Echinodermata

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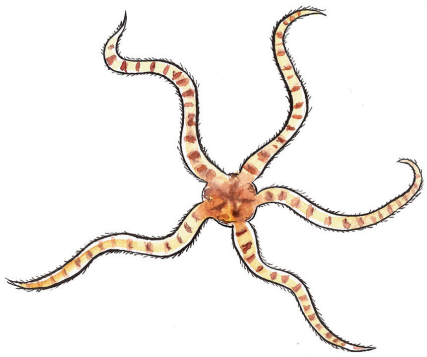

Chapter vignette artwork by Brigitte Baldrian.

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INTRODUCTION

The Echinoderm Body Plan

Echinoderms are a phylum of invertebrate deuterostomes that are morphologically characterized by a fivefold (pentameric) symmetric adult body plan. There are five extant subtaxa, Crinoidea (e.g., sea lilies and feather stars), Asteroidea (e.g., sea stars), Ophiuroidea (e.g., brittle stars), Echinoidea (e.g., sea urchins), and Holothuroidea (e.g., sea cucumbers) (Fig. 1.1). Studies of morphology and molecules (Janies et al. 2011) demonstrate the existence of two higher-order subphylum clades: Pelmatozoa (Crinoidea) and Eleutherozoa (the remaining classes). Echinodermata together with Hemichordata form the clade Ambulacraria (to which some authors add the enigmatic Xenacoelomorpha group). This grouping is the sister to the Chordata.

A series of autapomorphies defines the Echinodermata, including the pentameral body plan and the water vascular system (WVS). The WVS derives from the hydrocoel during develop-

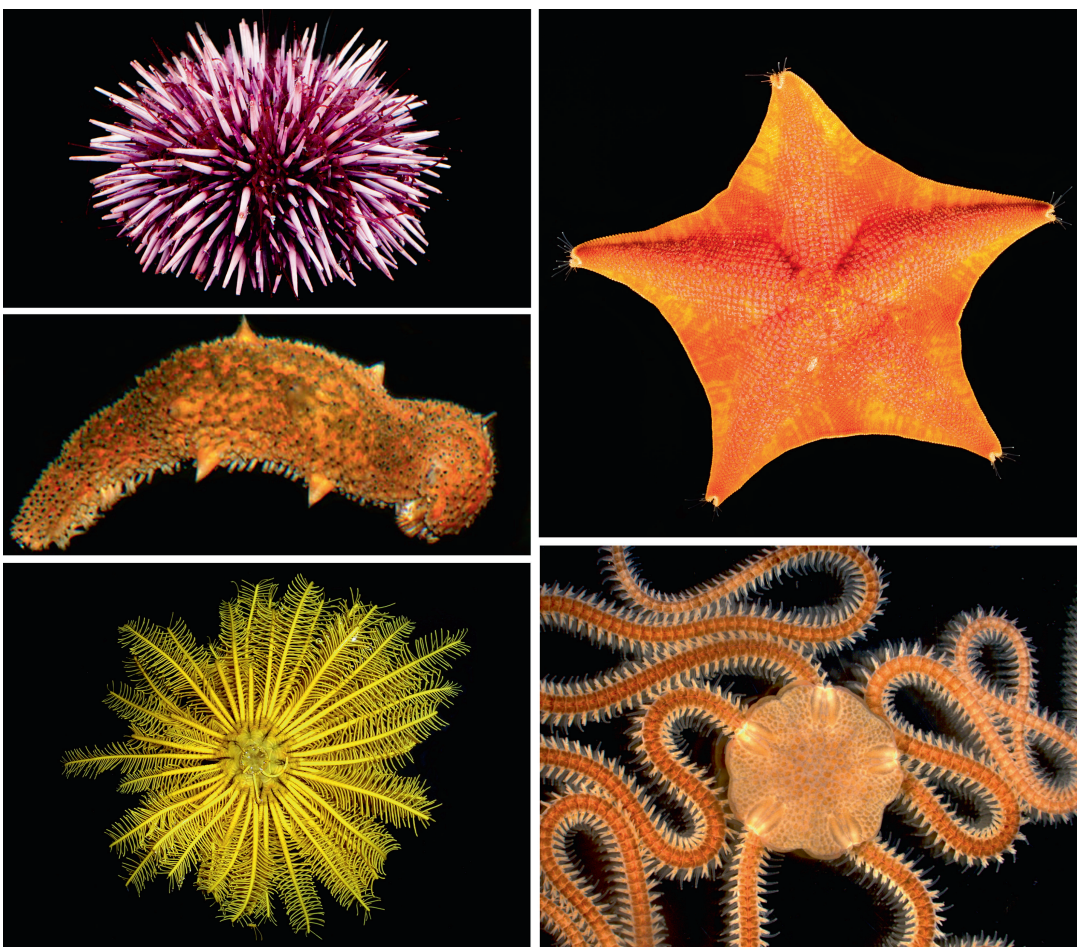


Fig. 1.1 Representatives of the five different classes of extant echinoderms. *Left column*, *top* to *bottom*: the echinoid *Strongylocentrotus purpuratus* (Courtesy of Mattias Ormestad), the holothuroid *Parastichopus parvimensis* (Courtesy of Peter Bryant), and the crinoid *Oxycomanthus intermedius* (Courtesy of Hisanori Kohtsuka). The *upper right image* shows the asteroid *Patiria miniata* (Courtesy of Mattias Ormestad) and the *lower right image* the ophiuroid *Amphiura filiformis* (Courtesy of Anna Czarkwiani and Paola Oliveri)

ment and consists of a system of fluid-filled canals and reservoirs that are used for locomotion and internal transport. Generally, the system consists of an oral water ring and five canals, each with small side branches to the locomotory tube feet and their ampullae.

