline membrane from the egg plasma membrane. He interpreted the formation of this fertilization envelope as a landmark of fertilization (Derbès 1847), and high school, community college and university practical courses still use fertilization envelope elevation as the first visible sign of sperm-mediated egg activation (Vacquier 2011). Ernest Everett Just proposed that fertilization envelope elevation occurs within one minute after sperm-egg fusion and acts as a mechanical block to polyspermy (Just 1919) (reviewed in Byrnes and Newman 2014). E. E. Just was an African American cell biologist and embryologist of international renown who can be considered an early ecological developmental biologist (Just 1939) (reviewed in Byrnes and Eckberg 2006). Fertilization envelope elevation is accomplished by the cortical granule reaction occurring at the egg's surface. Several organelles are present on the cortex of unfertilized eggs, including cortical granules (Vacquier 1975), acidic vesicles (Sardet 1984; Morgan 2011) and endoplasmic reticulum (Sardet 1984). Cortical granules, about 1 μm in diameter, are especially abundant and are present immediately beneath the cytoplasmic membrane. Following sperm entry, their content is released into the space between the cell membrane and the structured mesh of proteins that forms the vitelline envelope. This exocytosis process releases several biological compounds. A trypsin-like protease called cortical granule

serine-protease digests the proteins linking the cell membrane to the vitelline membrane and degrades the Bindin receptors, immediately removing any sperm (Haley and Wessel 1999). However, the cortical granules also release mucopolysaccharides, highly hydrophilic compounds generating an osmotic gradient that pumps water into the space between the cell membrane and the vitelline membrane, which swells and detaches from the egg (reviewed in the textbook Gilbert 2006). Finally, a peroxidase enzyme present in the cortical granules hardens the fertilization envelope by crosslinking the tyrosine residues of neighbouring proteins (Foerder and Shapiro 1977; Wong et al. 2004).

In 1876, Oskar Hertwig published the first observations indicating that only one sperm enters the egg during fertilization (Hertwig 1876; Fol 1879). Using the Mediterranean sea urchin *Paracentrotus lividus* (named at the time *Toxopneustes lividus*), he was also the first to observe the fusion of egg and sperm pronuclei (Hertwig 1876; Clift and Schuh 2013). Three years later, Hermann Fol further characterized the mechanism of sperm entry and made similar observations, primarily the gametes from the starfish *Marthasterias glacialis* (named at the time *Asterias glacialis*) and to a lesser extent *Paracentrotus lividus* (Fol 1879). Ever since, technical developments in optical microscopy have made it possible to refine these observations, and ultimately electron microscopy has enabled ultrastructural investigation of sea urchin fertilization. The surface of the fertilized egg changes abruptly during cortical granule exocytosis. Two minutes after insemination, actin filaments assemble and participate in the formation of the so-called fertilization cone (Tilney and Jaffe 1980). In *Arbacia punctulata*, the sperm passes through this structure, makes a 180° U-turn and comes to rest lateral to its penetration site (Longo and Anderson 1968). This process has been carefully documented using scanning electron microscopy (Schatten and Mazia 1976). The male pronucleus and its centriole separate from the mitochondria and flagellum, which then disassemble in the cytoplasm. According to Monroy (Monroy 1986), Friedrich Meves was the first to observe that sperm mitochondria do not proliferate in the egg, leading him to propose that embryonic mitochondria have a maternal origin (Meves 1912). After mitochondria and flagellum dissolution, the centriole localizes between the male pronucleus and the egg pronucleus. This centriole extends its microtubules to form an aster so that the two pronuclei migrate toward each other and occupy a central position in the egg, where karyogamy proceeds. DNA synthesis can occur during the migration of the two pronuclei or after their fusion into the zygote nucleus (Gilbert 2006). Centrosome inheritance in echinoderms is exclusively paternal (Zhang et al. 2004). The two sperm centrioles duplicate concomitantly with DNA synthesis and end up producing the centrosomes that will steer embryonic development (Longo and Plunkett 1973; Sluder 2016).

Embryonic development proceeds normally only if a single sperm enters the egg. Fertilization by two sperms leads to a triploid nucleus, where each sperm's centriole divides independently to form four centrosomes. Theodor

Boveri already observed in 1902 that dispermic sea urchin eggs develop abnormally or die, and based on these observations, he was the first scientist to speculate that malignant tumours could be the consequence of an abnormal chromosome constitution (Boveri 1902, translated in Boveri 2008; Maderspacher 2008; Scheer 2018). His contributions to the elucidation of the role played by chromosomes as the vectors of the genetic inheritance are widely acknowledged among cell biologists.

17.1.2 SEA URCHIN'S CONTRIBUTION TO OUR UNDERSTANDING OF THE ROLE PLAYED BY CALCIUM SIGNALLING DURING FERTILIZATION

The term “egg activation” designates the multiple changes—both biochemical and morphological—that transform the egg cytoplasm after sperm penetration and prepare the cell for mitosis. In sea urchins, egg meiotic maturation occurs in the female gonads before gamete spawning. Thus, the activation of the sea urchin egg, which already possesses a haploid pronucleus, is independent of meiotic maturation. The events triggered by the activation of the sea urchin egg can be classified as early responses, occurring within seconds, and late responses, taking place several minutes after fertilization (Allen and Griffin 1958; Gilbert 2006). The early responses include the fast block of polyspermy and the exocytosis of the cortical granules. Among the late responses, we can cite the activation of mRNA translation and the duplication of DNA. Strikingly, all these events can occur independently of fertilization and are also triggered by artificial activation or parthenogenesis, which was discovered in the sea urchin by Jacques Loeb (Loeb 1899; Monroy 1986). Analyzing the effect of ions on the sea urchin egg, he observed that a treatment with a hypertonic solution of $MgCl_2$ provokes the elevation of the fertilization envelope (Loeb 1899). As Monroy points out (Monroy 1986), Loeb's work prompted Otto Heinrich Warburg to use sea urchins to develop his work on oxygen consumption in living cells (Warburg 1908). He observed that the fertilization of sea urchin eggs resulted in a rapid and nearly six-fold increase in oxygen consumption. Refining this observation to the metabolic abnormalities of cancer cells, Warburg was awarded the Nobel Prize in 1931 for his discovery of the “nature and mode of action of the respiratory enzyme”. In the context of fertilization, a specific NADPH oxidase of the egg's surface uses oxygen and produces a burst of hydrogen peroxide (Wong et al. 2004; Finkel 2011). Rather than damaging the egg, the hydrogen peroxide hardens the fertilization envelope and contributes to blocking polyspermy. H_2O_2 is produced by a Ca^{2+} -dependent mechanism that involves the reduction of one molecule of oxygen and the oxidation of two proton donors. Parthenogenetic activation by A23187 ionophore is sufficient to trigger this oxidative burst by using free cytosolic calcium (Wong et al. 2004).

The hypothesis of Ca^{2+} release following sea urchin fertilization was first proposed in the mid-20th century (Mazia 1937). The Ca^{2+} ion is essential for egg activation in all metazoans but more specifically in marine invertebrate

deuterostomes, which has been extensively discussed (Runft et al. 2002; Whitaker 2006; Ramos and Wessel 2013; Costache et al. 2014; Swann and Lai 2016). Calcium release triggered by fertilization or ionophore treatment was first demonstrated in sea urchin eggs using the luminescent calcium sensor aequorin (Steinhardt and Epel 1974; Steinhardt et al. 1977). Two independent types of Ca^{2+} waves have been observed following fertilization in sea urchins. The first one, a small initial cortical flash, results from an action potential-mediated influx of extracellular Ca^{2+} . A second cytosolic wave, due to the release of Ca^{2+} from the intracellular stores, begins at the sperm entry point and travels throughout the cytoplasm to encompass the entire egg (Parrington et al. 2007; Whitaker and Steinhardt 1982). The initial cortical flash does not automatically provoke the second Ca^{2+} wave, which is a distinct process exclusively triggered by sperm arrival. Notably, fertilization elicits a single Ca^{2+} wave in the sea urchin, whereas it provokes multiple Ca^{2+} oscillations in ascidians and mammals (Whitaker 2006; Sardet et al. 1998; Dupont and Dumollard 2004).

Research into the mechanisms triggering the calcium wave in sea urchins has given rise to abundant literature (reviewed in Ramos and Wessel 2013). Just after fertilization, the Ca^{2+} rise occurs as a result of inositol 1,4,5-triphosphate (IP3)-mediated release of Ca^{2+} from the endoplasmic reticulum (Terasaki and Sardet 1991). Other intracellular second messengers, including nicotinic acid adenine dinucleotide phosphate (NAADP), cyclic guanosine monophosphate (cGMP), cyclic ADP-ribose (cADPR) and nitric oxide (NO), were shown to increase at fertilization and could trigger Ca^{2+} release (Kuroda et al. 2001). However, in contrast to IP3, none of these second messengers is indispensable to the fertilization wave in the sea urchin egg.

17.1.3 SEA URCHIN EGG ABUNDANCE AND SYNCHRONOUS EARLY EMBRYONIC DEVELOPMENT ARE OPTIMAL FOR BIOCHEMISTRY AND CELL BIOLOGY ANALYSES

Unfertilized sea urchin eggs are physiologically blocked at the G1 stage of the cell cycle. Fertilization thus triggers entry into the S-phase and completion of the first mitotic division. Thanks to the large number of cells that can be recovered from a single female and their embryonic mitotic division synchronicity, these gametes have been crucial for the development of biochemical approaches studying cell cycle progression and protein translation (Evans et al. 1983; Humphreys 1969).

Unravelling the mechanisms controlling protein synthesis has been a central area of research in the 20th century (Thieffry and Burian 1996). In the 1940s, it was generally admitted that thymonucleic acid (DNA) existed only in animals and zymonucleic acid (RNA) in plants. However, Jean Brachet was the first biologist to localize both nucleic acids first in sea urchin and then in other animals (Brachet 1941). This critical observation led him to conclude that both

nucleic acids could be present in all cells. To study this issue, Jean Brachet made several visits to the Biological Station of Roscoff, and he liked to describe the exciting atmosphere of this place in the early 30s (Brachet 1975). His results led him to suggest that there is a strong correlation between RNA levels and protein synthesis activity. Sea urchin eggs thus played a crucial role in demonstrating that RNAs are present in all cells and that they are implicated in the synthesis of proteins, as proposed by the central dogma of Francis Crick (DNA makes RNA, which in turn makes protein).

Sea urchin eggs permeability to radioactive precursors has helped elucidate the mechanisms controlling protein synthesis in relationships with the entry into mitosis in response to fertilization. Incorporation of exogenous amino acids into protein occurs only after fertilization in sea urchin. Indeed, RNA synthesis is negligible both before and after fertilization (Schmidt et al. 1948). Moreover, the inhibition of RNA transcription by actinomycin D alters neither protein synthesis rate nor the first mitotic divisions of early sea urchin embryos (Gross and Cousineau 1963), demonstrating that the zygotic genome activity is not required for early protein synthesis (Gross et al. 1964). These observations indicated for the first time that maternal mRNAs are already present in unfertilized eggs and strongly supported the notion that their translation is tightly controlled. Furthermore, the work of Hultin showed that the synthesis of specific proteins is required for mitosis entry (Hultin 1961), heralding the future discovery of Cyclins (Ernst 2011).

