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Chapter 1

Introductory Chapter: Sea Urchin - Knowledge and Perspectives

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http://dx.doi.org/10.5772/intechopen.70415

1. Introduction

This book is a collection of chapters aimed to provide an overview of some interesting fields of the current research on sea urchin. This magnificent marine world-wide diffused organism offers a myriad of opportunities for investigations on the most various topics, from ecology and toxicology, to aquaculture, as well as to marine, developmental, molecular and cellular biology. To this list of subjects, they must add new emerging biomedical and pharmaceutical applications that, on one side, make this organism even more interesting for those who do not know it from a scientific point of view, and on the other side, rejoins the trust of those who well know its suitability for the basic research, offering further opportunity, e.g. for applied biological investigations.

The sea urchin is an ancient seafloor-dwelling invertebrate, belonging to the phylum of Echinoderms (Figure 1), which appeared 520 million years ago, before the Cambrian explosion [1].

Historically, sea urchin has been a key model system in elucidating a variety of classic developmental problems, including fertilization, cleavage, gastrulation and regulation of differentiation in the early embryo [2]. Nowadays, the availability of modern methods of molecular biology allows to deepen specific aspects of the developmental mechanisms, leading to further significant results.

But what are the reasons for the enormous application of this organism for developmental, cellular and molecular studies? These are the most important ones: the gametes can be obtained easily, sterility is not required and the eggs and early embryos of many commonly used species are beautifully transparent. In addition, the early embryo development is highly synchronous. For all these reasons, biochemical and molecular studies result simplified and more feasible.

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This introductory chapter has been divided in sections, aimed to provide a panorama of the current knowledge and future perspectives offered by using this organism.

2. **Phylum and ecology**

Sea urchin belongs to the class of echinoid, organism of the Echinoderms *phylum*, divided into five main classes: crinoids, asteroids, ophiuroids, echinoids and holothurians (Figure 2). Recent molecular studies support the existence of a further class for this phylum, that is Asterozoa, an ophiuroid/asteroid clade [3].

Crinoids are considered the most primitive class, whereas echinoids and holothurians are the most advanced. Despite the various shapes, Echinoderms possess common characteristics: benthic lifestyle, adult radial symmetry, water vascular system and calcite endoskeleton with a three-dimensional structure. Their success, testified by the worldwide distribution in several oceanic environments at various depths, latitudes and temperatures, is related to the ability to survive in adverse conditions, by means of strategies such as a spiny defence structure, an immune defence system, a toxin-producing apparatus and an incredible regeneration.
capability [1, 4]. To these strategies, the skill of Echinoderms to face the constant exposure to environmental factors, such as predation, changes in temperature and pH, hypoxia, pathogens, metals, toxicants and pollutants, must be added [5].

Among the five classes of Echinoderms, echinoids are the most various and well-represented, from shallow waters to abyssal depths. They are radially symmetrical in adults, ranging from a few millimetres to more than a meter in diameter, and live with their mouth, downward-facing [6]. The group of echinoids includes the sea urchins, heart urchins and sand dollars. Echinoids are usually globular, discoidal or heart-shaped and have a skeleton made of many calcareous plates. Regular echinoids, such as sea urchin, can be distinguished easily from irregular ones by their spherical body, a pentaradial symmetry, and the central location of the anus, above the mouth. The spines are generally long and a jaw apparatus is present in all taxa. Irregular echinoids are elongate in the adult stage. This difference of shape and the posterior position of the anus, instead of dorsally like the regular echinoids, are the two most tell-tale differences setting the two types apart [7, 8].

The spines are used for locomotion, as a passive defense against predators and for trapping floating food particles. Tube feet are located among the spines and are used to move, capture food and attach to substrates. A stinging jaw (or small pincers), called the pedicellariae, is
also used for protection and to clutch food items. The mouth of the sea urchin is located on the underside of the organism and consists of a five-pointed jaw called Aristotle’s lantern [8].

For their evolutionary position, Echinoderms represent a link between invertebrates and vertebrates, thus occupying a strategic phylogenetic position. This evidence has strongly been sustained by fully sequencing the genome of *Strongylocentrotus purpuratus*: sea urchin is more closely related to humans than other invertebrate models [9, 10].

The echinoderm larval skeleton has been recently proposed as a possible model system for experimental evolutionary biology. It is well known that the evolution of various body plans results from the acquisition of novel structures and/or the loss of existing structures, by multiple and intermediate steps, adaptive or neutral. As the acquisition of a larval skeleton requires multiple steps, this mechanism provides a good model for reproducing intermediate evolutionary stages [11].

