Cell Adhesion and Communication: A Lesson from Echinoderm Embryos for the Exploitation of New Therapeutic Tools

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Abstract. In this chapter, we summarise fundamental findings concerning echinoderms as well as research interests on this phylum for biomedical and evolutionary studies. We discuss how current knowledge of echinoderm biology, in particular of the sea urchin system, can shed light on the understanding of important biological phenomena and in dissecting them at the molecular level. The general principles of sea urchin embryo development are summarised, mainly focusing on cell communication and interactions, with particular attention to the cell–extracellular matrix and cell–cell adhesion molecules and related proteins. Our purpose is not to review all the work done over the years in the field of cellular interaction in echinoderms. On the contrary, we will rather focus on a few arguments in an effort to re-examine some ideas and concepts, with the aim of promoting discussion in this rapidly growing field and opening new routes for research on innovative therapeutic tools.

1 Introduction

Echinodermata are among the most familiar marine invertebrates. The phylum, characterised by the great morphological variety of its members, belongs to a branch of the animal kingdom known as deuterostomes. The Echinodermata have been extensively studied, particularly because of some aspects such as the ample fossil record extending back to the Precambrian, their ecological importance in the marine environment, the interesting mor-

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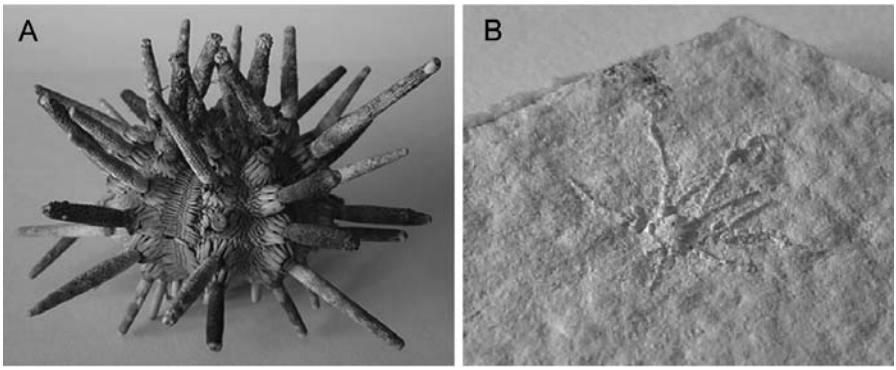


Fig. 1. **A** Living fossil, the pencil urchin *Eucidaris tribuloides*. **B** Presumptive crinoid fossil found in 1981, in the Black Forest, Germany

phology of adult organisms and the advantage of experimentally manipulable embryos. Approximately 13,000 echinoderm fossil species are known (Fig. 1), while living species number about 7,000 and fall into five well-defined classes:

- Crinoidea (sea lilies and feather stars), with about 600 living species. The body is oriented with the mouth facing up and they may or may not have a stalk.
- Asteroidea (starfishes or sea stars), the most familiar forms to man, with about 1,500 living species. Typically, they have their oral surface on the ventral side and usually have multiple "arms" surrounding a central disc.
- Ophiuroidea (basket stars and brittle stars), with five arms, typically flexible, radiating from a central disc, which are used for locomotion. The class includes about 2,000 living species.
- Echinoidea (sea urchins, sand dollars and sea biscuits), with a usually globular body, formed by the fusion of skeletal plates, without distinct arms. Some of them have bilateral symmetry, which occurred secondarily during evolution. About 1,700 living species are known.
- Holothuroidea (sea cucumbers), characterised by a cylindrical shape and a relatively soft body. About 1,100 living species are known.

The Concentricycloidea (sea daisies), with only two species, is an enigmatic group of echinoderms whose phylogenetic position remains elusive, although evidence suggests a relationship with asteroids (Pearse and Pearse 1994). The phylogenetic relationships among the five classes have been extensively controversial, but it seems generally accepted that the class of Crinoidea branched first and that the Echinoidea and Holothuroidea are sister clades (Fig. 2).

Although the main character of the body plan of adult echinoderms, the pentamerous symmetry, led the father of palaeontology, George Cuvier (1769–1832), to place them in the Radiata phylum, it is now widely accepted

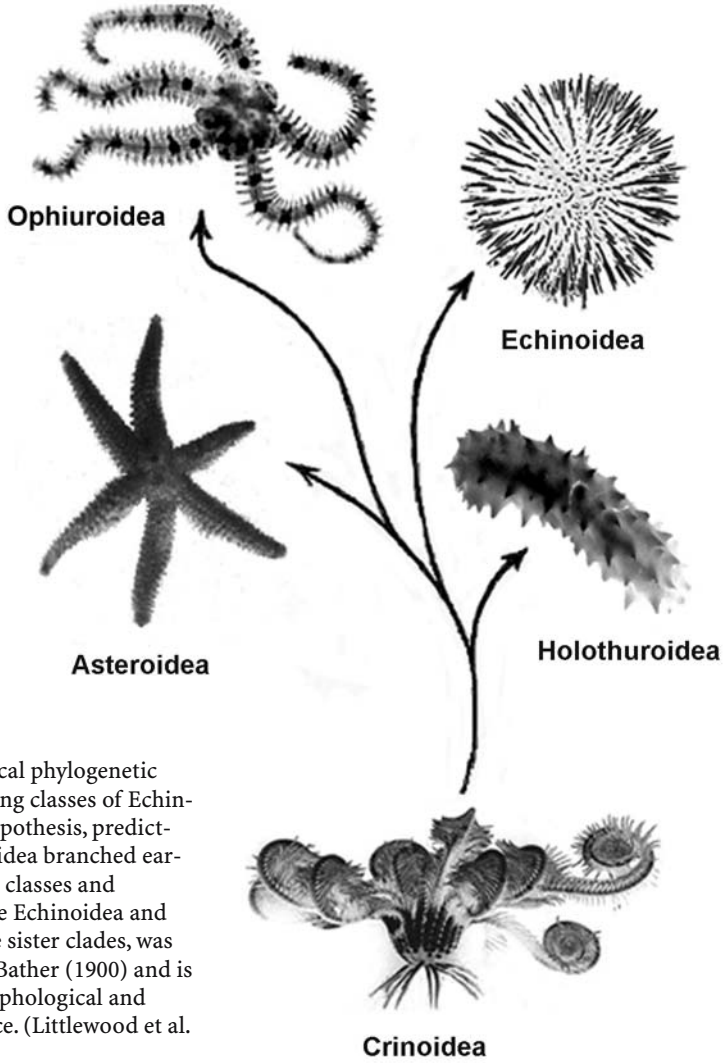


Fig. 2. Hypothetical phylogenetic tree of the five living classes of Echinodermata. This hypothesis, predicting that the Crinoidea branched earlier than the other classes and suggesting that the Echinoidea and Holothuroidea are sister clades, was first proposed by Bather (1900) and is supported by morphological and molecular evidence. (Littlewood et al. 1997)

that the phylogenetic proximity of echinoderms is to chordates. Among marine invertebrates, in fact, the echinoderm clade has a unique position in the evolutionary tree: during evolution it first appeared in the Metazoa phylum with features of deuterostomes, in direct evolutionary line with chordates and vertebrates, thus implying that there is less divergence between echinoderms and vertebrates than between echinoderms and other invertebrates. This is not surprising since, although the two phyla appear so dissimilar, molecular data and fossil records strongly support this grouping, even if the attempts to identify a common ancestor are highly speculative. As the eminent anthropologist Konrad Lorenz observed, "It is not a theory, but an irrefutable

historical fact, that the living world – since its origin – has evolved from 'below' to 'above'". Actually, it is becoming clearer that the genes encoding developmental regulatory proteins already identified in other phyla, the modified interactions among them and changes in their expression patterns are at the basis of the evolution of animal morphology (Raff 1996; Wray and Lowe 2000).

The name echinoderm is derived from the Greek word meaning spiny skin and it has been the original denomination for sea urchins, since spines are their main external characteristic. Echinoderms are exclusively marine invertebrates and, with a few exceptions, are all benthic organisms (bottom-dwellers). A number of lifestyles are typical among different classes, e.g. sea star and crinoids are predators and filter-feeders respectively, while sea urchins scrape algae from rocks, and holothurians, sand dollars and ophiuroids often feed on remains.

Echinoderms usually have separate sexes with no evident sexual dimorphism. Reproduction is typically achieved by external fertilisation, with eggs and sperm freely released into the seawater. The life cycle is usually complex. In most echinoderms the embryo develops into a planktonic larva with a bilateral symmetry, that in some species goes through metamorphosis, typically radical, before reaching the final adult morphology.

A number of morphological features are unique to the Echinodermata phylum, including:

- a pentaradial symmetry in adults (higher-order radial symmetry can be observed in several cases, but it is clearly a secondary modification);
- a water vascular system, consisting of a set of water-filled canals branching from a ring canal and leading to tube feet, which has many important functions, including locomotion, cellular respiration and feeding;
- a mesodermal endoskeleton composed of calcareous plates assembled in a meshwork;
- a complex subepithelial nervous system with radial nerves running under each of the ambulacra, the row of tube feet, but without a recognisable central part;
- sensory neurons located primarily within the ectoderm of podia, without specialised sense organs;
- a circulatory system, if present, consisting of a haemal system derived from coelomic sinuses.