In late July 1982, once the teaching in the Woods Hole Marine Station was over and the sea urchin season was coming to an end, Tim Hunt performed the critical experiment that led to the discovery of Cyclins (Hunt 2002). Cyclins form complexes with Cyclin-dependent kinases (CDKs), a family of conserved serine/threonine kinases that phosphorylate substrates throughout the cell cycle (reviewed in Malumbres 2014). Before working on cell cycle control, Tim Hunt was interested in the regulation of protein synthesis. He wanted to compare the protein synthesis rates observed in normally fertilized and parthenogenetically activated eggs, using the calcium ionophore A23187. For this purpose, he studied the sea urchin *Arbacia punctulata*. Adding [³⁵S] methionine to an egg suspension and separating proteins by gel electrophoresis, he produced an autoradiogram where one specific band, which was later identified as a Cyclin, showed an unexpected behaviour (Evans et al. 1983). Whereas most bands became stronger and stronger with time, this protein accumulated after fertilization but disappeared rapidly just before blastomere cleavage. In 2001, Tim Hunt shared the Nobel Prize in physiology or medicine with Leland Hartwell and Paul Nurse for discovering the key regulators of the cell cycle.

17.1.4 EMBRYONIC AND LARVAL DEVELOPMENT OF THE SEA URCHIN IN THE AGE OF MOLECULAR BIOLOGY

In the late 60s and early 70s, the rapid expansion of molecular biology was about to impact all the biology domains, including developmental biology. Notably, the first eukaryotic gene fragment isolated and introduced into the bacteria *E. coli*

was obtained from unfractionated DNA from *Lytechinus pictus* and *Strongylocentrotus purpuratus* (Kedes et al. 1975). These fragments encoded for histones, making these sea urchin genes the first protein-coding eukaryotic genes ever cloned (Ernst 2011).

The study of sea urchin has provided many descriptions of developmental gene regulatory networks (dGRNs). These logic structures depict the sequential regulatory events determining cell fate in different tissues and embryonic layers. The genes involved in dGRNs encode for transcription factors and components of signalling pathways but also for effector genes acting downstream of cell fate determinants and for different cell state-specific markers. The configuration adopted by dGRNs, based on empirical data, provides a dynamic picture of the genetic interactions controlling spatial and temporal aspects of development (Martik et al. 2016). dGRNs are thus predictive and testable models which help in understanding why and when developmental functions take place. The dGRN controlling the specification of *S. purpuratus* endodermal and mesodermal layers was originally described before its genomic sequence was available (Davidson 2002). However, with the completion of the sea urchin genome (Sea Urchin Genome Sequencing Consortium et al. 2006), these original descriptions have never ceased to be enriched with new components and functional data (Davidson 2006; Oliveri et al. 2008; Su et al. 2009; Saudemont et al. 2010; Peter and Davidson 2010; Li et al. 2014). Different diagrams of dGRNs are available on the E. H. Davidson's laboratory webpage (<http://grns.biotapestry.org/SpEndomes/>). Eric Davidson was a US developmental biologist working at the California Institute of Technology and an inspiring figure for the community of developmental biologists, particularly those working with multicellular marine organisms (Ben-Tabou de-Leon 2017). He is renowned for his pioneering work on the characterization of regulatory networks and their roles in body plan evolution.

Here, we will summarize the mechanisms that initiate cell specification and the establishment of the main layers of the sea urchin embryo. More complete descriptions of these dGRNs are available in reviews (Arnone et al. 2015; Martik et al. 2016; Ben-Tabou de-Leon 2016). In sea urchins, the embryonic body plan is rapidly established after fertilization. At the 16-cell stage, maternal inputs plus zygotic transcription determine at least three distinctive dGRN states that control ectoderm, endoderm, and micromere determination (Martik et al. 2016). Ectoderm emanates from the animal pole, and endoderm and mesoderm derive from the vegetal pole. The canonical Wnt- β -catenin signalling pathway is involved in primary axis formation and endoderm specification (Wikramanayake et al. 1998; Logan et al. 1999; Wikramanayake et al. 2004). β -catenin is active in the vegetal pole and controls polarization along the animal-vegetal axis. When β -catenin enters the nucleus, it forms an active complex with the transcription factor Tcf, which initiates the specification of endoderm in the sea urchin vegetal half. At the 16-cell stage, the future endoderm and mesoderm are still assuming a common endomesodermic identity. A Delta-Notch signal controls the separation of these two embryonic

territories. The Delta-ligand expression is activated indirectly by the β -catenin-Tcf input in the skeletogenic mesoderm (Oliveri et al. 2008). Cells receiving the Delta signal are specified as mesoderm, and the others acquire an endodermal fate (Sherwood and McClay 1997; Sherwood and McClay 1999; Sweet et al. 2002). The reception of Delta in the cells initiates the expression of the transcription factor GCM (*glial cell missing*) (Ransick and Davidson 2006; Croce and McClay 2010). Then, a triple positive feedback circuit involving GCM, GataE and Six1/2 is responsible for GCM expression maintenance in the mesoderm (Ransick and Davidson 2012; Ben-Tabou de-Leon 2016). Once the original endomesoderm and ectoderm GRN states are defined, further specification and signalling come into play to generate at least 15 different cell types, which are already distinguishable by early gastrulation (Peter and Davidson 2010; Peter and Davidson 2011; Martik et al. 2016).

For instance, the dorsal–ventral (DV) axis, also referred to as the oral–aboral axis, is morphologically distinguishable at the gastrula stage (Cavalieri and Spinelli 2015) and forms thanks to the activity of the Nodal and BMP ligands, which specify respectively ventral (oral) and dorsal (aboral) ectoderm (Duboc et al. 2004; reviewed in Molina et al. 2013). Nodal activates the expression of the BMP2/4 in the ventral ectoderm, but it also elicits the production of Chordin, which blocks the activation of the BMP receptors in this region. BMP2/4 of ventral origin can then diffuse to the dorsal side (Lapraz et al. 2009), where it specifies dorsal fates activating the phosphorylation of the transcription factor Smad1/5/8 (Floc’hlay et al. 2021).

In sea urchins, activation of the zygotic genome begins at the 16-cell stage. Thus, previous development is driven by maternal factors (reviewed in Kipryushina and Yakovlev 2020). Among the post-transcriptional processes involved in early embryonic development, mRNA translation regulation deserves particular attention (Morales et al. 2006; Cormier et al. 2016). By polysome profiling and RNA sequencing, the translome, which gives a complete picture of the polyosomal recruitment dynamics, has been investigated in the sea urchin *P. lividus* (Chassé et al. 2017). This translome represents the first step to an inclusive analysis of the translational regulatory networks (TRNs) that control the egg-to-embryo transition as well as the early events patterning the sea urchin embryo (Chassé et al. 2018). Future challenges for sea urchin embryology will include deciphering the molecular mechanisms linking TRN and dGRN activities after fertilization.

17.2 ECHINODERM PHYLOGENY

The echinoderms are an ancient and successful taxon of marine animals grouping together more than 10,000 living species. The first representatives of this phylum, which has left behind an extensive fossil record, have been found in the Cambrian Stage 3 (520 Mya) (Zamora et al. 2013). The echinoderms are deuterostome organisms belonging to the Ambulacraria clade, which also includes the Hemichordata (Figure 17.1). Estimates based on molecular clocks indicate

that these two taxa could have separated 580 Mya, during the Ediacaran (Erwin et al. 2011). The other branch of the deuterostomes, the Chordata, split even earlier and gave rise to the cephalochordates, the tunicates and the vertebrates (Lowe et al. 2015; Simakov et al. 2015).

The Paleozoic seas hosted at least 35 separate echinoderm clades presenting extremely diverse body plans. Most of them appeared during the Great Ordovician Biodiversification Event (GOBE), but only five of them made their way into the Mesozoic and have found a place in the modern faunas. These five clades correspond to the Crinoidea (sea lilies and feather stars), the Asterozoa (sea stars), the Ophiurozoa (brittle stars), the Holothurozoa (sea cucumbers) and the Echinozoa (sea urchins). Their representatives are all characterized by a typical pentaradial symmetry that is thought to have secondarily evolved from a bilateral ancestral form (Smith and Zamora 2013; Topper et al. 2019).

The ancient origin of the different echinoderm groups, which appeared during the Ordovician, has been a major obstacle to ascertaining their phylogenetic relationships. However, recent molecular phylogenies strongly support the so-called Asterozoan hypothesis that places the Ophiurozoa as the sister group of the Asterozoa (Reich et al. 2015; Telford et al. 2014; Cannon et al. 2014). According to these molecular phylogenies, Crinoidea appears as the basal branch of all the Echinoderms, with Holothurozoa being the closest relatives of Echinozoa (Figure 17.1). This last group underwent further diversification during the late Permian, the Mesozoic and the Cenozoic (Kroh and Smith 2010), producing a vast array of forms that have adopted remarkably different lifestyles and have adapted to all sorts of marine environments and climates.

The majority of the Echinozoa currently studied in the laboratory, including several edible species of commercial interest, belong to the order Camarodonta. The presence of this taxon in the fossil record has been dated back to the Miocene (Kroh and Smith 2010), but its different families may have originated earlier during the Middle Eocene and the Oligocene (45–23 Mya) (Láruson 2017). The recent characterization of the mitochondrial genomes and transcriptomes of several Camarodonta representatives (see Figure 17.2) have allowed researchers to establish the phylogeny of this group (Bronstein and Kroh 2019; Láruson 2017; Mongiardino Koch et al. 2018). At the same time, these molecular tools have provided an opportunity to develop comparative genomic approaches aimed at studying the molecular basis of the many anatomical, developmental, physiological and ecological specializations that characterize the different members of this taxon. Indeed, the density of available landmarks allows for the comparison of closely related species, such as the various Strongylocentrotidae representatives (divergence time estimated at 15–10 Mya) but also more distant species, such as the members of the Toxopneustidae, the Echinometridae, the Parechinidae or the Echinidae families (see Figure 17.2).

From a macroevolutionary perspective, these comparisons can nowadays be extended to other sea urchins