Different species of sea urchins live in a variety of marine environments of the world. Some usual places where they live are rock pools and mud, wave-exposed rocks, coral reefs in kelp forests and in sea grass beds, in order to protect themselves from large waves or currents. Sea urchins live in areas where they can find algae, sea grass, seaweed and other foods to consume. One other very important characteristic of these organisms is that they are nocturnal because of their light sensitivity. For this reason, they move spines in reaction to shadows. In order to protect themselves from the powerful ocean currents and waves, sea urchins lodge themselves into holes or crevasses. A common place where it is possible to find a sea urchin is in coral reefs in which it plays a key role in the maintenance of feeding integrity of corals [12]. But sea urchins have also been reported to cause erosion of reefs, so although they are important to the survival of an ecosystem, they can also become dangerous in great numbers [13–15]. In the first chapter of this book, by a comparative analysis among different species of sea urchin, several purposes for the cryptic behaviour are considered: source of food, mechanical defence, protection from sunlight and predators. The results suggest that a phylogenetic relationship provides a functional predictive tool for determining the purpose of the covering.

### 3. Fishery and aquaculture

Cultivation of sea urchin increasingly becomes a necessity, both for stock enhancement programs and as a means to meet market demand. Sea urchin culture has been practised on a large scale for many decades in Japan, where effective methods have been established. In the rest of the world, sea urchin cultivation is a more recent practice.

It must be consider that when wild stocks decline, the demand created in the market place raises the price of the product and, consequently, culturing is more likely to become economically profitable. Hence the future of the aquaculture industry is closely linked to that of the fisheries, whose fate will ultimately determine the market forces that will shape this growing industry [16]. But it would be desirable that the aquaculture systems would diffuse without waiting for depletion of wild stocks.
There are several examples of how a sea urchin fishery can be managed poorly. The most sobering example was the serial depletion of the Chilean fishery, the main producer and exporter country in the world before 2002. Smaller fisheries exist also in Europe, mainly supplying domestic markets. This fishery has undergone a major transformation to implement management strategies and to avoid a fishery collapse. There is a number of well managed and sustainable sea urchin fisheries around the world that tend to rely on a good overview of the biology of species present in the area as well as sound knowledge of the dynamics of the sea urchin populations. Comprehensive stock assessment and mapping also appears to be an integral part of successful fisheries management [17].

The commercial fisheries and destructive harvesting methods employed to meet market demand, caused, in the last decades, a dramatic depletion of Paracentrotus lividus, especially in the Mediterranean, leading to a complete disappearance of urchins from areas of former abundance. Strong aquaculture research programs currently exist in many countries. Several approaches have been tested to respond to the depletion of wild stocks [18–20], but the most challenging strategy could be the setting up of closed aquaculture systems. Several studies have been carried investigating different light/dark regimes, temperature and supply of artificial diets to ensure a rapid gonadal growth and to promote an effective maturation of gametes [21, 22].

In the chapter of Sartori and colleagues the usefulness of formulated diets rich in carotenoids [23–26] was analysed, considering different biological parameters. The authors report the most effective breeding conditions, in Recirculating Aquaculture System, to promote gonadal growth and sexual maturation. In this way, gaps in reproductive events of natural populations can be overcome, always having good quality gametes available for ecotoxico logical applications.

Another interesting and cost-effective option is to obtain an uniform quality of gonads, in combination with the systems that use the co-culture with other organisms [27]. As the diet quality can significantly influence the somatic and gonadal growth of sea urchins [28–32], in this book, Tenuzzo and coworkers analyse the morphological and biochemical profiles of the gonadal cycle of P. lividus, comparing wild-type organisms with bred ones. These data allow to define the best diet for sea urchins in view of the breeding set-up conditions, representing an important starting point for a scale production of P. lividus.

4. Developmental and molecular biology

Sea urchin typically has separate sexes and fertilization takes place externally. During the gametogenic cycle of sea urchin, gonads of both sexes undergo a series of structural changes in charge of the two major cellular populations of the germinal epithelium: (i) germinal cells, i.e. oogonia or spermatogonia and (ii) somatic cells, called nutritive phagocytes, present in both sexes, in which nutrient storage occurs. Nutritive phagocytes are somatic cells that provide a structural and nutritional microenvironment for germinal cells during gametogenesis [33]. The oocytes of sea urchin, differently from other Echinoderms and other animals, complete meiotic maturation before fertilization. During oogenesis, several processes occur to
ensure the correct formation of the mature eggs. Recent data have shown that the autophagic process is required for sea urchin oogenesis undoubtedly as a catabolic basal mechanism. The major concentration of vesicular acidic organelles is near the germinal vesicle. Interestingly, the mitochondria are more active in the same regions [34, 35]. The manifestation in the same areas of two catabolic mechanisms, oxidative phosphorylation and autophagy, is interesting as their coexistence could occur to ensure the final steps of the meiotic process.