2 Why Study Echinoderms?

There are some features that make echinoderms so interesting to study. First, their sensitivity to environmental changes in seawater ecosystems. It is well known that the disappearance of fragile species from certain geographic areas is in direct relationship with high contamination of seawater and sedi-

ments, and echinoderms are one such fragile species. In particular, embryos, juveniles and adults of the classes Echinoidea and Asteroidea are well utilised in studies on marine pollution, since they highly resent it. Second, their ability to regenerate parts of the body (particularly Ophiuroidea and Asteroidea), based on stem cell recruitment. This phenomenon makes a fundamental contribution to the adaptive capacities of the whole species. Third, their ability to cause remarkable transformations in submarine substrates. Since the echinoderms are one of the most important marine invertebrates that do not feed on filtration, as they graze on substrate (except for holothurians and crinoids), they can induce changes in the ratios and distributions of other marine species (fishes and others), and eventually cause segregation as well as speciation. These three features of echinoderms are just some examples of the huge number of ways in which echinoderms can be of help in the understanding of important biological phenomena, and in dissecting them at the molecular level.

2.1 Echinoderms for Biomedical Research: A Simple Model to Study Biological Events Occurring in Higher Organisms

Recently, a number of studies have offered new notions that non-mammalian models may represent important future directions for studies on human diseases, since the results obtained from these models are in many cases applicable to mammals including humans. Few people realise that research on simple marine organisms has led to some of our greatest medical advances, as well as to new insights into environmental pollution. One of the advantages of using echinoderms is that they produce thousands of virtually identical embryos, and that the morphological abnormalities are readily visualised in the live organism under the light microscope. In the following text, some aspects of the medical advances achieved from such studies will be briefly outlined.

2.1.1 Screening and Testing of Toxic Substances

Sea urchin gametes, embryos and larvae can be used for fast, low-cost and reliable screening and testing of toxic substances and for detailed studies on their mechanism of action. One example is the screening for the toxicity caused by retinoids utilised in dermatological practice, since it has been shown that foetal malformation is a major form of toxicity associated with some of them (Kahn et al. 1988; Sciarrino and Matranga 1995). The sea urchin embryo has also been used as a model for screening for suspected mammalian developmental neurotoxicants and for anticancer drug testing (Nishioka et al. 2003; Qiao et al. 2003). This test system is applicable also for exami-

nation of new and known pharmacologically active substances, including their adverse effects and potential antidotes. Interestingly, a method has been developed to study the invasive properties of metastatic cells and to test the differential effects of anti-tumoural substances on their invasive capacity, which makes use of the sea urchin embryo basement membrane (Livant et al. 1995; Dyer et al. 2002). This structure is selectively permeable and can be obtained intact with the associated extracellular matrix (ECM) from sea urchin embryos (Livant et al. 1995). It has been demonstrated that all metastatic tumour cells placed in contact with these basement membranes were able to invade them and the invasion was rapid and efficient; on the other hand, as expected, non-metastatic cells failed in the invasion. These results suggest that molecules participating in basement membrane recognition and invasion have been functionally conserved during evolution and that their constitutive activity may allow metastatic cells to escape their tissues of origin (Livant et al. 1995).

2.1.2
From Echinoderm Molecules to Mammalian Diseases: How Fundamental Research Points to Clinical Trials

The analysis of genome sequences led to the finding of novel non-traditional targets involved in disease pathogenesis, whose usage has the advantage of removing infection without inducing resistance. For example, it has been shown that novel polysaccharides present on echinoderm surfaces seem able to stimulate early host defence and microbial clearance, but not the later phases of inflammatory tissue injury associated with sepsis. These are the most promising alternative or integrative treatments for pneumonia that are under development (Cazzola et al. 2004).

In the last 10 years, a number of molecules with different effects on mammalian cells have been purified from echinoderms. These include compounds with anti-coagulant activity on human blood cells, such as the peptide "plancinin" isolated from the sea star (Koyama et al. 1998), a promising drug for anti-thrombotic therapy. Other compounds display considerable cytotoxicity against a small panel of human solid tumour cell lines, such as polyhydroxysterols and saponins isolated from the sea star (Wang et al. 2004) and the glycolipid A-5 extracted from sea urchin intestine (Sahara et al. 1997, 2002). The latter compounds have been suggested as useful drugs for cancer chemotherapy.

Recently, Meijer and Raymond (2003) have reviewed the steps that lead to the identification of new drugs; that are now under evaluation for therapeutic use against cancer, neurodegenerative diseases and cardiovascular disorders. This is an example of how results obtained from basic research, i.e. studies on the cell cycle in the starfish oocyte model, can be utilised in applied medical research and treatment. The starfish cyclin-dependent kinase CDK1/cyclin B

was initially identified as a universal M-phase-promoting factor and then used as a screening target to identify pharmacological inhibitors. From the first inhibitors discovered, a more selective one was optimised, which is now entering phase II clinical trials against cancers and phase I clinical tests against glomerulonephritis (Meijer and Raymond 2003).

2.1.3
Highly Conserved Proteins Associated with Important Biological Functions

The study of echinoderms also led to the identification of proteins with high levels of homology to vertebrate proteins expressed in particular syndromes or tumour cells. A sea urchin gene showing very strong sequence and structural homology with the gene coding for dystrophin, which is defective in Duchenne muscular dystrophy, has been identified. The partial characterisation of this gene helped in the construction of an evolutionary tree connecting the vertebrate dystrophin gene family with related genes in invertebrates (Wang et al. 1998). A novel protein homologue to the sea urchin fascin (an actin-bundling protein) has been found to be over-expressed in pancreatic ductal adenocarcinoma, suggesting its use as a tumour marker with potential diagnostic and therapeutic implications for pancreatic carcinoma (Maitra et al. 2002).

Sea urchin sperm homologues of polycystin-1 and polycystin-2, the proteins mutated in autosomal-dominant polycystic kidney disease, have been sequenced (Mengerink et al. 2002; Neill et al. 2004). Both proteins have been shown to co-localise exclusively to the plasma membrane over the sperm acrosomal vesicle, where they may function as a cation channel mediating the sperm acrosome reaction. These data provide the first suggestion for the role of a polycystin-1 protein in a specific cellular process (Mengerink et al. 2002).

Recently, a fasciclin-I-like protein has been purified from sea urchin ovaries and, by in vitro assays, it has been shown to be active in promoting HT1080 human fibrosarcoma cell attachment (Sato et al. 2004). Fasciclin-I is a neuronal cell adhesion molecule and up to now various proteins belonging to the family have been identified in different species, including bacteria, plants and vertebrates, and in the sea urchin embryo and eggs (Brennan and Robinson 1994; Wessel et al. 2000), although their biological function had not been characterised. However, latest findings indicate that the protein is highly conserved in evolution and suggest important biological roles (Sato et al. 2004).

There are also examples of the isolation of new human genes whose function has been hypothesised on the basis of their high homology to already known and characterised echinoderm genes. A novel human homologue of the gene coding for echinoderm microtubule-associated protein (EMAP) has been isolated from a locus of Usher syndrome type 1, an autosomal recessive genetically heterogeneous disorder. The finding of its high level of homology to the echinoderm cytoskeletal component EMAP, especially at the micro-

tubule-binding domain, and the proposed cytoskeletal nature of Usher disease, define the human EMAP as a good candidate for the USH1a syndrome origin (Eudy et al. 1997).

3
Use of Echinoderm Embryos to Study the Basic Mechanisms of Communication Among Cells

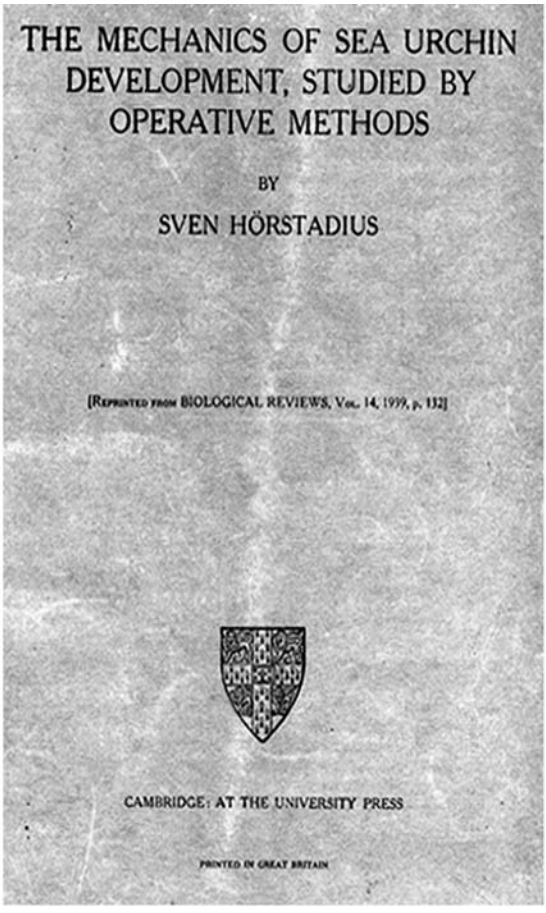
Developmental biology is a discipline studying the mechanisms regulating embryogenesis and it is one of the most attractive fields in rapid expansion among the biological sciences. Its study is becoming essential for the comprehension of any other fields in biology, since it combines molecular and cellular biology, physiology, anatomy, immunology, research on cancer and also evolution and ecology. Development is mainly devoted to the production and organisation of all the different cellular types constituting the adult organism. The generation of different types of cells is a process known as differentiation, while the organisation of differentiated cells in tissues and organs is performed during morphogenesis.