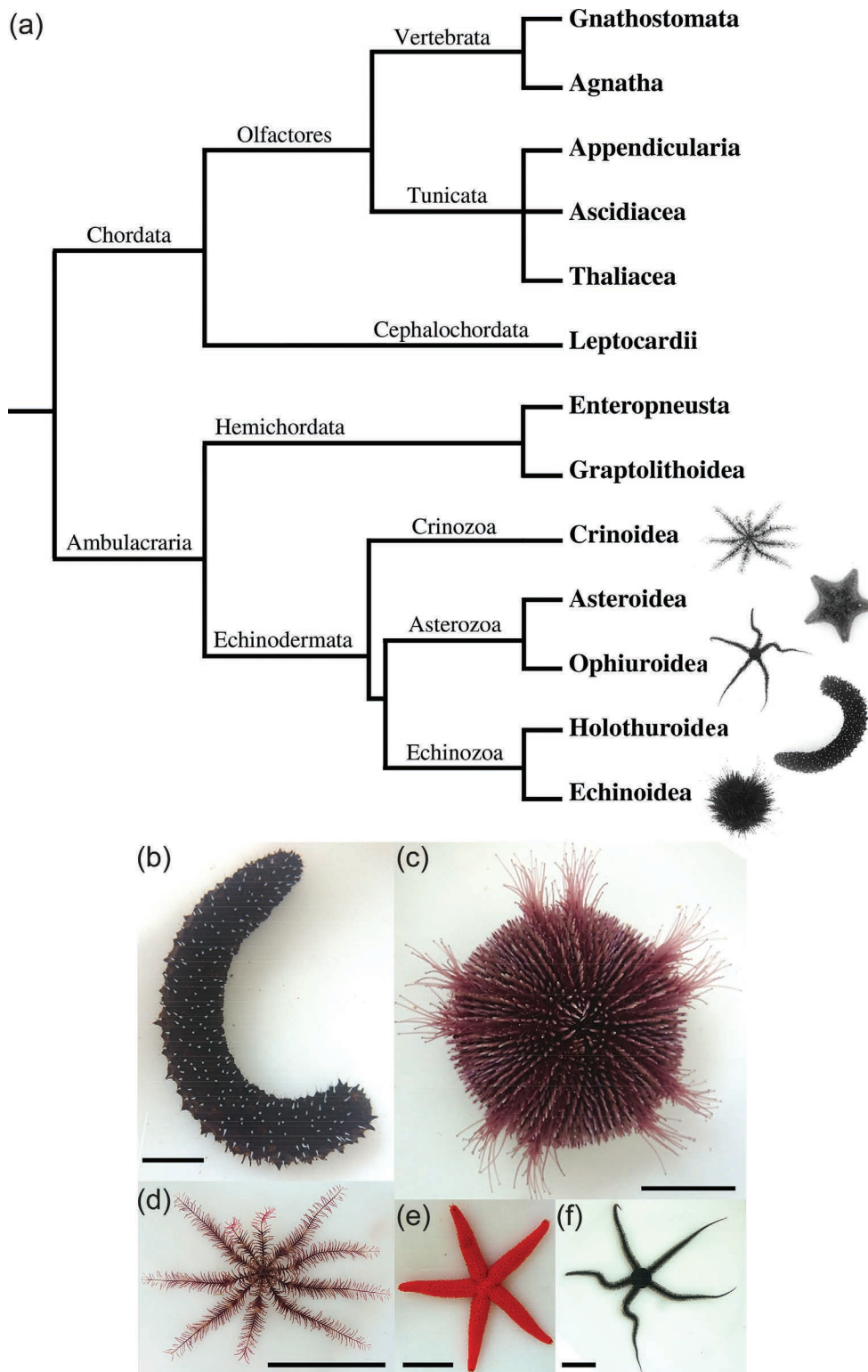


FIGURE 17.1 (a) Deuterostome group taxonomy: The deuterostome group includes two main lineages, Chordata and Ambulacraria. Chordata include cephalochordates, vertebrates and tunicates. Vertebrates are subdivided into Agnatha (e.g., myxines, lamprey) and Gnathostomata, which include Chondrichthyes (e.g., sharks, sawfish) and Osteichthyes (e.g., ray-finned fish, tetrapods). Tunicates are represented by ascidians, larvaceans (appendicularians) and thaliaceans. Ambulacraria include hemichordates and echinoderms, which are subdivided into five classes (crinoids, asteroids, ophiuroids, holothuroids and echinoids). Nodes and branches represent splits between taxons without any relative time reference. Each class of echinoderms is represented by black and white unscaled photographs. (b–f) Living adult representative echinoderms. (b) The holothuroid *Holothuria forskali*; (c) the echinoid *Sphaerechinus granularis*; (d) the crinoid *Antedon bifida*; (e) the asteroid *Echinaster sepositus*; (f) the ophiuroid *Ophiocomina nigra*. Animals were collected and maintained by the Roscoff Aquarium Service at the Roscoff Marine Station, France. Animals are shown at different scales and bars positioned at the bottom represent 5 cm.

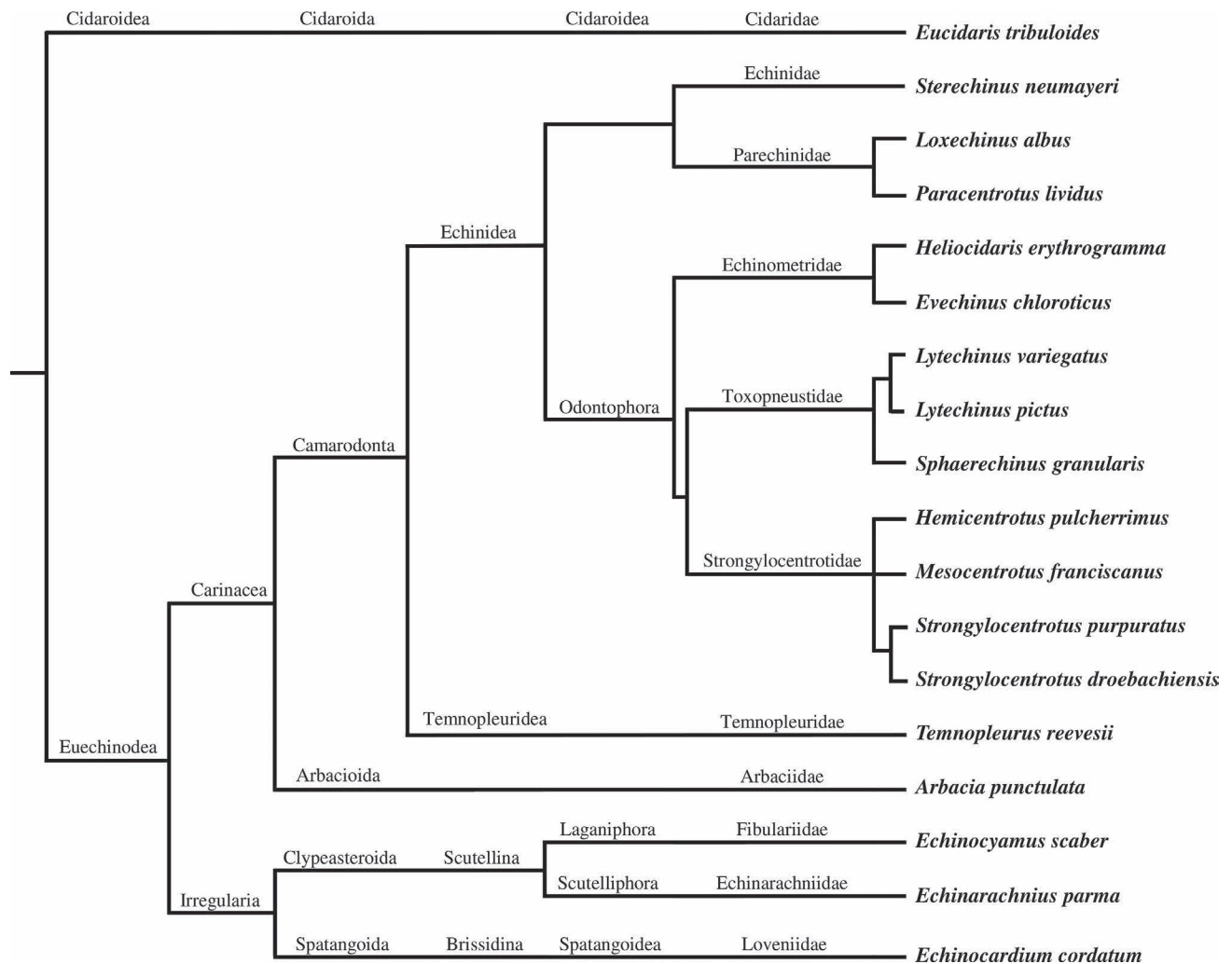


FIGURE 17.2 Echinozoa classification. The taxon of Echinozoa is mostly represented in laboratories by the Camarodonta order, but studies are also made on irregular sea urchins (Irregularia) and the most distant group Cidaroida. Nodes and branches represent speciation without any relative time reference. Each column corresponds to the classification (subclass, infraorder, order, infraorder, superfamily, family, species) identified on the World Register of Marine Species (WoRMS). (From Schwentner et al. 2018.)

commonly used in the laboratory that belong to more distant orders, such as the different members of the genus *Arbacia*, the Scutellina *Echinarachnius parma* (sand burrowing sea urchin) and the primitive forms of the order Cidaroida, a basal group that separated from the rest of Echinozoa during the late Permian (250 Mya) (Kroh and Smith 2010). Notably, a draft of the genomic sequence of the Cidaroida *Eucidaris tribuloides* is also available, allowing comparisons with the two fully sequenced Camarodonta *Strongylocentrotus purpuratus* and *Lytechinus variegatus* (Kudrarkar and Cameron 2017).

17.3 GEOGRAPHICAL LOCATION OF ECHINODERMS

Echinoderms, with their large diversity of species (>2,000 Asterozoa, >2,000 Ophiurozoa, >600 Crinozoa, >4,000 Echinozoa and >1,700 Holothurozoa species) inhabit all

the oceans and seas of the planet (see Figure 17.3). This group is exclusively marine and is absent from freshwater, although some species can be found in brackish waters (Pagett 1981).

Echinoderms are benthic, and some are considered sub-soil species, since they can burrow a few tens of centimetres in the sand (e.g., *Echinocardium cordatum*). Echinoderms have managed to adapt to a wide variety of environments, ranging from the warm waters of the tropics to the coldest waters of the poles (McClintock et al. 2011). For instance, all five classes of echinoderms are present in the Arctic Ocean (Smirnov 1994), and the Antarctic Ocean hosts the sea urchin *Sterechinus neumayeri*, which is studied for its biological mechanisms adapted to sub-zero temperatures (Pace et al. 2010). Echinoderms are also found at all depths, with some sea urchins inhabiting environments as deep as 7,300 meters (Mironov 2008), starfish and brittle stars at 8,000 m (Mironov et al. 2016) and feather stars at 9,000 m (Oji et al.

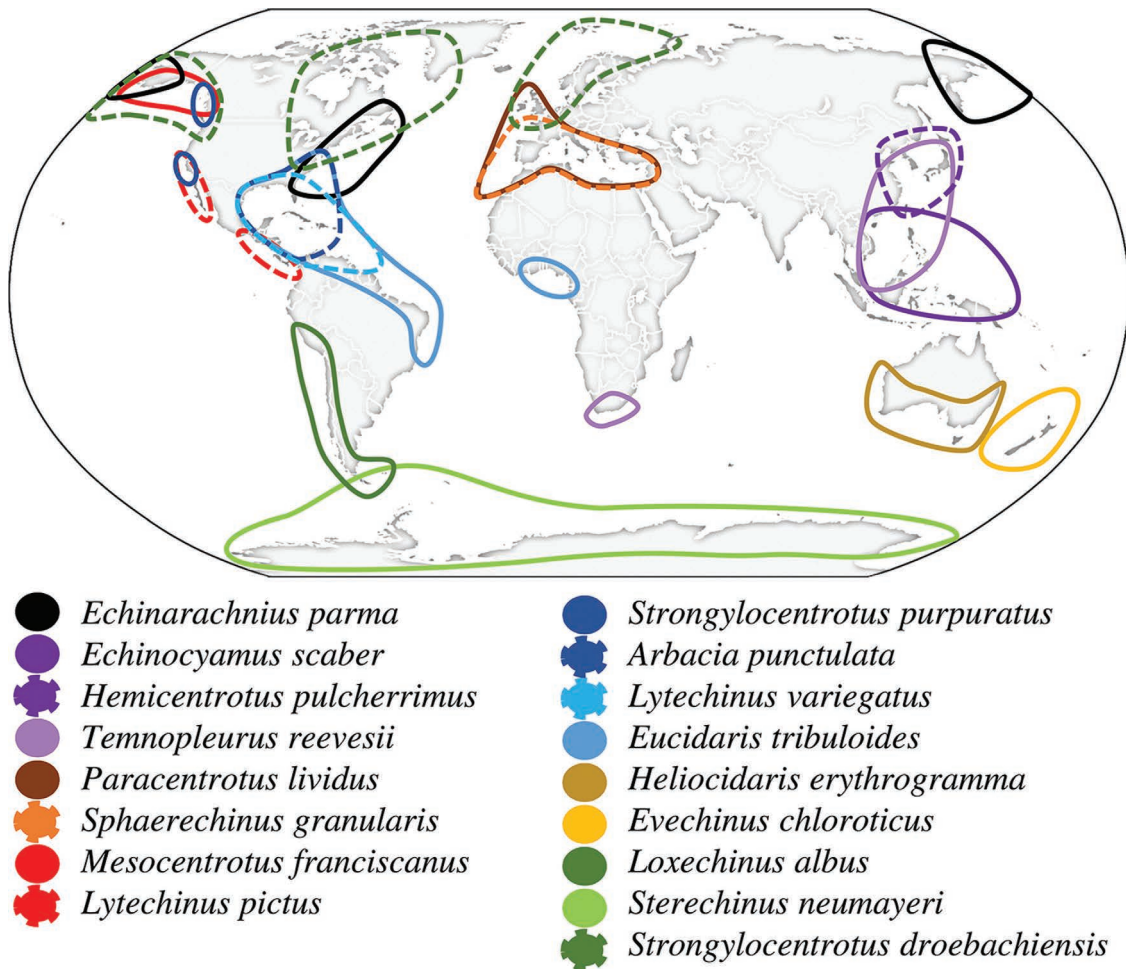


FIGURE 17.3 Geographic distribution of the main sea urchin species cited in this chapter. Geographical locations represent the major place where each species is found. Particular single occurrences in other areas can be found in the World Register of Marine Species (WoRMS). The cryptic species *Echinocardium cordatum* is widely distributed on the planet (not shown on the figure for clarity), divided into four distinct geographical lineages: one in the north-east and north-west Atlantic Ocean, one in the Mediterranean Sea and the north-east Atlantic Ocean, one in the Mediterranean Sea and one in the North and South Pacific Ocean avoiding the equatorial zone. Close colour shapes do not represent a taxonomic relationship between species but are here to help distinguish between species in the same region. (From Horton et al. 2020; Chenuil and Feral 2003; Egea et al. 2011.)