Spawning of gametes occurs in the spring and early summer, when water and food conditions are favourable. Females will release several million eggs into the water which will unite with sperms released from the males. Embryo development, till pluteus stage, can be followed continuously in real time by microscopy, because the embryos are optically transparent and no histological preparation is required.

After hatching and gastrulation, the free swimming larvae, with a planktonic existence which lasts several months, undergo metamorphosis, before they settle to the bottom. Sexual maturity is reached at 4–10 years, with a diameter of 1.8–2.5 cm. As sea urchin embryo develops rapidly (15- to 60-min cell cycles depending on the species), synchronously and predictably, large cultures can be cultivated, treated and dissociated, while individual embryos can be injected or manipulated to test important features of their mechanisms of development. The micromeres obtained after the fourth cell division are the major signalling centre of the embryo [2, 36]. The mechanism behind this induction is poorly understood, but the micromeres do acquire nuclear β-catenin shortly after their formation, and evidence suggests Notch-Delta and Wnt8 signalling as candidates for this inductive process [37]. Perturbation of the transcription factors and signals provided the means for assembling models of the gene regulatory networks used for specification and the control of the subsequent morphogenetic events. By this tool, it was learned that ectoderm provides a series of patterning signals to the skeletogenic cells and as a consequence the skeletogenic cells secrete a highly patterned skeleton based on their ability to genotypically and locally decode several signals [38].

The ability to trace the entire trajectory of specification of a cell type until it terminally differentiates is now a realistic goal. In its current form, the sea urchin genes regulatory network includes more than 100 transcription factors and a number of signalling pathways, and in most cases, multiple laboratories have validated each connection in different sea urchin species, such as *Lytechinus* and *Paracentrotus*, starting from *S. purpuratus*. Using a similar tool, it is now possible to analyse and understand how a cell type arises and works in detail. This will be valuable in understanding the entire history of neurogenesis, and in determining the mechanisms by which neurons diversify toward different neurotransmitter cell types [39].

5. Defence mechanisms: a success guarantee!

Sea urchin is considered a good bioindicator of marine health, as well as a good tool for testing the toxicity of environmental and chemical molecules. Currently, it is one of the organism considered by the European Agency for Alternative models and in full respect of the 3Rs objectives (reduction, refinement and replacement of animal experiments); thus, it could be a
suitable organism for toxicological studies as alternative to vertebrates. Sea urchin is able to detect both biotic and abiotic stress and to recognize, transform and eliminate many potentially harmful materials to protect itself.

The immune defence of sea urchin, appeared early in the evolution, is mediated by a vast repertoire of molecules [5]. Human require both adaptive and innate immune responses, whereas sea urchin require only innate immune functions, as revealed by the several innate immunity gene families discovered [40]. The immune cells from the adult *P. lividus*, which have been introduced in the past 15 years as sentinels of environmental stress and for toxicological testing, are valid tools to discover basic molecular and regulatory mechanisms of immune responses [5]. Echinoderm immune cells, also known as coelomocytes, are a heterogeneous population of freely moving cells found in all coelomic spaces, including the perivisceral cavities and the water vascular system. They are also present sparsely in the connective tissue and among tissues of various organs [41–44]. A rapid increase of the number of the red amoebocytes has been shown in samples taken from polluted sea water, whereas petaloid cells are actively involved in the phagocytosis and phylopodial cells trigger the clot formation [5].

A recent and promising search path concerns the antimicrobial peptides (AMPs), important effector molecules in innate immunity. Echinoderms live in a microbe-rich marine environment and are known to express a wide range of AMPs. Recently, two novel AMP families from coelomocytes of sea urchins have been found: strongylocins and centrocins [45].

Another defence strategy employed by some species of sea urchin is represented by toxins. The spines of the species *Tripneustes gratilla* are considered toxin-producing organs. Upon being stung by this sea urchin, humans and animals could be poisoned, with difficulty breathing, muscle paralysis, convulsion and other symptoms [46].

Several toxicological studies analyse the possibility that a number of environmental molecules and xenobiotics interfere with embryogenesis and cause developmental anomalies [47–49]. The effects of environmental anthropogenic factors on reproduction of the sea urchin *Staphylococcus intermedius* are considered in the second chapter of this book. The results of long-term studies in wild populations, located in the north-western Sea of Japan and with different levels of anthropogenic pressure, indicate that the principal external factor determining a shift in timing of spawning is the phytoplankton concentration, closely related to the chronic eutrophication.