It is well known that cells do not behave as single entities, but rather their association in multicellular structures requires precise co-ordination between release and uptake of signals. Communication and interaction among living cells are, in fact, fundamental events required for the proper development of tissues and organs. Living cells continuously receive inputs from the environment and modify their behaviour throughout a complex network of signalling pathways. This communication occurs at various levels and, to obtain co-ordinated responses, the integration of exchanged information is essential. Malfunctioning of this network of signalling pathways is often connected with pathological conditions ranging from abnormal proliferation to cell death. Understanding the molecular basis of communication among living cells is a fundamental challenge for biologists, since, besides providing better understanding of the processes controlling growth, differentiation and death, it may increase the number of discoveries of new therapies against diseases caused by inappropriate signalling.

Historically, within the phylum of Echinodermata, the sea urchin embryo has been an excellent experimental system for investigating the cellular basis of development, principally because of its relatively simple organisation and because of its optical transparency that makes the observation of morphogenesis in vivo possible. Pioneering studies on development date back to 1892, when Driesch, following the experimental approach to embryology proposed by Roux about 10 years before, utilised the sea urchin embryo in his studies with important outcomes for embryology (Driesch 1892). On the basis of his results, Driesch proposed the very modern concept of nucleus–cytoplasm interaction as an essential event for development (Driesch 1894). Later, from 1928 to 1935, Hörstadius performed some of the most remarkable experi-

ments in the history of embryology using the sea urchin embryo (Figs. 3, 4). First, he separated each blastomere of the early embryo and followed their fates; then he was able to recombine different series of blastomeres and, from the results obtained, to propose the well-known theory on the existence of graded properties within the unfertilised egg and the early embryo (Hörstadius 1939).

The sea urchin system was also one of the first in which time-lapse microscopy was exploited extensively. For example, the classic studies of Gustafson and Wolpert (1967) led to the identification of many of the basic behaviours exhibited by cells in the embryo during morphogenetic movements. At the time, these authors remarked, "we are, however, still ignorant about the final steps in the casual chain between the genes and the shapes they control" (Gustafson and Wolpert 1967). In the past 35 years, our knowledge of the molecular basis of developmental processes and the relationship between molecules and cell behaviour has advanced considerably. The classic studies


THE MECHANICS OF SEA URCHIN DEVELOPMENT, STUDIED BY OPERATIVE METHODS

BY

SVEN HÖRSTADIUS

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Fig. 3. Cover of the original review by Hörstadius (1939) on development of the *Paracentrotus lividus* sea urchin embryo (Kindly provided by M. Delarue, Laboratoire Biologie et Multimédia, UPMC-P6, www.snv.jussieu.fr/bmedia/sommaires/dvpt.htm)

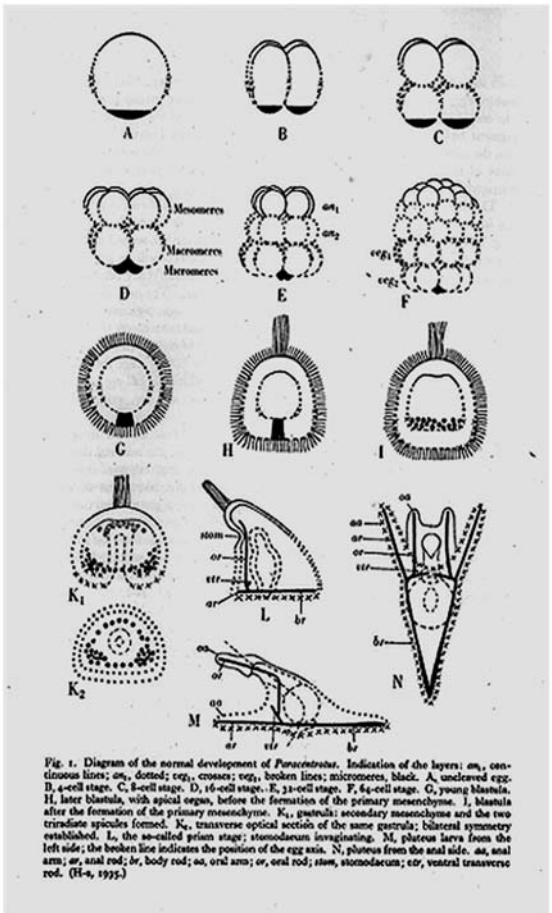

Fig. 1. Diagram of the normal development of *Paracentrotus*. Indication of the layers: en_1, continuous lines; en_2, dotted; veg_1, crosses; veg_2, broken lines; micromeres, black. A, uncleaved egg. B, 4-cell stage. C, 8-cell stage. D, 16-cell stage. E, 32-cell stage. F, 64-cell stage. G, young blastula. H, later blastula, with apical organ, before the formation of the primary mesenchyme. I, blastula after the formation of the primary mesenchyme. K_1, gastrula: secondary mesenchyme and the two triradiate spicules formed. K_2, transverse optical section of the same gastrula; bilateral symmetry established. L, the so-called prism stage; stomodaeum invaginating. M, pluteus larva from the left side; the broken line indicates the position of the egg axis. N, pluteus from the anal side. *aa*, anal arm; *ar*, anal rod; *br*, body rod; *oa*, oral arm; *or*, oral rod; *stom*, stomodaeum; *ttr*, ventral transverse rod. (H-a, 1935.)

Fig. 4. Diagram of the normal development of the *P. lividus* sea urchin embryo published in 1939 by Hörstadius (Kindly provided by M. Delarue)

of Hörstadius have been extended by the lineage studies performed by Davidson and colleagues, providing a more detailed picture of the establishment of tissue territories in the early embryo (see reviews by Cameron and Davidson 1991; Cameron et al. 1991). More recently, many other laboratories in the world, thanks to new technologies developed in the field of molecular biology, have succeeded in the identification and characterisation of control genes and their key target sites and the determination of their functional significance in the early embryo, allowing the proposal of complex gene regulatory networks (see review by Davidson et al. 2002). An equivalent remarkable progress has not been seen in the cell biology field, particularly in the characterisation of the proteins involved in cell communication and cell adhesion, although some studies date back to the early 1970s.

In the following text, molecules of the sea urchin embryo involved in cell communication, such as cell–matrix and cell–cell adhesion proteins (*Pl-*

nectin, fibronectin, laminin, collagen, integrin, toposome, cadherin), growth factors (BMP2/4, univin), signal transduction molecules (kinases), and their genes (when characterised) will be described. Our intention is not to review all the work done over the years in the field of cellular interaction in echinoderms, but rather we will focus on a few arguments on the assumption of re-examining some ideas and concepts.

3.1 Sea Urchin Embryo Transparency: A Living Laboratory for Studying Development and Morphogenesis

To facilitate the reader in the following discussion on the relationship between adhesion/signalling molecules and cell differentiation occurring during embryogenesis, the development of the Mediterranean sea urchin species *Paracentrotus lividus* will be briefly described. The time scale and embryo morphology apply, with very little modifications, to many other sea urchin species.

Fertilisation is the first event that gives rise to development and triggers instantaneously several changes within the egg. A transient decrease in the membrane potential, a slight increase in the intracellular pH and free calcium ions activate several metabolic processes, causing, among other things, the exocytosis of cortical granules and the consequent elevation of the fertilisation membrane (Fig. 5A). Cleavage stages begin within 1 h after fertilisation and are characterised by a number of cell divisions occurring about every 30 min (Fig. 5B,C). While the first three divisions give rise to eight equivalent blastomeres, the fourth one produces 16 cells of different sizes: eight mesomeres at the animal pole, four macromeres and four micromeres at the vegetal pole (Fig. 5D). Cameron and colleagues were able to describe extensively the cell lineage for each of the 16 blastomeres; in addition, they showed that most of the lineage founder cells for many tissues of the later embryo are established at the 64-cell stage (Cameron and Davidson 1991; Davidson et al. 1998). It is noteworthy to underline that the lineage boundaries are detected well before the appearance of any morphological difference in the embryo and the spatially restricted patterns of gene expression already described agree with these lineage boundaries. As cell divisions proceed, the embryo develops into a blastula, which consists of one layer of epithelial cells, with a typical apical–basal polarity, surrounding a filled cavity, the blastocoel (Fig. 5E). Later, the embryo hatches from the fertilisation envelope to become a free-swimming blastula. From this stage another phase of development starts, which is characterised by a number of morphogenetic movements that will completely rearrange the embryo. The first cells to move are the primary mesenchyme cells (PMCs) that detach from the vegetal plate and ingress into the blastocoel, where they migrate using short filopodia (mesenchyme blastula stage; Fig. 5F). Some PMCs migrate towards two ventro-lateral sites and