2009). Holothurians are though the record holders, as some specimens have been observed below 10,000 m (Mironov et al. 2019).

The most popular species in the laboratories (*A. punctulata*, *P. lividus*, *S. purpuratus*, *L. variegatus*, *L. pictus*, *H. pulcherrimus*) come mainly from the northern hemisphere. However, sea urchin species have been described in all oceans, including the Indian Ocean, the deep Pacific and the Arctic (Smirnov 1994; Price and Rowe 1996; Rowe and Richmond 2004; Filander and Griffiths 2017; Mironov et al. 2015; Mulochau et al. 2014). Other species cited in this chapter (e.g., *S. granularis*, *H. erythrogramma*, *S. neumayeri*, *M. franciscanus*, *S. droebachiensis*) illustrate different aspects of sea urchin diversity and facilitate the study of many biological questions, from phylogeny, adaptation and evolution to species conservation, community interactions and ecology.

17.4 SEA URCHIN LIFE CYCLE

Sea urchins are gonochoric with an average sex ratio of 1:1. Both sexes release their gametes (eggs or sperm) directly into the water column once a year, although in some species, a second period of spawning has also been reported, such as in *Paracentrotus lividus* (González-Irusta et al. 2010). In this animal, the mature season varies from January to June, and the spring equinox usually marks the height of the breeding season. In all echinoderms, the reproductive cycle and time of breeding (Table 17.1) may fluctuate, based on geographical (Figure 17.3) and local conditions. For example, *P. lividus* is found in the Mediterranean Sea and in the eastern Atlantic Ocean, from Scotland and Ireland to Southern Morocco and the Canary Islands. This species lives mainly in areas where winter water temperatures range from 10–15°C and summer temperatures from

TABLE 17.1
Breeding Season and Egg Diameter in Different Echinoidea Species

Echinoidea Species	Breeding Season	Egg Diameter	References
<i>Eucidaris tribuloides</i>	—	94 µm	(McAlister and Moran 2012; Lessios 1988; Lessios 1990)
<i>Sterechinus neumayeri</i>	September–November	180 µm	(Bosch et al. 1987; Stanwell-Smith and Peck 1998)
<i>Paracentrotus lividus</i>	May–September (AO) April–June and September–November (MS)	75 µm	(Hamdoun et al. 2018; Ouréns et al. 2011; Rocha et al. 2019; Byrne 1990)
<i>Heliocidaris erythrogramma</i>	November–February (SEA) February–May (SWA)	400–450 µm	(Binks et al. 2012; Foo et al. 2018; Raff 1987)
<i>Evechinus chloroticus</i>	November–February	—	(Delorme and Sewell 2016)
<i>Hemicentrotus pulcherrimus</i>	January–March	—	(Kiyomoto et al. 2014)
<i>Mesocentrotus franciscanus</i>	June–September	130 µm	(Bernard 1977; Bolton et al. 2000)
<i>Strongylocentrotus purpuratus</i>	November–March	80 µm	(Bolton et al. 2000; Hamdoun et al. 2018)
<i>Strongylocentrotus droebachiensis</i>	March–May	145 µm	(Himmelman 1978; Levitan 1993; Meidel and Scheibling 1998)
<i>Lytechinus variegatus</i>	May–September	100 µm	(Hamdoun et al. 2018; Lessios 1990, 1988; Schatten 1981)
<i>Lytechinus pictus</i>	May–September	120 µm	(Hamdoun et al. 2018)
<i>Sphaerechinus granularis</i>	April–June (Brittany) June–November (MS)	100 µm	(Guillou and Lumingas 1998; Guillou and Michel 1993; Vafidis et al. 2020)
<i>Temnopleurus reevesii</i>	July–January	100 µm	(Hamdoun et al. 2018)
<i>Arbacia punctulata</i>	June–August	69 µm	(Bolton et al. 2000; Gianguzza and Bonaviri 2013)
<i>Echinarachnius parma</i>	March–July (NWP)	110–135 µm	(Costello and Henley 1971; Drozdov and Vinnikova 2010; Summers and Hylander 1974)
<i>Echinocardium cordatum</i>	May–July (NWP) April–October (MS) May–October (AO)	110 µm	(Drozdov and Vinnikova 2010; Egea et al. 2011; Hibino et al. 2019)

AO: Atlantic Ocean, MS: Mediterranean Sea, NWP: North-West Pacific Ocean, SWA: South-West Australia, SEA: South-East Australia

18–25°C. Several factors, like temperature, photoperiod, resource availability and water turbulence contribute to the regulation of gametogenesis in these populations (Gago and Luís 2011). On the other hand, records from the North Pacific, Arctic and North Atlantic Oceans show that spawning of the sea urchin *Strongylocentrotus droebachiensis* may also be synchronized with the spring phytoplankton increase (Himmelman 1978; Starr et al. 1990). However, the main environmental factors triggering spawning and the molecular mechanisms that mediate this response are not yet known.

During the reproductive cycle, *P. lividus* gonads go through different development stages, which have been exhaustively characterized (Byrne 1990). Observation of gametogenesis in *P. lividus* through histological examinations allows us to classify the annual reproductive cycle of this species in six developmental stages: 1) recovery, 2) growing, 3) premature, 4) mature, 5) partly spawned and 6) spent. In turn, in *Strongylocentrotus droebachiensis*, four stages have been recognized by examining the activity of the two main cell populations composing the germinal epithelium (Walker et al. 2007, 2013). These populations are the germinal cells, which are either ova in the ovary or spermatogonia in the testis, and a group of somatic cells called nutritive phagocytes (NPs), which are functionally equivalent to the vertebrate Sertoli cells and are present

in both sexes. Stage 1, called inter-gametogenesis, occurs directly after spring spawning and lasts for about three months. Residual reproductive cells are present, but otherwise, the gonads look empty. Toward the end of this stage, NP cells increase in number and resume nutrient storage, doubling their size by the end of this phase. In addition, reproductive cells begin to appear. NPs are involved in the phagocytosis of residual ova and spermatozoa, and thus participate in the recycling of derived nutrients. Stage 2 is called pre-gametogenesis and NP renewal. This stage begins in summer and lasts for approximately three to four months. Reproductive cells, present at the periphery of the gonad, increase both in number and size. Stage 3, gametogenesis and NP utilization, takes place during five winter months. The reproductive cells continue to develop and migrate into the centre of the gonad. Conversely, the NPs cells shrink, and their number decreases. Stage 4 corresponds to pre-spawning and spawning. This stage occurs in late winter and lasts around three months. The lumen of the gonad is packed with fully differentiated gametes, and the NP cells are barely observable. At the end of stage 4, spawning occurs, and gametes are released from the gonads by the gonopores.

Several holistic approaches have been generated to understanding the molecular mechanisms of gametogenesis and the events of the life cycle. Whole-genome and Q-PCR

data have been obtained to identify genes expressed by *S. purpuratus* during oogenesis (Song et al. 2006). A general picture of protein abundance changes occurring during *P. lividus* gonad maturation has been generated by the proteomic approach (Ghisaura et al. 2016).

In Figure 17.4, we show the life cycle of the sea urchin *Sphaerechinus granularis*, which can be found at high densities in some locations of Brittany, such as the Glénan Islands and the Bay of Concarneau (Guillou and Michel 1993). However, captured adults maintained in appropriate conditions can release a large number of gametes from September to early July. Consequently, the availability of mature adults during most of the year makes this species a choice organism for cellular and biochemical studies (Feizbakhsh et al.

2020; Chassé et al. 2019). In the laboratory, gamete spawning may be induced artificially using several methods, such as intracoelomic injection of 0.1 M acetylcholine or of 0.5 M KCl. During the breeding season, when adults are mature, the expulsion of a small number of gametes may be obtained by a gentle shaking or by weak electrical stimulation, which facilitates the sexing of different individuals.

17.5 SEA URCHIN EMBRYOGENESIS

Sea urchins were one of the first animals to be used for embryological studies, that is, the development of a multicellular organism from a single cell (the fertilized egg) (reviewed in Etensohn 2017). Therefore, the particular