Environmental stress usually causes severe effects on embryogenesis, but sea urchin is able to detect stressful insults and to activate a response. It is well known that embryos of different species, exposed to different kind of stress, temporarily suspend their development and activate several protective strategies, including programmed cell death. Sea urchin activates several cellular strategies of defence, such as synthesis of heat shock proteins, apoptosis and autophagy, to face different stress and survive in adverse conditions. The protective action of heat shock proteins is not always sufficient to block the toxic action of a stressor. When the cell damage is too great, mechanisms of programmed cell death, such as apoptosis and autophagy, may occur and contribute to the elimination of the irreversibly damaged cells in order to maintain the integrity of the embryos (Figure 3).
A xenobiotic able to cause a strong activation of all embryonic defence mechanisms reported in (Figure 3) is cadmium, an heavy metal [47, 50–53]. It must be underlined that such defence mechanisms are basally activated in physiological conditions and that their extent highly increases in stress conditions.

In the last chapter of this book, the authors draw attention on the validity of sea urchin embryo as a model to test the developmental effects and toxicity responses against various environmental contaminants and chemical compounds.

The evaluation of the defensive responses of sea urchin is of high interest to researchers and marine organizations for the management of biological resources and ecosystems [54].

6. New perspectives

Like many other marine organisms, Echinoderms have been, and continue to be, examined as a source of biologically active compounds with biomedical applications [16]. Multiple species of sea urchin were used anciently in the traditional Chinese medicine for treating several diseases. The shells of yellow sea urchin, everyday consumed, are discarded, although they could be employed by pharmacologists for discovering the medicinal components. Several examples of Echinoderms derived substances that may have biotechnological value and therapeutic
application have been reported, e.g. antibiotic, antifouling and anticancer molecules [55]. The major chemical and bioactive constituents of sea urchin have recently been summarized: pigments, proteins, toxins, polysaccharide, minerals, sterols, vitamins, fatty acids and amino acids. Modern medical studies have proved that shells, spines, gonads and other parts of sea urchins have a good medicinal value. Ulcer disease, otitis media, cervical lymph node tuberculosis and pain are some examples of pharmaceutical applications. The toxic, haemolytic and hypotensive functions of white sea urchin spines have been used to develop neuromuscular blocking drugs [56]. For all these reasons, sea urchin clearly represents a fairly unexploited source in the identification of new and useful drugs. These bioactive molecules were produced in the course of millions of years of evolution in order to protect the individual, the cells or the entire organism from possible competitors, are continuously evolving molecules that interfere with signalling or are simply toxic for other cells [57]. A novel and promising search path concerns antimicrobial peptides (AMPs). The echinodermal AMPs are crucial molecules for the understanding of immunity, and their potent antimicrobial activity makes them potential precursors of novel drugs [45].

In 2006, it has been shown that 7077 sea urchin genes are conserved in humans, and many of these may correlate with cancer, Huntington’s disease, muscular dystrophy, atherosclerosis and other illnesses [9]. The last chapter of this book offers an interesting overview of the contribution of sea urchin embryo as a simple model to study ageing and age-associated neurodegenerative diseases, as well as the pathways involved in cell survival and death.

7. Conclusions

Since the beginning of cell biology, the Echinoderms have been employed as embryological model systems to study basic phenomena, such as mitosis, cell division, differentiation and organ formation, by the famous cell biologists in the nineteenth and twentieth centuries, such as Boveri, Heilbrunn, Mazia, Monroy, Wilson, Hertwig and Brachet. Today, sea urchin continues to be the model of choice for several cellular and molecular biologists, contributing to significant advance in the fields of evolutionary and developmental biology and leading to the discovery of complex gene regulatory networks. In the same time, its usefulness as a sensitive system for toxicological test and environmental studies continues to increase.

Although at present, sea urchins seem to be in no immediate danger of disappearing or becoming endangered; in general, they have shown mass mortality caused by an increased pollution in the marine environment, an increased amount of fishing by humans, as well as a rise in the temperature of water due to global warming. As sea urchins are very susceptible to changes, they may become endangered in the future; thus, the use of aquaculture systems is desirable.

Furthermore considering the current interest for novel drugs discovery, the urgent need for novel antibiotics against the bacterial resistance and the need of new strategies in cancer research, sea urchin represents an important resource to exploit in the biotechnology and medicine field.
Acknowledgements

A special thanks to Prof. Maria Carmela Roccheri and Anna Maria Rinaldi, the main guides in my research path on sea urchin.

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