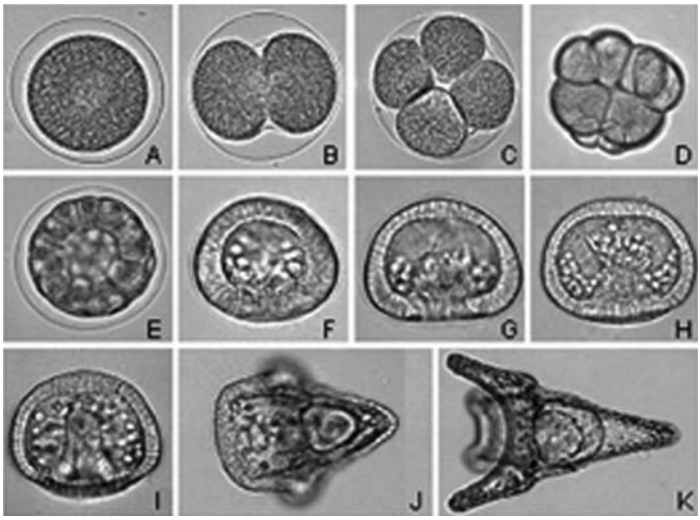

Fig. 5A–K. Development of the sea urchin embryo *Paracentrotus lividus*. **A** Fertilised egg; **B** two blastomeres; **C** four blastomeres; **D** 16 blastomeres; **E** early blastula; **F** mesenchyme blastula; **G** early gastrula; **H** middle gastrula; **I** late gastrula; **J** early pluteus; **K** pluteus

form clusters that are connected by other PMCs in a form of a subequatorial ring. The morphology of PMCs during migration was described for the first time by Gustafson and Wolpert using time-lapse microscopy (1961, 1967). Shortly after PMC ingression, the vegetal plate epithelium invaginates to form the archenteron, which is the future intestine. It is possible to distinguish at least three gastrula stages during the elongation of the archenteron towards the animal pole: early, middle and late gastrula (Fig. 5G–I). PMCs produce the skeleton, first in the form of triradiate spicules, which elongate and pattern in a complex species-specific manner. Skeleton is formed by calcium and magnesium carbonate, which deposit on an organic matrix constituted by a number of well-known spicule-specific proteins. In the laboratory, the larval stage of pluteus is obtained after 48 h post-fertilisation: it shows four arms, a complex patterned skeleton and a tripartite intestine, encircled by muscles undergoing peristaltic contractions (Fig. 5J,K). At this point, the larva, if correctly fed, is ready to continue development and form the juvenile sea urchin through metamorphosis.

From the developmental processes outlined above it is evident that cell–cell and cell–ECM contacts play a fundamental role in morphogenetic movements occurring during embryogenesis. Knowledge of the key actors involved as well as understanding of their complex interactions would eventually lead to the unravelling of the developmental machinery. Furthermore, it should be noted that cells forming the blastula stage monolayer exhibit a structural asymmetry of the cytoplasm and the plasma membrane is compartmen-

talised into distinct apical, basal and lateral domains, with characteristic lipid and protein compositions. Embryogenesis and morphogenesis are characterised by cell movements and complex cell rearrangements, which require appropriate interactions of cells with the underlying ECM by means of specific membrane receptors. For this reason, interest in the identification, purification and functional studies of ECM components, along with their ligands and other molecules involved in cell–matrix adhesion, has increased in recent years. In the following text, after a brief description of the appearance of the ECM upon embryo development, protein molecules found in different plasma membrane compartments of blastula cells will be described.

3.1.1
ECM Patterning in Echinoids During Embryo Development

In recent years, it has been shown that the ECM of the sea urchin embryo is a very complex structure, consisting of a number of layers organising during different developmental steps. Using specific antibodies, different storage compartments of the ECM components, like granules and vesicles, have been identified in the unfertilised egg cytoplasm. The protein contents of these compartments are exocytosed and assembled in a highly regulated fashion at different moments after fertilisation (for a review see McClay et al. 1990). The early event after sperm entry is the elevation of the fertilisation envelope above a water-filled perivitelline space (Fig. 5A; for more detailed description on ionic events occurring at this stage, see the chapter by Angelini et al., this Vol.). Then, a new ECM is secreted on the outer surface of the zygote, which gives rise to different layers, the most acknowledged of them being the apical lamina and the hyaline layer. During cleavage, other ECM molecules are also released into the newly forming basal lamina, which will underlie the blastocoelic cavity from the stage of blastula (Fig. 5E). A great number of in vitro and in vivo studies suggest that ECM has an important role during morphogenesis, serving as a mechanical support to the embryo as well as a substrate for cell movements, and providing both spatial and temporal information to adherent cells. Thus the ECM is not a fixed structure but rather is able to respond to and effect changes in its local microenvironment.

Changes in ECM composition and regulated matrix remodelling are common events associated with embryogenesis and are carried on by matrix metalloproteases (MMPs). These molecules are a growing family of metalloendopeptidases that act by cleaving the protein components of the ECM and thereby regulating its composition (for a review see Stamenkovic 2003). For many years MMPs were believed to function in facilitating cell migration simply by removing barriers such as collagen. However, recent discoveries have shed new light on the role of MMPs in embryology, physiology and disease. It is becoming increasingly clear, in fact, that MMPs are also involved in the functional regulation of non-ECM molecules, including growth factors and

their receptors, cytokines and chemokines, adhesion receptors and cell-surface proteoglycans, and a variety of enzymes. MMPs therefore play an important role in the control of cellular interactions with their environment, promoting tissue turnover, both physiological, such as normal development, and pathological, such as inflammation and cancer. The sea urchin embryo was found to express a dynamic pattern of gelatinase activities associated with the external ECM, some expressed only in later stages of development, others expressed in the unfertilised egg and persisting throughout the course of embryonic development (Mayne and Robinson 1996; Robinson 1997; Flood et al. 2000). Their substrate specificity and metal ion requirements suggest that these molecules are members of the MMP class of ECM remodelling enzymes.

In recent years, a new family of molecules in the animal kingdom has been described, called ADAMs (A Disintegrin And Metalloprotease). These are multidomain transmembrane proteins containing a metalloproteinase and a disintegrin domain and all or some of the following structures: a signal peptide, a propeptide, a cysteine-rich and an epidermal growth factor (EGF)-like domain, a transmembrane region, and a cytoplasmic tail. As a consequence, ADAMs could have four potential functions: proteolysis, adhesion, fusion and intracellular signalling (Stone et al. 1999). Although the number of ADAM genes has grown rapidly, the biological functions of most members are still unclear. However, they seem to be key regulators of the cell–cell and cell–ECM interactions (see review by White 2003). Recently, a sea urchin SpADAM gene has been sequenced and the deduced protein sequence includes all the characteristic domains of vertebrate ADAMs. The structure and the types of cells in which SpADAM orthologues are expressed are then apparently conserved in deuterostomes (Rise and Burke 2002).

4
Apical Cell Surface

It has been reported that the first ECM structure developing soon after fertilisation of the egg is the external one. As viewed by electron microscopy after ruthenium staining (Lundgren 1973), the presence of at least two distinct layers is evident: an inner apical lamina more tightly associated with the apical plasma membrane, which is the thickest, and an outer layer, the hyaline layer, associated with the tips of microvilli (see Fig. 6).

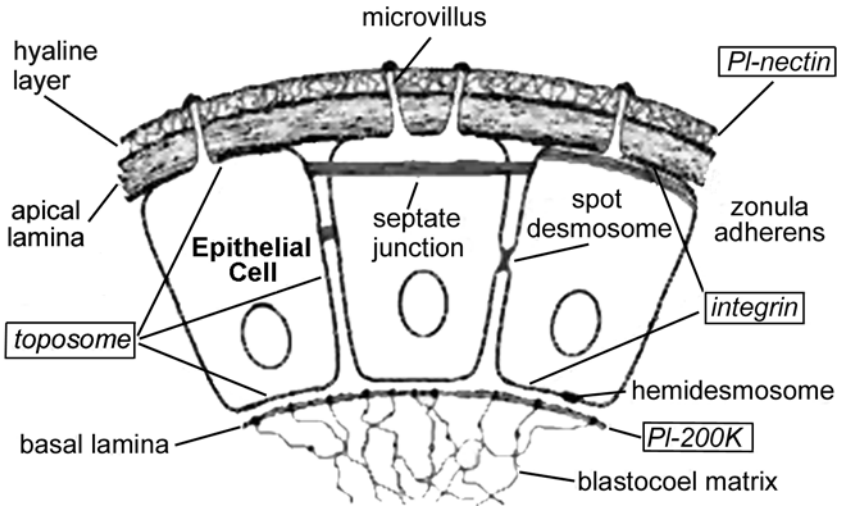

Fig. 6. Schematic diagram of the ultrastructure of the epithelium of the blastula embryo. (Adapted from Hardin 1996)

4.1 The Apical Lamina

The apical lamina is a fibrous layer surrounding the embryo, which remains on the apical surface after removal of hyalin from the hyaline layer (Hall and Vacquier 1982; Burke et al. 1998), and is composed principally of fibropellins (Bisgrove et al. 1991; Burke et al. 1991). The gene coding for one of the three known fibropellins has been sequenced and, like other extracellular molecules, it contains a series of up to 20 epidermal growth-factor-like repeats (Delgadillo-Reynoso et al. 1989; Grimwade et al. 1991; Bisgrove and Raff 1993). In vitro experiments showed that dissociated cells adhere to affinity-purified fibropellins in a temperature-, time- and dose-dependent manner, suggesting the role as a substrate for cell adhesion (Burke et al. 1998). Their in vivo functional role has been investigated using monoclonal antibodies, which were shown to interfere with the initial phase of gastrulation (Nakajima and Burke 1996). At this time no further proteins have been described as belonging to this layer.