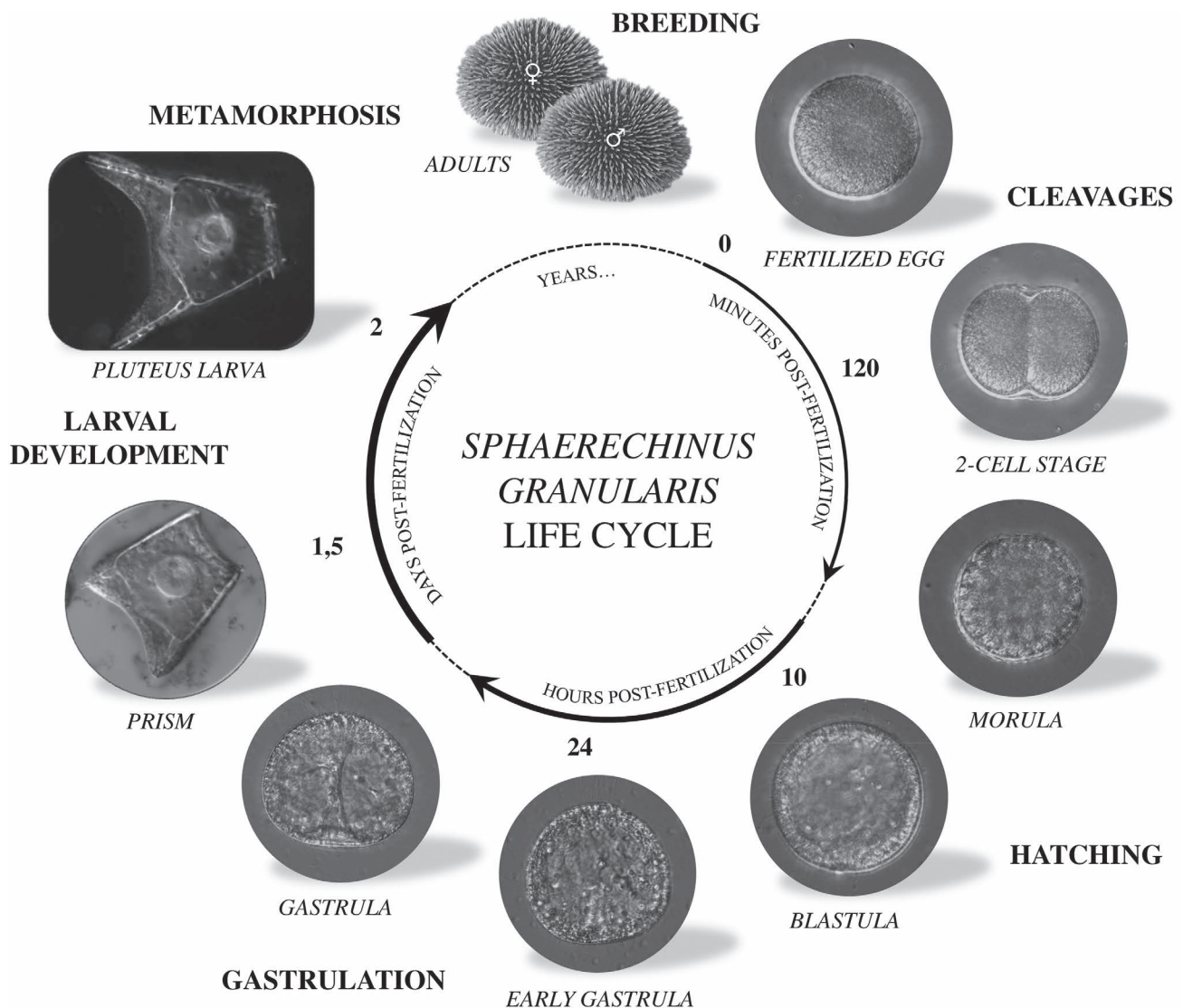


FIGURE 17.4 Life cycle of the sea urchin *Sphaerechinus granularis*. The sea urchin life cycle is composed of three periods of time with embryology (cleavages, hatching, gastrulation) taking minutes to hours, larval development taking days and growing individuals following the metamorphosis taking years. *Sphaerechinus granularis* development is synchronous, and times are noted. Microscopy pictures of *S. granularis* stages were taken with DIC filter on a Leica DMi8 microscope. Fertilized egg diameter is around 100 μm ($\times 20$ objectives) and slightly increases to prism and pluteus larva stage ($\times 40$ objectives). (From Delalande et al. 1998.)

development of a large set of species has been characterized in detail (see Table 17.2 and reviewed in Arnone et al. 2015; Hamdoun et al. 2018).

Sea urchin eggs are typically 80–200 µm in diameter and present an evenly distributed yolk (isolecithal; see Table 17.1). When released through the female gonoducts, unfertilized eggs are blocked at the G1 stage of the cell cycle, having completed their meiotic maturation in the ovary. Unfertilized eggs are polarized along a primordial axis, the animal–vegetal axis (A-V), which is specified during oogenesis and consequently is maternally established (Goldstein and Freeman 1997). Classically, the position of the animal pole corresponds to the extrusion site of the polar bodies. In some batches of *Paracentrotus lividus* eggs, a pigment band, initially described by Theodor Boveri (Schroeder 1980) and corresponding to a subequatorial accumulation of pigment granules, can be used as a visible marker of A-V polarity (Sardet and Chang 1985). A surface blister marking the animal pole has also been described in *Echinocardium cordatum* (Sardet and Chang 1985).

Bisection of an unfertilized egg through the equator, followed by independent fertilization of the two halves, results

in an animal half that gives rise to an undifferentiated epithelial ball and a vegetal half that develops into a relatively normal pluteus (Horstadius 1939; Maruyama et al. 1985). The fates of the two halves are explained by the presence of genetic determinants in the vegetal pole and the subsequent participation of regulative interactions that implement the formation of the missing animal blastomeres in the vegetal half (reviewed in Angerer and Angerer 2000; Kipryushina and Yakovlev 2020).

Sea urchin embryos exhibit holoblastic cleavages; that is, they undergo a complete partition subdividing the whole egg into separate blastomeres. Cleavages are radial: the division planes form a right angle with respect to the previous division. The cleavage rate and the development speed usually depend on temperature. At 18°C, *Sphaerechinus granularis* zygotes reach the first division by 120 minutes, and each subsequent division occurs at regular intervals of nearly 60 minutes. In *Paracentrotus lividus*, the first cleavage is faster, occurring at 70–90 minutes post-fertilization. The first cleavage (Cl.1, 2 cells) is meridional (in the polar axis) and divides the egg into two equally sized blastomeres (Figure 17.5). The second cleavage (Cl.2, 4 cells) is perpendicular to the first but also

TABLE 17.2

Availability of Omics in Different Echinoidea Species and Their Main Research Thematics

	Omics Data	Main Research Thematics	References
<i>Eucidaris tribuloides</i>	G./T. available (Echinobase/NCBI)	Embryogenesis, Development, Global changing	(Erkenbrack et al. 2018)
<i>Sterechinus neumayeri</i>	T. available (NCBI)	Toxicity, Fertilization, Genetics, Global changing	(Dilly et al. 2015)
<i>Loxechinus albus</i>	T. available (NCBI)	Ecology, Genetics, Global changing	(Gaitán-Espitia et al. 2016)
<i>Paracentrotus lividus</i>	G. in progress (European consortium); T./Trl. available (NCBI)	Ecology, Toxicity, Fertilization, Embryogenesis, Development, Global changing, Economy	(Chassé et al. 2018; Gildor et al. 2016)
<i>Heliocidaris erythrogramma</i>	T. available (NCBI)	Fertilization, Embryogenesis, Development, Global changing	(Wygoda et al. 2014)
<i>Evechinus chloroticus</i>	T. available (NCBI)	Ecology, Toxicity, Global changing	(Gillard et al. 2014)
<i>Hemicentrotus pulcherrimus</i>	G./T. available (HpBase/NCBI)	Toxicity, Fertilization, Embryogenesis, Metabolism, Development, Genetics	(Kinjo et al. 2018)
<i>Mesocentrotus franciscanus</i>	T. available (NCBI)	Ecology, Fertilization, Genetics	(Wong et al. 2019)
<i>Strongylocentrotus purpuratus</i>	G./T. available (Echinobase/NCBI)	Toxicity, Fertilization, Embryogenesis, Development, Genetics, Global changing	(Kudtarkar and Cameron 2017; Sea Urchin Genome Sequencing Consortium et al. 2006; Tu et al. 2014)
<i>Strongylocentrotus droebachiensis</i>	Transcriptome available (NCBI)	Toxicity, Fertilization, Metabolism, Development, Global changing	(Runcie et al. 2017)
<i>Lytechinus variegatus</i>	G./T. available (Echinobase/NCBI)	Ecology, Toxicity, Fertilization, Development, Global changing	(Davidson et al. 2020; Hogan et al. 2020)
<i>Lytechinus pictus</i>	G. in progress; Transcriptomes available (Echinobase/NCBI)	Toxicity, Fertilization, Embryogenesis, Development	(Nesbit et al. 2019)
<i>Sphaerechinus granularis</i>	T. from ovaries available (Echinobase/NCBI)	Toxicity, Fertilization, Embryogenesis	(Reich et al. 2015)
<i>Temnopleurus reevesii</i>	See chapter 18	Genetics	(Suzuki and Yaguchi 2018)
<i>Arbacia punctulata</i>	T. available (NCBI)	Toxicity, Fertilization, Embryogenesis, Metabolism, Development	(Janies et al. 2016)
<i>Echinarachnius parma</i>	T. from ovaries available (Echinobase/NCBI)	Toxicity, Fertilization, Development	(Reich et al. 2015)
<i>Echinocardium cordatum</i>	T. available (NCBI)	Ecology, Toxicity, Development	(Romiguier et al. 2014)

G: Genome, T: Transcriptome, G./T: Genome and Transcriptome, Trl: Translatome

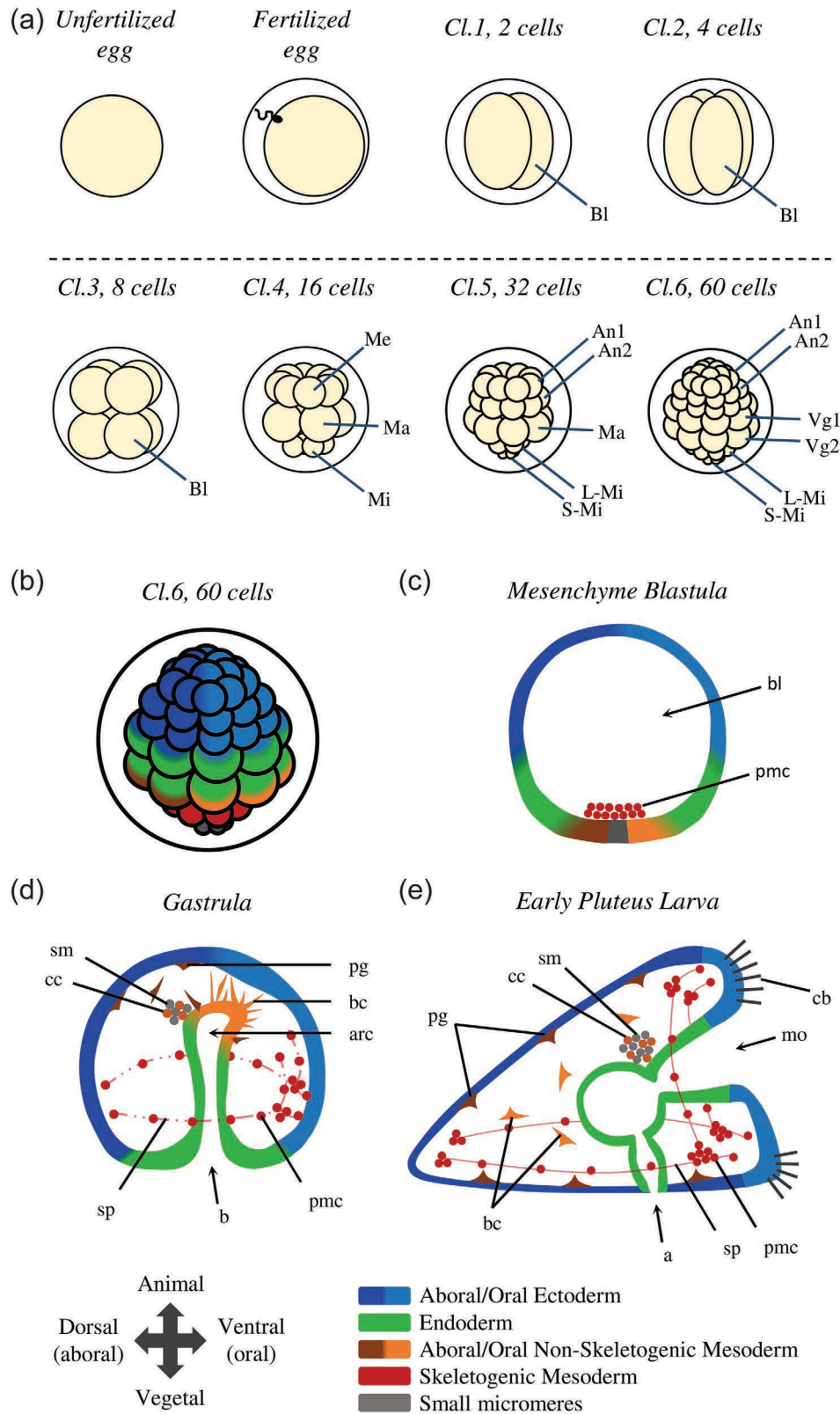


FIGURE 17.5 Diagrams of sea urchin embryo development. (a) The six first cleavages of a sea urchin embryo. (b) Diagram of the 60-cell stage, (c) mesenchyme blastula, (d) gastrula and (e) pluteus larva stage with the colouration of presumptive cell fates. See embryogenesis text part for more details on cleavage axis, cell fate and migration. (Cl: cleavage, Bl: blastomeres, Me: mesomeres, An: animal, Ma: macromeres, Vg: vegetal, Mi: micromeres, S/L-Mi: small/large micromeres, bl: blastocoel, pmc: primary mesenchyme cells, b: blastopore, arc: archenteron, cb: ciliary/ciliated bands, pg: red-pigmented cells, bc: blastocoel cells, cc: coelomic cells, sm: small micromeres, sp: larval spicules, mo: mouth, a: anus.)

occurs in a meridional plane, resulting in the production of four equally sized blastomeres. The third cleavage (Cl.3, 8 cells) is equatorial (at right angles of the polar axis), resulting in four upper and four lower blastomeres, all of equal sizes. The fourth cleavage (Cl.4, 16 cells) exhibits a complex and characteristic pattern that reveals the basic A-V polarity of the embryo. In the vegetal pole, the four blastomeres divide asymmetrically and horizontally, forming four small cells placed in the egg's pole (the micromeres) and four larger cells situated above (the macromeres). In the animal pole, the four blastomeres divide meridionally and symmetrically, resulting in eight equally sized cells (the mesomeres). At the fifth cleavage (Cl.5, 32 cells), the eight mesomeres at the animal half divide equatorially and symmetrically, resulting in two layers of cells called "an1" for the upper one and "an2" for the lower one. In the vegetal pole, the four macromeres instead divide meridionally, forming a tier of eight cells. The four micromeres divide horizontally and asymmetrically, resulting in four small micromeres at the extreme vegetal pole hemisphere and four large micromeres above. At the sixth cleavage (Cl.6, 60 cells), all the cleavage furrows are equatorial. The macromeres divide, giving rise to two eight-cell tiers called "vg1" and "vg2". The large micromeres divide as well, but not the small micromeres. In total, the 60-cell embryo shows, from top to bottom, 16 "an1" cells distributed in two layers of 8 cells each, 16 "an2" cells forming also two layers of 8 cells each, 8 "vg1", 8 "vg2", 8 large micromeres and 4 small micromeres.

The macromeres producing the "vg1" and "vg2" cells are the endomesoderm progenitors. The large micromeres contribute instead to the skeletogenic mesenchyme, and the small micromeres to the primordial germ cells (PGCs) (Okazaki 1975; Yajima and Wessel 2012). In the Echinoderm phylum, micromeres are only observed in echinoids and are thus considered a derived character. Asymmetric cell division is directed by the control of spindle and furrow cleavage position and by uneven repartition of molecules. Although the precise molecular mechanisms that orchestrate these asymmetric divisions are still poorly understood, it has been shown that the AGS/Pins proteins (activator of G-protein signalling/partner of Inscuteable) are required for normal asymmetrical division during micromere formation (Voronina and Wessel 2006; Poon et al. 2019).

As soon as the eight-cell stage is reached, a small central cavity forms in the centre of the embryo. As cleavage proceeds, this space enlarges and forms the blastocoel. A morula appears roughly six hours after fertilization, but at the 120-cell stage, the smooth-surfaced blastula becomes a continuous spherical monolayer surrounded by an outer hyaline layer. The epithelium sits on an inner basal membrane; cell adhesion is mediated by tight junctions. Cilia develop on the surface of the blastula, and their coordinated action triggers the rotation of the blastula within the fertilization envelope. Ten hours after fertilization, the blastula is composed of about 600 cells. Cell division rates decrease as the cell cycle lengthens. At the end of segmentation, the blastula is covered by cilia, presents a conspicuous apical ciliary tuft

in the animal pole and starts secreting a hatching enzyme that digests the fertilization envelope. The synthesis of this hatching enzyme takes place in the animal-most two-thirds of the blastula and is likely to be restricted to the presumptive ectoderm territory (Lepage et al. 1992a, 1992b). Finally, a swimming blastula is released into the sea.

The blastula wall thickens at the vegetal pole, forming the vegetal plate. In the central region of this vegetal plate, the micromere descendants display pulsatile movements and start developing filopodia in their basal face. These cells lose their affinity for the outer hyaline structure and gain affinity for the fibronectin present in the basal lamina and the extracellular matrix lining the blastocoel (Fink and McClay 1985). Eventually, they detach from the epithelium and enter the blastocoel, forming the primary mesenchyme (Peterson and McClay 2003). As these cells are the first ones to ingress into the blastocoel, they are called primary mesenchyme cells (PMCs) (Burke et al. 1991). Adhering to the blastocoel matrix, these cells progress from the vegetal pole toward the animal pole and then reverse their trajectory. Finally, the PMCs reach an area located between the vegetal pole and the equator and form a ring pattern consisting of two ventrolateral cell clusters and dorsal and ventral interconnected chains of cells (Malinda and Etensohn 1994). Then their filopodia coalesce, and the characteristic syncytial bridges of the larval skeleton appear. The primary mesenchyme cells of the sea urchin represent one of the best developmental models for studying mesodermal migration (Anstrom 1992; Etensohn 1999; Etensohn and Sweet 2000; Peterson and McClay 2003), and the cellular basis of skeletogenic cells has been characterized in detail (Okazaki 1975; Etensohn and McClay 1988; Armstrong and McClay 1994). Moreover, the gene regulatory network (GRN) that controls their formation has also been described not only in species of the order Camarodonta (Oliveri and Davidson 2004; Oliveri et al. 2008) but also in other echinoid orders (Minokawa 2017).

In euechinoids, the ingression of the PMCs marks the onset of gastrulation. The invagination of the Vg2 territory in the blastocoel gives rise to the archenteron (primitive gut), opened to the outside by a circular blastopore (the future anus). Invagination of the vegetal plate, a universal feature of echinoderm gastrulation, is traditionally divided into "primary" and "secondary" invagination (Gustafson and Kinnander 1956). The "primary" invagination corresponds to an initial phase of gut extension that involves extensive extracellular matrix remodelling and cell shape changes (reviewed in Kominami and Takata 2004). Three hypotheses have been advanced to explain the "primary" invagination (reviewed in Etensohn 2020). First, according to the so-called apical constriction hypothesis, a ring of vegetal plate cells become bottle shaped, compressing their apical ends (Kimberly and Hardin 1998). This cell shape modification causes the cells to pucker inward. However, bottle cells could be a specialized feature of euechinoids and not a general characteristic of all echinoderms (Etensohn 2020). A second hypothesis proposes that invagination could be

driven by changes in extracellular matrix composition (Lane et al. 1993). In fact, the hyaline layer is made up of two layers: an outer lamina composed of hyalin protein and glycoproteins and an inner lamina composed of fibropellin proteins (Hall and Vacquier 1982; Bisgrove et al. 1991). After PMC ingression, the vegetal plate cells secrete chondroitin sulfate proteoglycans into the inner lamina of the hyaline layer. As these chondroitin sulfate proteoglycans capture abundant water, the inner layer expands even if the outer layer remains stiff. The result is a force pushing the epithelium toward the blastocoel (Lane et al. 1993). A third hypothesis suggests another force arising from the concerted movement of cells toward the vegetal pole that may facilitate the invagination by drawing the buckled layer inward (Burke et al. 1991).

The “secondary” invagination ensues after a brief pause. During this stage, the archenteron extends and produces a long thin tube. The cells of the archenteron, which are organized as a monolayered epithelium, move over one another and flatten (Ettensohn 1985; Hardin 1989). In *Lytechinus variegatus*, gastrulation has been analyzed at a high resolution by live imaging and using transplantation techniques (Martik and McClay 2017). In this species, the process of archenteron elongation is mainly driven by the elongation of Vg2 endoderm cells. In fact, even if oriented cell divisions also contribute to gut elongation, cell proliferation inhibition does not preclude gastrulation, indicating that cell proliferation is not essential for this process (Stephens et al. 1986; Martik and McClay 2017).

The oral ectoderm of the gastrula flattens as the gastrula becomes roughly triangular, forming the prism larva. The embryonic radial symmetry is gradually replaced by a bilateral symmetry. An early sign of this transformation consists in the aggregation of primary mesenchyme cells into two clusters that develop in the opposite posterolateral–ventral angles of the prism larva. The cells of the primary mesenchyme form then a syncytium, in which two calcitic spicules develop. These spicules, flanking the primitive digestive tract, will constitute the endoskeleton of the pluteus larva. For this, the primary mesenchyme cells endocytose seawater from the larval internal body cavity and form a series of vacuoles where calcium can concentrate and precipitate as amorphous calcium carbonate (Kahil et al. 2020).

Once the archenteron reaches about two-thirds of its final length, the third and last stage of archenteron elongation begins (Hardin 1988). This phase is driven by the secondary mesenchyme cells, which extend filopodia through the blastocoel cavity to reach a specific area in the inner surface of the blastocoel roof (Hardin and McClay 1990). These filopodia pull the archenteron toward the animal pole and contact the region where the mouth will form. The mouth forms in the future ventral side of the larva after the fusion of the archenteron and the ectoderm epithelium. Typical of the deuterostomes, the mouth and the archenteron create a continuous digestive tube that joins the blastopore, which coincides with the anus.

During the processes of archenteron elongation, the secondary mesenchyme cells spread into the blastocoel fluid,

where they form at least four non-skeletogenic mesoderm cells (Ettensohn and Ruffins 1993). Early in gastrulation, a population of red-pigmented cells forms (Gustafson and Wolpert 1967; Gibson and Burke 1985). It is interesting to note that independent knock out of the genes encoding for polyketide synthase, flavin monooxygenase family 3, and the glial cells missing (*gcm*) protein results in the disappearance of red-pigmented cells throughout the body of the larva (Wessel et al. 2020).

Later in gastrulation, a group of cells coming from the tip of the archenteron moves into the blastocoel and adopts a fibroblast-like morphology: they are the so-called basal cells (Cameron et al. 1991), or blastocoel cells (Tamboline and Burke 1992). At the end of gastrulation, two coelomic cavities appear as a bilateral out-pocketing of the foregut (Gustafson and Wolpert 1963). Afterwards, secondary mesenchymal cells move out of these coelomic cavities and produce the circumesophageal musculature of the pluteus larvae (Ishimoda-Takagi et al. 1984; Burke and Alvarez 1988; Wessel et al. 1990; Andrikou et al. 2013). While the right coelomic pouch remains rudimentary, the left coelomic pouch undergoes massive development to build many of the structures of the future adult sea urchin. The left side of the pluteus contributes to the formation of the future oral surface of the sea urchin adult (Aihara and Amemiya 2001). The left pouch splits into three smaller sacs. A duct-like structure, the hydroporic canal, extends from the anterior left coelomic pouch to the aboral ectoderm where the hydropore forms (Gustafson and Wolpert 1963). This hydroporic canal is covered by cilia and could be an excretory organ of the larvae (Hara et al. 2003) and later differentiates into a part of the adult water vascular system (Hyman 1955). The hydroporic canal formation constitutes the first morphological signature of left–right asymmetry in the pluteus larva (Luo and Su 2012). An invagination from the ectoderm fuses with the intermediate sac to form the imaginal rudiment, from which the pentaradial symmetry of the adult body plan is established (Smith et al. 2008). To facilitate the observation and the study of complex phases of development, a larval staging schematic of *Strongylocentrotus purpuratus* has been proposed (Smith et al. 2008). This schematic subdivides larval life into seven stages: 1) four-arm stage, 2) eight-arm stage, 3) vestibula invagination stage, 4) rudiment initiation stage, 5) pentagonal disc stage, 6) advanced rudiment stage and 7) tube-foot protrusion stage.

In the late gastrula, primary germ cells located in the archenteron tip incorporate into the imaginal rudiment. Skeletogenic mesenchyme cells penetrate the rudiment to produce the first skeletal plates of the future adult endoskeleton (Gilbert 2006). The rudiment separates from the rest of the larva during metamorphosis, reorganizes its digestive tract and then settles on the ocean floor, where the miniature sea urchin juvenile starts a benthic life.