4.2 The Hyaline Layer

Historically, the first function assigned to the hyaline layer has been to hold blastomeres together (Osanai 1960; Vacquier and Mazia 1968). In agreement with this hypothesis, Citkowicz (1971) showed that the hyaline layer forms a

shell that remains structurally intact after removal of epithelial cells using hyperosmotic solutions, demonstrating that it is a structure independent from cells that lie underneath. A number of proteins constituting the hyaline layer have been identified and characterised. The major component is hyalin, a high molecular weight glycoprotein secreted by the cortical granules, which can be isolated by successive steps of solubilisation and precipitation through removal and re-addition of Ca^{2+} to seawater or other osmotically balanced media (Kane 1970). Hyalin has been extensively studied both structurally and functionally in sea urchin and sea star embryos (Vacquier 1969; Kane and Stephens 1969; Spiegel and Spiegel 1979; Adelson et al. 1992) and partial cDNAs have been isolated by screening expression libraries with monoclonal antibodies to hyalin (Wessel et al. 1998). Its sequence contains consensus calcium-binding motifs, in agreement with its capacity to interact with calcium biochemically (Robinson et al. 1992), and modular repeats similar to that known in other large ECM molecules (McClay 1991). Hyalin has been shown to support cell adhesion in vitro (McClay and Fink 1982) and to be involved in sea urchin morphogenesis in vivo (Adelson and Humphreys 1988). Interestingly, a recent study has demonstrated that two mammalian cortical granule envelope proteins share common antigenic epitope(s) with echinoderm hyalin and, like hyalin, play a role in early embryogenesis (Hoodbhoy et al. 2000).

4.2.1
Pl-Nectin: An Example of Signal(s) from Outside to Inside the Embryo

About 10 years ago, with the aim of purifying fibronectin from the sea urchin embryo, we isolated and characterised another ECM protein localised in the hyaline layer, namely *Pl*-nectin (Fig. 6). Although the protocol used for its purification was that originally developed for fibronectin, the molecular weight and other features indicated that this protein was a new molecular species. *Pl*-nectin has been isolated from the species *Paracentrotus lividus* (Matranga et al. 1992) and *Temnopleurus hardwickii* (*Th*-nectin; Yokota et al. 1994) as a collagen-binding molecule and, like other ECM components, it has been found stored in specific cytoplasmic vesicles, recently named nectosomes, in the unfertilised egg (Kato et al. 2004). The genesis and distribution of the nectosome during oogenesis, its translocation to the cortex and gradual secretion into the hyaline layer after fertilisation have recently been fully documented by using immunoelectron microscopy in the Japanese species *T. hardwickii* (Kato et al. 2004). At later stages, *Pl*-nectin has been found to be localised on the apical surface of ectoderm and endoderm cells. By in vitro assays, the protein has been shown to mediate cell-substrate adhesion in a dose-dependent fashion, suggesting a functional role during development (Matranga et al. 1992). Thus it was crucial to investigate the in vivo *Pl*-nectin function by morphogenetic assays in which embryos were cultured in the

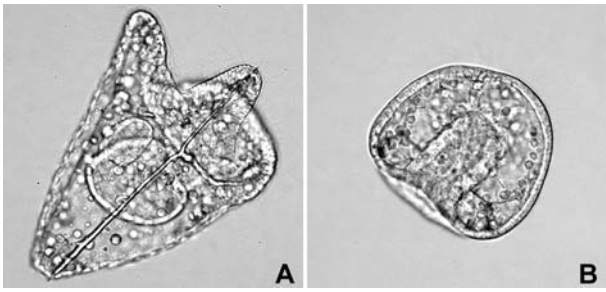

Fig. 7A, B. Skeleton defects obtained after culturing embryos in the presence of high concentrations of monoclonal antibody to *Pl*-nectin. **A** Control embryo cultured in the presence of unrelated IgGs; **B** treated embryo showing strong skeleton defects

presence of monoclonal antibodies to *Pl*-nectin. To our surprise, after this treatment, we observed the presence of a high number of embryos with dramatic skeleton defects, but with normally developed ectoderm and endoderm structures (Fig. 7; Zito et al. 1998, 2000). In addition, depending on the amount of antibody used, it was possible to obtain embryos with different degrees of skeleton defects, which were classified on an arbitrary scale (Zito et al. 2003). These data suggested that outer ectoderm–*Pl*-nectin interaction is indirectly involved in inner skeleton formation. For more detailed description on this issue, see Section 10. The partial sequence of the coding region of the *Pl*-nectin gene (accession no. AJ578435) has revealed its similarity with another component of the hyaline layer, namely echinonectin, purified from the species *Lytechinus variegatus*. This protein has a lectin-binding activity that allowed its chromatographic purification and, like *Pl*-nectin, it is an adhesive substratum for cells (Alliegro et al. 1988; 1990). Although in a previous report no cross-reaction between the two molecules was found, it is very likely that *Pl*-nectin and echinonectin belong to the same family of ECM components.

4.2.2
Other Apical ECM Components

Concerning the presence of fibronectin-like molecules in the sea urchin embryo, first evidence was obtained by immunofluorescence experiments using antibodies against human fibronectin, even if the cross-reactive material was described as being differently distributed in the embryo, i.e. on the outer cell surface of the epithelial layer (Spiegel et al. 1983), on the basement membrane (Spiegel et al. 1980; DeSimone et al. 1985), and also on the surface of migrating PMCs (Katow et al. 1982). A sea urchin fibronectin-like molecule has been purified for the first time from ovaries of *Pseudocentrotus depressus* by Iwata and Nakano (1981, 1983). The same authors have also demonstrated that this protein is involved in migration, adhesion and spicule synthesis of in vitro-cultured micromeres (Miyachi et al. 1984). Later, fibronectin-like proteins were purified from five different species living in Mediterranean and

Pacific seawaters and their biochemical and immunological relationships have been analysed for the purpose of comparative phylogenetic studies (Matranga et al. 1995). These proteins differed in their binding affinity to gelatin but shared different epitopes, suggesting that they are members of a sea urchin fibronectin superfamily. Furthermore, analysis of the different features of fibronectin-like proteins and sea urchin nectins, i.e. *Pl*-nectin and *Th*-nectin, has been carried out, since essentially the same method was utilised to purify both groups of molecules (Yokota et al. 1994). It has been shown that these two groups of proteins belong to different ECM protein families since they differ in their affinity to collagen as well as in their localisation inside the embryo.

A gene coding for a new ECM protein has been sequenced in the direct developer *Heliocidaris erythrogramma*. By the use of polyclonal antibodies raised against the protein, it has been demonstrated that it is localised on the apical surface of ectoderm, in tight association with the plasma membrane. The protein has been named apextrin and it has been proposed to be involved in apical cell adhesion (Haag et al. 1999).

Although a reasonable amount of information is currently available on the hyaline layer and apical lamina components, the spatial relationship between them is still unclear. An approach to such studies could come from experiments of co-immunoprecipitation after transient cross-linking of surface molecules in live embryos. Alternatively, electron microscopic observations of rotary shadowed purified molecules in diluted solutions, or in combination with other potential partners, would serve to solve the intricate meshwork that surrounds the embryo.

5
Basal Cell Surface

As previously reported, the basal lamina develops when new ECM molecules are released, from the blastula stage, at the basal surfaces of epithelial cells, into the constituting blastocoelic cavity (Fig. 6). A discrete number of reports have described the formation of this layer and its function in sea urchin development (Okazaki and Nijima 1964; Kawabe et al. 1981; Galileo and Morrill 1985; Amemiya 1989).

Different experimental approaches have shown the presence of few types of collagens in the sea urchin embryo, in contrast to about 20 different types of the protein found in higher metazoan phyla. By immunofluorescence, Wessel et al. (1984) showed collagen localisation on the basal lamina of the embryo, using antibodies against known vertebrate ECM components, i.e. collagen types I, III and IV. Partial biochemical characterisation of collagen-like molecules has been carried out from in vitro-cultured micromeres (Pucci-Minafra et al. 1975; Benson et al. 1990; Shimizu et al. 1990). Collagen genes have been characterised in several species of sea urchin. Using mouse type IV cDNAs,

genomic clones have been isolated from *S. purpuratus* and characterised (Venkatesan et al. 1986). A putative collagen gene has also been cloned from a *P. lividus* genomic library using a *C. elegans* collagen gene as a probe (D'Alessio et al. 1989, 1990; Exposito et al. 1992). It has also been demonstrated that fibrillar collagen types I and II and non-fibrillar collagen type IV are expressed only by PMCs and secondary mesenchyme cells (SMC) (Angerer et al. 1988; D'Alessio et al. 1989; Wessel et al. 1991; Lethias et al. 1995).