This mode of development, however, is not universal among echinoids (reviewed in Raff 1987). Indeed, many sea urchins endowed with large eggs bypass the pluteus stage and directly form a non-feeding larva. For instance, *Peronella*

japonica, a species that possesses 300- μm -diameter eggs, produces a partial pluteus with a variable skeleton but no larval gut. *Heliocidaris erythrogramma* produces from a 450- μm -diameter egg a free-floating larva but lacks any relic pluteus structure except for the vestibule. The sea urchin *Abatus cordatus*, with a 1,300- μm -diameter egg, undergoes direct development in a brood chamber placed inside the mother.

17.6 ANATOMY OF THE ADULT SEA URCHIN

A regular adult sea urchin resembles a sphere densely covered with spines. Animal size usually varies between 5 and 12 cm, but *Echinocyamus scaber*, an irregular echinoid, is the smallest known species (6 mm in size). The largest one is the red sea urchin *Mesocentrotus franciscanus* (syn. *Strongylocentrotus franciscanus*), with a body diameter of 15 to 17 cm and spines up to 30 cm.

Adult sea urchins exhibit a pentaradial symmetry with five equally sized parts radiating out of a central axis. The body is divided into radial (= ambulacral) and interradial (= interambulacral) alternate sectors. The mouth is present in the ventral side, and the anus appears in the dorsal—or aboral—region. The body plan is therefore organized around an oral–aboral axis, with no cephalic structures. Irregular echinoids, which include many species used for biological studies (Hibino et al. 2019), deviate from this regular pattern and belong to different clades such as the cidaroids (Order Cidaroida), the clypeasteroids (also known as sand dollars; Order Clypeasteroida) and the spatangoids (also known as heart urchins; Order Spatangoida). In these species, the anus and often also the mouth are no longer present in the two poles of the animal, generating a bilateral symmetry. Whereas regular sea urchins live often on rocky or sandy substrates, most of the irregular sea urchins are burrowing animals that dig in the sediment thanks to their specialized spines.

Sea urchins—like other echinoderms—have a dermaskelton, which is a thin shell consisting of separate plates of hard calcite that is produced by mesenchyme cells of mesodermal origin. This dermaskelton, called the test, is made of living cells surrounded by both organic and inorganic extracellular matrices. This calcium carbonate shell (mainly formed by CaCO_3) displays a specific three-dimensional organization known as stereom (an echinoderm synapomorphy). The cells constituting the stroma fill the open spaces of these stereomic structures with their mineral secretions. In echinoids, the plates forming the test are tightly apposed and bound together by connective tissue, generating a resistant armoured structure. A thin dermis and epidermis cover the dermaskelton, which often bears protruding tubercles and rows of spines (in fact, the term echinoderm means in Greek “spiny skin”). The form and size of these spines are extremely variable. The base of the spine is attached by different sets of muscles capable of orienting the spine in different directions. The spine base contains a collagen matrix that can reversibly change its configuration

and become flexible or rigid, which allows immobilizing the spine in one particular direction.

The ambulacra of most echinoderms, including echinoids, consist of longitudinal rows of tube feet (podia) protruding out of the test. Sea urchin adoral podia are highly specialized organs that have evolved to provide an efficient attachment to the substratum. These feet generally secrete in their tips a series of adhesive proteins sticking to different supports. Podia are the external appendages of the water vascular system and consequently can be hydraulically extended or contracted. This sophisticated hydraulic system consists of five radially arranged channels connected to a central ring channel surrounding the mouth. Water enters the system through the madreporite, a plate with a light-colored calcareous opening placed on the aboral side. The madreporite filters the seawater, which passes over a short stone channel and joins the ring channel. The tube feet are connected to five main radial channels by a network of lateral branches. Feet have two parts: the ampulla and the podium. The ampulla is a water-filled sac located inside the test and is flanked by circular and longitudinal muscles. The podium protrudes out of the test and is surrounded by a sheet of longitudinal muscles. When the muscles around the ampulla contract, water flows into the connected podium, inducing elongation. On the contrary, when the podia muscles contract, water returns to the ampulla and the podia retract. Differing from adoral podia, peristomal podia are not involved in adhesion and locomotion and have a sensory role. A large family of genes predicted to act in both chemo- and photoreception is expressed in tube feet or pedicellariae and reveals a complex sensory system in sea urchins (Sea Urchin Genome Sequencing Consortium et al. 2006). Pedicellariae are small claw-shaped structures found on the echinoderm endoskeleton, particularly in Asterozoa and Echinozoa. In some taxa, they are presented as cleaning appendages thought to keep the animal’s surface free of parasites, debris and algae. Four primary forms of pedicellariae can be found in sea urchins: globiferous, triphyllous, ophicephalous and tridactylous. They typically present a claw shape consisting of three valves that have inspired the production of micro-actuated forceps (Leigh et al. 2012). Appendages, including tube feet, spines, pedicellariae and gills, are all present on the surface of the sea urchin. They present a broad diversity of shapes and offer a fantastic and strange spectacle under a simple dissecting microscope (for an excellent illustration of the different appendage types classified according to Hyman 1955, see Figure 4 of Burke et al. 2006).

These appendages are richly innervated sensory organs allowing sea urchins to interact with their environment (Yoshimura et al. 2012). Like other echinoderms, the sea urchin nervous system is dispersed, but it cannot be reduced to a loose neuron network. Although the adult is not cephalized, the radial nerve presents a segmental organization. The adult sea urchin nervous system is composed of five radial cords. They extend underneath the ambulacra and join their base by commissures that form the circumoral nerve ring, placed around the oesophagus next to the

mouth (for a review, see Burke et al. 2006; Yoshimura et al. 2012). The radial nerve produces a series of extensions that pass through the test's pores and innervate the base of each appendage. Almost all tissues, including the viscera, are innervated (Burke et al. 2006), but the echinoderm nervous system is one of the least well studied among metazoans. Until the publication of a genomic view of the sea urchin nervous system (Burke et al. 2006), our knowledge about the echinoid nervous system relied exclusively on morphological studies. Now new lines of investigation have opened (Garcia-Ararras et al. 2001). The sea urchin genome encodes for all the regulatory proteins involved in neuronal specification, and many potential neuro-modulators, neuropeptides and growth factors have been described, indicating that the echinoids use these modes of cell communication and regulation (Wood et al. 2018). While tube feet are non-ocular appendages, they do show localized expression of a set of retinal genes and many chemoreceptors, suggesting that they could be involved in light perception (Burke et al. 2006) and a wide range of other sensory modalities.

The digestive tract of echinoids is classically subdivided into different sections: mouth, buccal cavity, pharynx, oesophagus, stomach, intestine, rectum and anus (Hinman and Burke 2018). Sea urchins are benthic animals and eat organic matter that settles down from the column water, mainly preferring kelp, algae and sponges present in their habitat. Most of the irregular sea urchins, which live within the sediment, feed on its organic fraction. Sea urchins living in seaweed meadows graze and ingest macroalgae, including associated epibionts and microbiota (Burke et al. 2006). Echinoids possess a very sophisticated chewing apparatus, the lantern of Aristotle, which encircles the mouth opening and the pharynx. The lantern is composed of a pentamerous skeleton, including five teeth animated by a well-developed musculature (Ziegler et al. 2010). Sea urchins have an open circulatory system with an extensive body cavity filled with coelomic fluid. Passive gas exchange in the coelomic fluid takes place through gill-like appendages located around the mouth. Coelomocytes are free cells that are found in coelomic fluid and also among the tissue of various body parts. These cells are believed to play different functions, including nutrient transport and immune defence (Hakim et al. 2016).

In regular echinoids, sexes are separated, but the external morphology of males and females is indistinguishable. In the case of spatangoids, sexual dimorphism is apparent in the genital papillae; however, observing these structures is challenging, as they hide between the spines forming the apical system (Stauber 1993). The most prominent structures of the internal cavity of sea urchins are their five gonads (ovary or testis). These organs differentiate from a group of cells—the gonadal primordium—located in the dorsal mesentery of the newly metamorphosed juvenile (Chia and Xing 1996; Houk and Hinegardner 1980). The gonads are distinct organs delimited by a peritoneum; their innermost tissue layer contains the germinal epithelium. Each gonad

forms a gonoduct joining the genital pore, an opening in the genital plates present on the aboral side of the animal. At the spawning period, eggs or sperm are released through these five genital pores. The group of Gary Wessel has extensively studied germ cell formation during echinoid development (for reviews, see Wessel et al. 2014; Swartz and Wessel 2015). The specification of these cells seems to be regulated by a conserved set of genes that include several classic germline markers such as *Vasa*, *Nanos* and *Piwi*. The germline cells derive from the small micromeres (Yajima and Wessel 2011), which appear early in embryogenesis during the fifth cleavage.

17.7 GENOMIC DATA OF ECHINODERMS

Strongylocentrotus purpuratus was the first fully sequenced echinoderm (Sea Urchin Genome Sequencing Consortium et al. 2006). It was also the first non-chordate deuterostome genome, allowing the characterization of gene family evolutionary dynamics within the Bilateria and Deuterostomia. The sea urchin genome contains roughly 23,300 genes representing nearly all vertebrate gene families without extensive redundancy. Some genes previously considered vertebrate exclusive were found in the sea urchin genome, tracing their origin back to the deuterostome lineage. Since its first release, the genome's assembly has been improved, and the latest release in 2019 is the v5.0 genome. Other echinoderm genomes have been sequenced following this pioneering work, including different representatives of each class. The echinoderm genomes available in the NCBI genome dataset are listed in Table 17.3.

The genome dataset is completed by a vast amount of RNA-Seq data that are accumulating at a steady pace. There are currently over 4,000 Echinodermata high-throughput datasets archived in the NCBI Sequence Read Archive (SRA) database. They are organized in 345 BioProjects (search in November 2020) concerning both nuclear and mitochondrial genomes and are useful for phylogenomic analysis, transcriptome analysis of developmental stages and adaptation to stress or climate change. Table 17.2 presents the omics availability in the different Echinoidea species listed in the biogeographic map and phylogenetic tree shown previously.

An important resource for biologists working on echinoderms is the Echinoderm genome database EchinoBase (www.echinobase.org, and its former version at legacy.echinobase.org; Kudtarkar and Cameron 2017). Originally set up for the annotation of the *S. purpuratus* genome, it has incorporated data for several other echinoderm species, and nowadays, it constitutes a crucial tool for studies on gene regulation, evolution and developmental and cellular biology.

Other useful databases are HpBase (devoted to the Asian sea urchin *H. pulcherrimus*; cell-innovation.nig.ac.jp/Hpul; Kinjo et al. 2018, and EchinoDB, comparative transcriptomics on 42 species of echinoderms; echinodb.uncc.edu; Janies et al. 2016).