The first evidence of the presence of a vertebrate laminin-like molecule as a component of the embryonic sea urchin basal lamina came from studies that utilised an monoclonal antibody specific to laminin (McCarthy and Burger 1987). Later, the isolation and characterisation of a cDNA clone encoding a region of the carboxy terminal globular domain (G domain) of the alpha-1 chain of laminin from the sea urchin *S. purpuratus* was reported (Benson et al. 1999).

Among echinoderm-specific ECM components, pamlin has been isolated from *H. pulcherrimus* embryos (Katow 1995). The author showed that PMCs isolated from mesenchyme blastulae bound exclusively to pamlin, which stimulates their migration in in vitro functional assays. Analysis of the molecular image of pamlin by transmission electron microscopy showed that the protein is composed of three subunits, which can aggregate in a complex fashion, giving rise to a large supramolecular network (Omoto and Katow 1998).

Other ECM molecules have been identified as part of the basal lamina surrounding the blastocoel (Fig. 6). A novel protein has been purified from the sea urchin species *P. lividus* and *H. pulcherrimus*, and its biochemical characterisation and functional features have been described (Tesoro et al. 1998). The protein has been named *Pl*-200K or *Hp*-200K, respectively, because of the species from which it was isolated, and, as assessed by affinity chromatography columns, it shows different binding affinities to type I collagen and heparin. *Pl*-200 K has been shown to function as an adhesive substrate, and to be involved in the regulation of sea urchin embryo skeletogenesis (Tesoro et al. 1998).

By means of specific monoclonal antibodies, a molecule with multiple calcium-binding domains, ECM3, has been shown to be arranged in fibres on the basal surface of the ectoderm. These studies established ECM3 as a strong candidate for a PMC substrate molecule and point to several possible mechanisms by which interactions between PMC filopodia and ECM3-containing fibers could provide guidance information to migrating PMCs (Hodor et al. 2000).

As in the case of external ECM, it would be interesting to know the relationship among all the ECM proteins described so far and their spatial organisation.

6
The Blastocoel Matrix

It is useful to remember that the blastocoel is not an empty cavity, but it becomes filled with a matrix whose composition is still not well known (Fig. 6). Several methods have been used to visualise and study the structure of the blastocoel matrix that forms at the blastula stage. Earlier electron microscope studies described this matrix as a "meshwork of fibrous and granular material" which is an extension of the basal lamina (Katow and Solursh 1979). More recent studies have revealed an oriented meshwork, formed by fibrillar sheets interconnected by finer filaments, that completely fill the blastocoelic cavity, in contact with the basal lamina (Cherr et al. 1992). Although detailed information about the structure of the blastocoel matrix is available, in this case little is known about the protein molecules constituting it (for review see Solursh 1986).

7
Receptors to ECM Molecules

ECM molecules are clearly important, but equally significant are the receptors by which cells interact with it, the major class being the integrin superfamily (for a review see Berman et al. 2003; Danen and Sonnenberg 2003). More than 20 of these receptors have been identified so far in different vertebrates: they are heterodimers of two transmembrane chains, α and β, and are widely expressed among the animal kingdom (Bokel and Brown 2002). The different combination of α and β subunits generates integrins with differing ligand specificity (Hynes 1992). Due to their structure, integrins can interact with ECM by their extracellular domains and transduce external signals to the inside by their cytoplasmic tails, which interact with the cytoskeleton, signalling molecules and other cellular proteins, resulting in regulation of many biological functions. These include adhesion, motility, shape, polarity, growth and differentiation (Yamada 1997). Although a reasonable number of ECM proteins have been described in the sea urchin embryo, little is known about their putative receptors. In recent years, however, it has been shown that this system expresses integrins structurally similar to those characterised in other animals (Burke 1999). Marsden and Burke (1997) reported evidence of three novel β integrin subunits that are expressed during early development of *S. purpuratus*. The full-length cDNA sequence for one of them, βG, shows 58 % similarity to vertebrate integrins, particularly to the cytoplasmic domain in which amino acids of the human β1 subunit involved in cell adhesion and signalling are conserved (Marsden and Burke 1997). Functional assays using antibodies and Fab fragments against another sea urchin β integrin, βL, demonstrated its involvement in the initial phase of gastrulation and in the organisation of actin filaments (Marsden and Burke 1998). Our preliminary

results, in collaboration with R.D. Burke, indicate that a β integrin serves as a receptor for *Pl*-nectin. In fact, by immunodetection using confocal microscopy, we found that both molecules are localised on the apical cell surface of gastrula embryos. In addition, we were able to show, by immunoprecipitation and affinity chromatography, a calcium-dependent binding between the two molecules, in agreement with what is known for vertebrate integrins (unpubl. results).

An α integrin subunit has been recently cloned from *S. purpuratus* and it was found to exhibit 74–77 % sequence similarity to mammalian α_5, α_8, α_{IIb} and α_v integrins, but its function or its putative ligand(s) have not been investigated (Susan et al. 2000). In contrast, an α integrin similar to the α_5 subgroup of vertebrate integrins, namely alphaSU2, has been described from *L. variegatus*, whose ligand has been identified (Hertzler and McClay 1999). In fact, this integrin appears to be involved in the binding of epithelial cells to laminin, as it has been demonstrated by its localisation on the basal cell surface of epithelia at the mid-blastula stage and by transfection experiments using an α-integrin-deficient CHO cell line. Transfected alphaSU2-expressing CHO cells have been shown to bind to isolated sea urchin basal lamina and to purified laminin, while they bind weakly or not at all to fibronectin, type I collagen and type IV collagen (Hertzler and McClay 1999).

A hypothetical integrin β_5 subunit has been recognised in an RGDS peptide-binding receptor, FR-1R. The molecule localises to the basal side of the ectoderm and to PMCs in sand dollar embryos and it is involved in PMCs migration and gastrulation (Katow and Sofuku 2001).

8
Lateral Cell Surface

From as early as 1900, it was documented that sea urchin embryos can be dissociated into single cells after a simple treatment with Ca^{2+}-free seawater (Fig. 8A; Herbst 1900). Later, based on Herbst's observation, Giudice (1962) observed that dissociated cells were able to spontaneously reaggregate and differentiate into structures closely resembling normal larvae if Ca^{2+} and Mg^{2+} were restored in the seawater (Fig. 8B). The possibility of obtaining normal bipinnaria larvae from dissociated cells has been also demonstrated in the starfish *Asterina pectinifera* (Dan-Sohkawa et al. 1986).

8.1
Cell–Cell Adhesion and Communication: The Discovery of Toposome

The ease of dissociating and reaggregating cells of the sea urchin embryo offered an exceptional model for studies on molecules involved in cell–cell adhesion and for the development of methods for their isolation. In the late

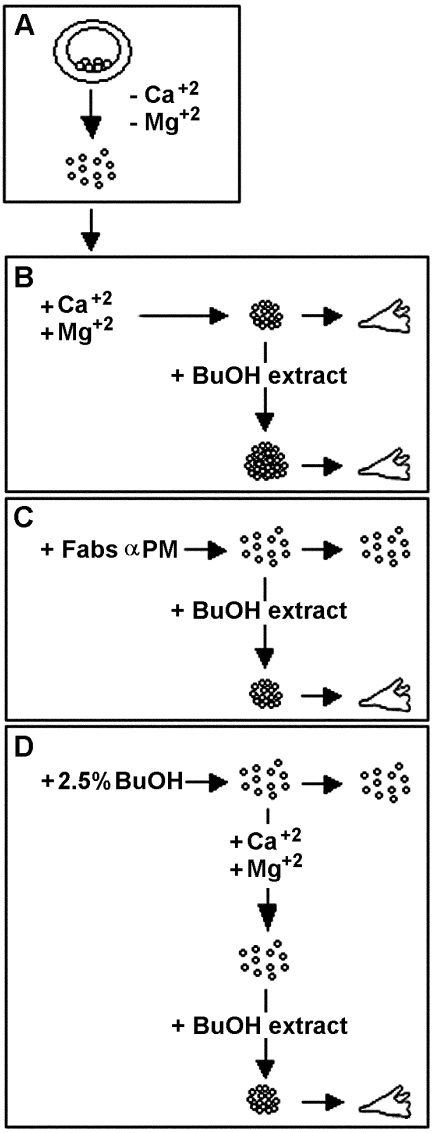

Fig. 8A–D. Schematic drawing illustrating dissociation and reaggregation experiments leading to identification of the active component mediating cell adhesion in the sea urchin embryo. *BuOH* n-Butanol; *PM* plasma membrane

1970s, it was first shown that Fab fragments (Fabs) from antibodies to plasma membranes purified from blastula embryos were able to prevent reaggregation of dissociated cells (Fig. 8C). The inhibition was reversed if soluble proteins extracted with n-butanol from purified membranes were added (Fig. 8C). In addition, these n-butanol extracts were able to strongly stimulate the rate of reaggregation of dissociated cells, suggesting the presence of some aggregating-factor(s) (Fig. 8B; Noll et al. 1979). In agreement with this

hypothesis, it was observed that the exposure of dissociated cells to n-butanol completely removed the protein(s) responsible for reaggregation, since cells were not able to reaggregate even in the presence of Ca^{2+} and Mg^{2+} (Fig. 8D). However, the treatment with n-butanol did not affect the viability of the cells, since both reaggregation and embryonic development were completely restored by readdition of extracted proteins to the butanol-treated cells (Fig. 8D; Noll et al. 1979). As a consequence, an easy and low-cost procedure, i.e. the non-cytolytic treatment of live dissociated cells with diluted n-butanol, allowed the preparation of large quantities of crude extracts, from which the isolation, purification and characterisation of the active component could be attempted. In fact, the biochemical identity and biological activity of a large and oligomeric glycoprotein complex, called toposome, was later achieved (Noll et al. 1985; Matranga et al. 1986). Toposome is a 22S complex consisting of six 160-kDa subunits that are processed proteolytically as development proceeds. Cuts are revealed only after analysis by SDS-PAGE, since nicks introduced by specific enzymes, probably a cathepsin B-like protease (Yokota and Kato 1988), do not cause fragmentation of the native protein, thus securing the embryonic integrity. The need for toposome processing has been explained by postulating that a limited number of subunits could be generated by this strategy. Their differential association could then give rise to various molecular populations, each specifying a positional code guiding the cell in the embryo; from this comes the name toposome (Noll et al. 1985). Supporting this interesting hypothesis, toposome-specific monoclonal antibodies have been shown to stain cell surface structures in a pattern consistent with a positional code. The biological activity of the whole toposome complex, or parts of it, in mediating cell adhesion of dissociated cells has been tested and it was found that the oligomeric integrity of toposome is essential for its function (Matranga et al. 1986; Scaturro et al. 1998). The ultrastructural localisation of toposome has been investigated by electron microscopy of immunogold-labeled eggs and hatched blastulae (Gratwohl et al. 1991). Toposomes were seen on the surface of the egg, as well as stored in yolk granules and in the electron-dense lamellar compartment of the cortical granules. In the hatched blastula, toposomes modified by limited proteolysis in the yolk granules have been found associated with the plasma membranes, while unmodified 160-kDa toposomes, originating from the cortical granules, have been found on the outside of the hyaline layer. The latter distribution suggests that these toposomes function by attaching the apical lamina to the surface of the microvilli and thereby to the cytoskeleton of the growing embryo. Therefore, the authors proposed a different function for the two differently localised populations of toposomes (Gratwohl et al. 1991).

An important aspect of dissociation and reaggregation experiments is the effect on the transduction of signals from the exterior of the cell to the nucleus, ultimately involving DNA synthesis. An intriguing paradox is that in the sea urchin embryos, a “no-contact inhibition” was found. In fact, dissociated cells stop DNA synthesis until cell contacts are re-established, i.e. in the

formation of the reaggregates (Sconzo et al. 1970; De Petrocellis and Vittorelli 1975). Later, we found that toposomes were responsible for the required signal transduction since reaggregation-inhibiting Fab restored DNA synthesis in dissociated cells (Vittorelli et al. 1980). Apparently, the binding of Fabs to the contact sites mimics cell–cell adhesion and thus stimulates DNA synthesis, in the same way that binding of Fabs to the receptors of epidermal growth factor or insulin mimics the action of those hormones (Kahn et al. 1978; Schreiber et al. 1981).

Further analysis on toposome molecules led to the characterisation of its precursor from sea urchin coelomic fluids of both male and female adults (Cervello and Matranga 1989). The authors produced, for the first time, evidence that the so-called vitellogenin (Vg), found in the coelomic fluid of both male and female sea urchin adults, and its intermediate form, the so-called major yolk protein (MYP) present in granules of unfertilised eggs, are both unprocessed precursor forms of toposome. Both proteins promote cell reaggregation of dissociated blastula cells, suggesting that processing is not required for the cell-adhesion function, but rather directs their localisation during development (Cervello and Matranga 1989). Historical work on the 22S particle assigned to the protein a nutritional role due to its accidental occurrence in granules of the egg and to the need for the non-feeding embryo to develop soon. Similarly, the so-called vitellogenin, although found in both male and female sea urchins, has been recognised as the bona fide precursor to yolk protein, asking for genetic and functional analogies with the vertebrate homologue. It was then important to find the coding sequence for the protein. Previous efforts to gain decisive evidence of a yolk-related nutritional role by cloning the gene for the 22S glycoprotein particle from *S. purpuratus* failed because they resulted in the isolation of only a short cDNA and genomic DNA fragments (Shyu et al. 1986, 1987).

Recently, full-length cDNAs from four different sea urchin species have been reported. They include the Hawaiian *Tripneustus gratilla* toposome mRNA complete coding sequence (cds), submitted back in 2001 and released in 2003 (accession no. AY026514) and the Mediterranean *P. lividus* cds submitted in 2003 and released in 2004 (accession no. AY274929). Strikingly, it has been discovered that the protein, also ironless, is a member of the transferrin family (H. Noll et al., pers. comm., work in prep.), in agreement with sequence data from reports on *Pseudocentrotus depressus* (Unuma et al. 2001), *S. purpuratus* (Brooks and Wessel 2002) and *H. pulcherrimus* (Yokota et al. 2003) cDNAs. Other ironless members of the transferrin family continue to be discovered, two of which are also membrane-associated (Morabito and Moczydlowski 1994; McNagny et al. 1996). Their functions, however, remain unknown.

8.2 Other Cell–Cell Adhesion Molecules

Among cell–cell adhesion molecules, cadherins have been fully characterised in vertebrate organisms (see review by Koch et al. 2004). These proteins are transmembrane glycoproteins that mediate homophilic calcium-dependent cell–cell adhesion in a number of cellular junctions, and their function has been shown to be critical both in normal development and in the development of the invasive and metastatic phenotype (see reviews by Wheelock and Johnsony 2003a; Hazan et al. 2004). Several pathways are activated by cadherin-mediated cell–cell interactions and numerous studies are in progress to elucidate the complex relationships among them (Wheelock and Johnsony 2003b). Although recent work on sequence analysis has shed new light on the molecular basis of cadherin adhesion, understanding the specificity of these interactions remains a major challenge (Patel et al. 2003). The first immunological evidence for the presence of a cell adhesion protein similar to the mouse E-cadherin in the sea urchin embryo has been shown in *P. lividus* (Ghersi and Vittorelli 1990; Ghersi et al. 1993). Furthermore, the use of polyclonal antibodies raised against a cloned sea urchin cadherin, which recognises at least three major polypeptides, and the cloning of a novel sea-urchin-specific cadherin molecule support the hypothesis for the presence of several cadherins in this system (Miller and McClay 1997).

8.3 Cell Junctions

The presence of cell junctions in the sea urchin embryo has been described since the 1960s. The blastular wall has the structure of a simple epithelium, similar to that of vertebrates. At their apical surfaces, the cells are joined by typical junctional complexes, including zonulae adherens or belt desmosomes, septate junctions and spot desmosomes, while hemidesmosome-like structures appear localised at their basal surface (Fig. 6; Wolpert and Mercer 1963; Spiegel and Howard 1983). Septate junctions have also been observed at the four-blastomere stage (Chang and Afzelius 1973), although they constitute a continuous layer only later in development (Gilula 1973). At least three types of desmosome, two types of septate junction and a tricellular junction have been described (Spiegel and Howard 1983). Studies on the osmotic and structural properties of the blastular wall date back to 1940, when a decrease in the permeability of the blastula wall to small molecules such as sucrose (Moore 1940) was observed, coinciding with the formation of junctional structures and with an apparent increase in the adhesion between cells.

9
Signalling Pathways

A key feature of both cell–ECM and cell–cell adhesion is the linkage of receptors or other transmembrane molecules to the cytoskeleton, which activates specific intracellular pathways. In particular, cadherins and integrins have been described to modulate and to be modulated by multiple signalling transduction pathways (Thiery 2003). These are complex networks involving a number of steps, including the production of second messengers, the activation of protein kinases and their subcellular distribution that brings them into contact with the appropriate targets. Although each adhesion protein activates a particular pathway, i.e. the tyrosine kinases and mitogen-activated protein (MAP) kinase cascade components activated by integrins and the Wnt pathway influenced by cadherins, it is becoming increasingly clear that cell adhesion proteins may share much of the same basic components of signal transduction machinery. Cell adhesion proteins, in addition to their well-established role in providing positional information, have been demonstrated to regulate also the cellular response to other extracellular stimuli, such as soluble growth factors. However, much remains to be learned concerning the mechanisms that co-ordinate positional and biochemical signals (for a review see Aplin et al. 1998). Some of the components of such signalling pathways have been described in echinoderm embryos and some pathways are going to be elucidated, but this issue will not be further extended in this chapter.