TABLE 17.3
Echinodermata Genomes Available at the NCBI Genome Database (www.ncbi.nlm.nih.gov/datasets/)

	Genome Size (Mbp)	NCBI Latest Assembly	Year	Other Database
Echinoidea				
<i>Strongylocentrotus purpuratus</i>	921	Spur_5.0	2019	Echinobase: www.echinobase.org*
<i>Lytechinus variegatus</i>	1061	Lvar_3.0	2021	www.echinobase.org*
<i>Eucidaris tribuloides</i>	2187	Etri_1.0	2015	legacy.echinobase.org*
<i>Hemicentrotus pulcherrimus</i>	568	HpulGenome_v1	2018	HpBase: cell-innovation.nig.ac.jp/Hpul/**
Holothuroidea				
<i>Actinopyga echinites</i>	899	ASM1001598v1	2020	—
<i>Apostichopus japonicus</i>	804	ASM275485v1	2017	—
<i>Apostichopus leukothele</i>	480	ASM1001483v1	2020	—
<i>Australostichopus mollis</i>	1252	assembly_1.0	2020	—
<i>Holothuria glaberrima</i>	1128	ASM993650v1	2020	—
<i>Paelopatides confundens</i>	1128	ASM1131785v1	2020	—
<i>Stichopus horrens</i>	689	UKM_Sthorr_1.1	2019	—
Asteroidea				
<i>Acanthaster planci</i>	384	OKI-Apl_1.0	2016	Echinobase: www.echinobase.org*
<i>Asterias rubens</i>	417	eAstRub1.3	2020	www.echinobase.org*
<i>Patiria miniata</i>	811	Pmin_3.0	2020	www.echinobase.org*
<i>Patiriella regularis</i>	949	assembly_1.0	2017	—
<i>Pisaster ochraceus</i>	401	ASM1099431v1	2020	—
Ophiuroidea				
<i>Ophionereis fasciata</i>	1185	assembly_1.0	2017	—
<i>Ophiothrix spiculata</i>	2764	Ospi_un_1.0	2015	legacy.echinobase.org*
Crinoidea				
<i>Anneissia japonica</i>	589	ASM1163010v1	2020	Echinobase: www.echinobase.org*

*(Kudtarkar and Cameron 2017);

** (Kinjo et al. 2018)

17.8 FUNCTIONAL APPROACHES: TOOLS FOR MOLECULAR AND CELLULAR ANALYSES

Their external fertilization, the large number of gametes, the easy access to all stages of embryogenesis and the transparency of both eggs and embryos make echinoderms suitable organisms for different approaches in cellular biology, biochemistry and molecular biology. The availability of genome and transcriptome data (see genomic resources section) has facilitated gene expression analysis and manipulation in many sea urchin species and other echinoderms.

Spatial and temporal localization of mRNAs has been investigated by *in situ* hybridizations in several sea urchin species (Erkenbrack et al. 2019), as well as in other echinoderms (Fresques et al. 2014; Dylus et al. 2016; Yu et al. 2013). Localization of proteins at the cellular and embryonic levels by immunolocalization is often dependent on the availability of cross-reacting antibodies directed against vertebrate homologs of the protein of interest. Many commercial antibodies against mammalian proteins have indeed helped to decipher different molecular processes in sea urchins, such as microtubule dynamics and Cyclin B/CDK1 complex activity during embryonic divisions (see Figure 17.6). However, some specific antibodies directed against sea urchin proteins have also been developed in many laboratories (Venuti et

al. 2004). The function of many molecular players and signalling pathways has also been investigated using different pharmacological inhibitors or activators (Mulner-Lorillon et al. 2017; Molina et al. 2017; Feizbakhsh et al. 2020). Finally, labelling of eggs and embryos with radioactive and non-radioactive precursors allows for the monitoring of metabolic activities (for example, protein synthesis; Chassé et al. 2019).

Manipulation of gene function and/or expression during embryogenesis is achieved by the microinjection of various reagents, such as exogenous mRNA coding for native proteins and dominant-negative forms, morpholinos that interfere with the translation or splicing of endogenous mRNAs and, more recently, CRISPR-Cas9 reagents permitting gene knock-out. Microinjection represents, thus far, the only way to efficiently introduce reagents into the sea urchin eggs or blastomeres. Several recently published methods have described microinjection techniques and applications (von Dassow et al. 2019; Molina et al. 2019; Chassé et al. 2019).

The genome-editing CRISPR/Cas9 technology has been successfully implemented in sea urchin to efficiently knockout developmental genes. So far, the genes targeted by CRISPR/Cas9 were selected because of a visible F0 phenotype: disruption of dorsoventral patterning for Nodal knockdown (Lin and Su 2016) or albinism as a visual readout for polyketide synthase 1 (Oulhen and Wessel 2016). Recently, the successful

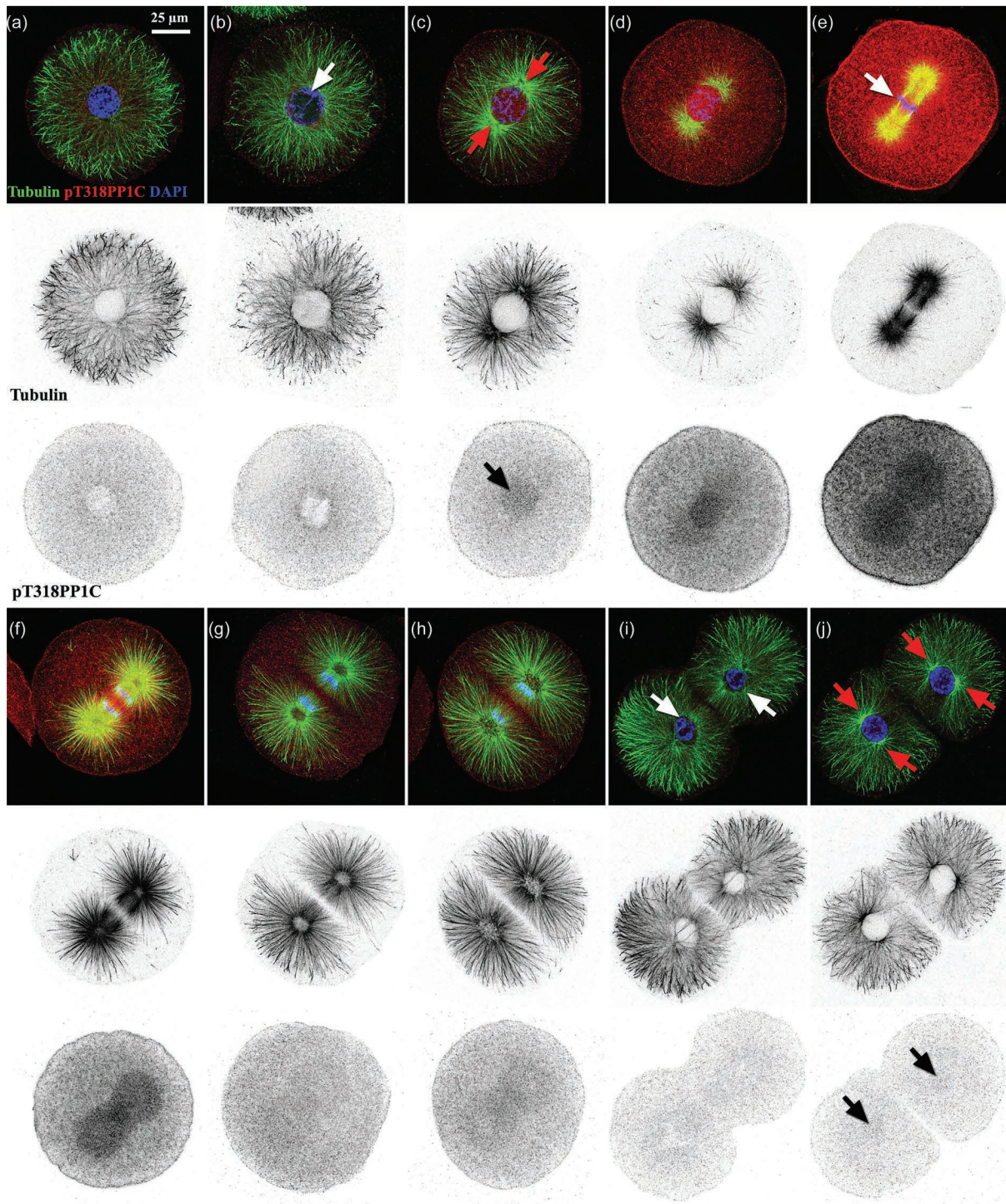


FIGURE 17.6 First mitotic division in *Sphaerechinus granularis* embryos. (a–j) Confocal micrographs describing progression through the first mitotic division in *S. granularis*. Embryos belonging to consecutive stages were labelled with anti-tubulin (shown in green, top panels; b/w, middle panels) and with an antibody against the T318 phosphorylated form of the phosphatase PP1C (red, top panels; b/w, bottom panels). The levels of this phospho-epitope reflect the activity of the Cyclin B/CDK1 complex (Chassé et al. 2016; Feizbakhsh et al. 2020). Nuclear DNA was labelled with DAPI (blue, top panels). (b) Chromatin condensation starts during early prophase (white arrow). (c) Later on, the pT318PP1C signal starts to accumulate in the nucleus (black arrow). The position of the MTOCs also becomes visible (red arrows). (d) Following the collapse of the microtubule radial network, the mitotic spindle begins to form. (e) During metaphase, the pT318PP1C levels reach their maximum, and the chromosomes align in the metaphasic plate (white arrow). The nuclear envelope has disappeared. (f–h) As sister chromatids separate during anaphase, the astral microtubules fill the entire cytoplasm. In parallel, the levels of pT318PP1C decrease dramatically. (i) Chromatin de-condensation begins in early telophase (white arrows). (j) By late telophase, the MTOCs of each daughter cell are apparent (red arrows). A faint pT318PP1C signal in the nuclei heralds the second mitotic division (black arrows).

production of a homozygous F2 mutant using the CRISPR-Cas9 system was obtained in *Temnopleurus reevesii*, which takes advantage of the relatively short life cycle of this species (Yaguchi et al. 2020; see also Chapter 18). This breakthrough gives us the possibility to implement genetic analyses in the sea urchin model (in species with short generation time) and study the function of many maternal factors and mRNAs.

17.9 CHALLENGING QUESTIONS

During their long evolutionary trajectory, echinoderms have adapted to all sorts of climatic conditions and have colonized most of the ocean floor, from the intertidal areas to the deep-sea benthos. The study of this adaptation capacity has just begun and should foster many exciting discoveries. Moreover, it has become evident that echinoderms constitute a valuable biological system to analyze the potential of marine species to adapt to anthropogenic disturbance. Their population densities are very sensitive to climate change, ocean acidification, eutrophication, overfishing, predatory removal and the introduction of alien species (Uthicke et al. 2009). In addition, this group of animals plays a crucial role in many marine habitats and food webs, and several members of this clade have been recognized as “keystone species” in different ecosystems (Power et al. 1996). Echinoderms have thus acquired an essential place in experimental marine ecology.

As detailed previously, many genomic resources are available nowadays for the researchers studying this clade, in particular for those interested in the analysis of the echinoids. These resources have greatly facilitated the development of comparative approaches aimed at understanding the genetic basis of adaptive traits. The density of available landmarks, including closely related species but also different groups separated by increasing phylogenetic distances, allows dissection at the molecular level of both micro- and macroevolutionary processes.

In echinoderms, many studies have focused on the acquisition of evolutionary novelties and the diversification of life strategies. For instance, it has been shown that several species have significantly accelerated their life cycles, reprogramming their ancestral planktotrophic larvae into non-feeding lecithotrophic forms (Raff and Byrne 2006). These evolutionary transitions have obvious adaptive roles. In lecithotrophic species, the life cycle becomes independent of fluctuations in plankton levels since their development relies on the nutrients supplied by their mothers. Indeed, it has been argued that the disturbance of planktonic food chains could contribute in the near future to the decline of planktotrophic species (Uthicke et al. 2009). At the same time, these developmental transitions can now be analyzed in great detail both at the cellular and molecular levels.

For instance, it has been shown in the *Heliocidaris* genus that the eggs of lecithotrophic species have undergone an outstanding increase in size, driven by a thorough remodelling of their oogenesis program (Byrne et al. 1999). Moreover, the comparison of gene regulatory networks controlling early development, like in the lecithotrophic

Heliocidaris erythrogramma and the planktotrophic *Heliocidaris tuberculata*, provides important hints about the identity of the molecular players participating in evolutionary change (Israel et al. 2016). These approaches have greatly benefited from the deep knowledge of developmental networks acquired thanks to the study of early development in *Strongylocentrotus purpuratus* and other echinoderm species (Cary and Hinman 2017).

Echinoderm biology stands now at the intersection between ecology, cell and developmental and evolutionary biology and should greatly profit from this privileged position.

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