10
Growth Factors

Growth factors, like the ECM, have been shown to fulfil vital developmental roles in many embryonic systems. Among them, transforming growth factor-beta (TGF-β) superfamily members appear to have diversified greatly with the evolution of vertebrates, but, although they are believed to be widely distributed among the animal kingdom, only a few invertebrate deuterostome TGF-β molecules have been identified so far. Sequence comparisons suggest an early origin and an evolutionary conservation of the molecular conformation of the members of TGF-β superfamily (see reviews by Hogan et al. 1994; Kingsley 1994; Chin et al. 2004). Among echinoderms, the first gene encoding a member of the TGF-β superfamily to be reported was identified in the sea urchin embryo, and it was named *univin* (Stenzel et al. 1994). Sequence comparisons placed *univin* in the bone morphogenetic protein (BMP) group of the TGF-β superfamily along with the vertebrate BMPs, decapentaplegic protein from *Drosophila* and Vg-1 from *Xenopus*. Recently, we have shown the involvement of this growth factor in the ectoderm inductive signals needed for the skeleton morphogenesis in the sea urchin embryo (Zito et al. 2003). In this system, skeletogenesis is a fully documented example of ecto-mesoderm

induction, since PMCs, although already determined to only synthesise the skeleton (Okazaki 1975), need signals from the surrounding ectoderm to direct skeleton growth and patterning (Armstrong et al. 1993; Ettensohn and Malinda 1993; Guss and Ettensohn 1997). As previously mentioned, we found that the inhibition of *Pl*-nectin interaction with ectoderm cells produced perturbed embryos, in which only skeleton elongation and patterning were specifically affected. Interestingly, we observed that *univin* expression was strongly inhibited in skeleton-defective embryos, while its misexpression, obtained by *univin* mRNA injection, rescued skeletogenesis. These findings are in agreement with the hypothesis of the involvement of diffusible molecules in the ecto-mesoderm induction suggested by other researchers (Kiyomoto and Tsukahara 1991; Page and Benson 1992; Ettensohn and Malinda 1993; Guss and Ettensohn 1997). Our data, in particular, support a model in which some ectodermal cells secrete processed *univin* or a related growth factor into the blastocoel, where it signals PMCs to synthesise the spicule matrix proteins required for spicule growth (Fig. 9). The ability of ectodermal cells to produce the signal depends on their association with *Pl*-nectin on the apical ECM. Since by our preliminary results the interaction between ectodermal cells and *Pl*-nectin seems mediated by an integrin receptor, it is reasonable to hypothesise the involvement of one of the signalling pathways already known for integrins in other systems. Studies aimed at the identification of such a pathway are in progress in our laboratory. Furthermore, this model predicts

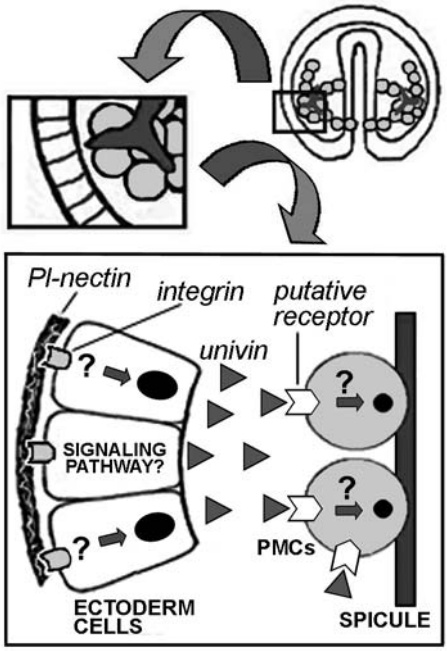

Fig. 9. Model to explain ecto-mesoderm induction in the sea urchin embryo. Ectoderm cells properly interacting with the outer *Pl*-nectin secrete into the blastocoel the growth factor *univin*, which signals PMCs to synthesise the spicule. The interaction of ectoderm cells with *Pl*-nectin, possibly mediated by an integrin receptor, activates a yet unknown signalling pathway. The model predicts also the expression of a putative TGF-β receptor on PMCs and thus a signalling pathway

that PMCs should express a TGF-β receptor. Recently, one similar to vertebrate type I receptor, Alk2, has been found to be expressed in ingressed PMCs of *S. purpuratus* embryos, which can mediate both activin and BMP signals (L. Angerer, unpubl. observ.). However, whether this receptor mediates the skeleton-promoting signal is not yet known.

Two very recent papers have shown the involvement of a MAP kinase signalling pathway in the development of the micromere lineage and mesenchyme differentiation (Fernandez-Serra et al. 2004; Rottinger et al. 2004). Whether a MAP kinase signalling is required for the ecto-mesoderm induction guiding skeletogenesis remains to be demonstrated.

Other BMPs homologues have been recently cloned in the sea urchin embryo, specifically *Sp*BMP5–7 (Ponce et al. 1999), *Tg*BMP2/4 (Hwang et al. 1999) and *Lv*BMP2/4 (Angerer et al. 2000), and *At*BMP2/4 in the starfish (Shih et al. 2002). Their developmental expression patterns have been described by Northern blotting and in situ hybridisation experiments. Functional studies altering *Lv*BMP2/4 mRNA levels showed the involvement of this growth factor in regulating cell fate allocation along the sea urchin animal–vegetal embryonic axis (Angerer et al. 2000). Evidence of the presence of growth-factor-like molecules belonging to other families than TGF-βs has also been reported. In 1987, a cDNA clone whose protein product displayed striking homology to the EGF family of proteins was identified and characterised in the sea urchin embryo (Hursh et al. 1987). The presence of other growth factors in echinoderms has been shown by indirect methods. Platelet-derived growth factor-BB (PDGF-BB) and TGF-α have been reported to be involved in development by using human recombinant proteins, which rescued sea urchin embryo abnormalities induced by ECM disrupters (Ramachandran et al. 1993), or using anti-human PDGF-B and TGF-α antibodies which affected embryo development (Govindarajan et al. 1995). Antibodies against mammalian receptors for PDGF and EGF, or a dominant/negative RNA for PDGF receptor, have been reported to be involved in early differentiation and morphogenesis of the embryo (Ramachandran et al. 1995, 1997). A new member of the fibroblast growth factor receptor (FGFR) family has been cloned in *S. purpuratus* and it has been shown to contain a series of domains characteristic of the family, as three immunoglobulin-like motifs, an acid box, a transmembrane domain, a relatively long juxtamembrane sequence, a split tyrosine kinase domain and two conserved intracellular tyrosine residues (McCoon et al. 1996). These authors detected *Sp*FGFR protein only in muscle cells of the embryo, suggesting that its function may be required to support the proliferation, migration and/or differentiation of myoblasts, rather than being involved in commitment to a muscle fate (McCoon et al. 1998).

11 Concluding Remarks

In the last 30 years, a number of adhesion molecules have been identified in the echinoderm model system, some homologous to already known vertebrate adhesion molecules, while others are specific to the phylum. However, it is noteworthy to remark here that some of the echinoderm-specific cell-adhesion proteins share epitope(s) with mammalian proteins. These include, for example, hyalin, whose homology has not been found in vertebrates, but some of its antigenic epitopes are shared with two mammalian cortical granule envelope proteins (Hoodbhoy et al. 2000). Taken all together, the findings described up to now reveal all the possible scenarios, such as a great conservation of some basic processes as well as the development of new genes and processes during evolution. We can find cell-adhesion proteins developed early in metazoan evolution that have been conserved and have conserved their developmental roles. There are other cases in which only some of the functional epitopes have been conserved and new proteins have been assembled from new arrangements of old domains. Eventually, entirely new proteins evolved, which do not show any close equivalent in invertebrates, and examples can be found in some uniquely vertebrate functions such as vascular biology, neurobiology and neural crest migration (Hynes and Zhao 2000). Nevertheless, one message still remains clear: that is, the phylogenetic closeness of echinoderms to chordates. Support for this relationship, other than by molecular data, is provided by fossil records. A new group of small fossils have just been discovered in China (Shu et al. 2004). These fossils, dating back to the Lower Cambrian (about 520 million years ago), have been interpreted to be the most primitive echinoderms yet known, and have been supposed to be the bridge linking echinoderms and other deuterostomes.

All the topics discussed up to now imply as a consequence that the knowledge of the functional genome of echinoderms might lead to the definition of basic pathways involved in development as well as in diseases. Further, important outcomes for the understanding of echinoderm and chordate evolution will be obtained from the comparative analysis of the expression patterns of regulatory genes in Echinodermata (Wray and Lowe 2000). Currently, a systematic analysis of the echinoderm genome, namely that of the sea urchin *S. purpuratus*, is in progress (http://sugp.caltech.edu/), which is expected to provide new understanding on the organisation of the genome, as well as new insights into the possible application of echinoderm genes in biotechnology and human therapy. An analogous analysis of the genome of the Mediterranean species *P. lividus* has recently started within the NoE Marine Genomics Europe, joining together the European scientific laboratories (www.marine-genomics-europe.org).

It is hoped that studies on the Echinodermata will result not only in important cell biological insights, but also in development of further therapeutic tools.

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