The echinoderm body is formed from radial (= ambulacral) and interradial (= interambulacral) regions with the side housing the mouth defining the oral surface and the opposite side the aboral surface. The ambulacra of most echinoderms have a radial water canal which gives rise to the tube feet. The water vascular system in echinoids, asteroids, and ophiuroids opens to the exterior through the madreporite: a special skeletal plate on the body surface. In crinoids the WVS system communicates with the external medium through minute pores in the body wall. Holothuroids have an isolated WVS system that does not directly communicate with the external medium. There are a series of small madreporitic plates attached to the oral water ring. Overall, the composition of the echinoderm coelomic fluid is similar to that of seawater and also includes coelomocytes and dissolved proteins. The perivisceral coelom also functions as internal transport system. The hemal system, a diffuse extracellular matrix, is situated between the basal membranes of the epithelia of epidermis, coeloms, and gut.

The echinoderm endoskeleton is made from calcite and the skeletal elements (ossicles, plates, or spicules) have a unique porous, lattice-like organization called the stereom (another echinoderm apomorphy). Each element is a crystalline unit that develops as a stereomic structure with cells (the stroma) filling the open spaces. In echinoderms where the apposition of the plates is tight (e.g., echinoids), the body is rigid, with the plates interconnected by connective tissue ligaments. In other groups, the plates are more loosely associated and embedded in connective tissue. Some connective tissue structures are of a special type, the so-called "mutable" form, which changes their mechanical properties through nervous control (Wilkie 1984; Birenheide et al. 1998; Byrne 2001). The skeleton derives from the mesoderm and is secreted by mesenchymal cells in the embryo.

As in other "radially organized" animals, the nervous system does not coalesce into an anterior centralized structure (e.g., brain). This may allow echinoderms to interact with the environment in all directions (Yoshimura et al. 2012), although bilateral tendencies in locomotion are also reported (Ji et al. 2012). The major nerves are the circumoral nerve ring around the esophagus and the radial nerves along the ambulacra. The nerves are composed of two tissue regions: the outer ectoneural and the inner hyponeural system. A basement membrane runs between these nerve cord regions and neurons connect the two systems along the cord (Cobb 1995; Hoekstra et al. 2012). The functions of these ecto- and hyponeural systems are poorly understood. Sensory receptors are scattered around the body and are restricted to simple epithelial structures innervated by a nerve plexus of the ectoneural system (e.g., Hendler and Byrne 1987). These receptors respond to touch, chemicals, water currents, and light (see, e.g., Ullrich-Luter et al. 2011).

The gut is complete from mouth to anus, except where the anus has been lost secondarily as in all ophiuroids. No excretory systems have been described although the axial organ is usually interpreted as an excretory (although not osmoregulatory) organ. Echinoderms are mostly gonochoristic.

The origin of the germ cells in development has been determined for echinoids, where these cells have been identified to express conserved specific germ line genes – e.g., *nanos* (Wessel et al. 2013). The so-called genital rachis – associated with the hemal system – is thought to be the site where the gonads originate from. The gonads are distinct organs covered by a peritoneum on the outer side and with a germinal epithelium as the innermost tissue layer. Most taxa (exceptions being the Crinoidea and many ophiuroids) have gonoducts that open through gonopores in the genital plates, although these are not always distinctive.

The mechanisms of germ line determination in echinoderms are diverse. While echinoids appear to use an inherited mechanism of germ line formation, the sea stars appear to use an inductive mechanism (which involves the interaction with

neighboring cells; a mechanism most probably used by the majority of echinoderms; see Wessel et al. 2013; Fresques et al. 2014).

Echinoderm Diversity

Echinoderms live in all climatic zones, from shallow coastal waters to the abyssal depths. Recent surveys of the global diversity of species have revealed that there are more than 7,000 extant (nominal) species of echinoderms living on earth (Appeltans et al. 2012). All of them are marine, with most individuals, as adults, forming part of the benthos. The distribution of genera and species within the five commonly recognized echinoderm classes is shown in Table 1.1. Detailed studies of museum collections and molecular analyses suggest that there are a substantial number of species that remain undescribed; for asteroids, see (Mah and Blake 2012). The recently compiled register of marine species (Appeltans et al. 2012) estimates the total number of extant echinoderm species to range between 9,617 and 13,251.

Table 1.1 Total number of genera and species known for all echinoderm classes

Class	No. of genera	No. of nominal species described	Source of the tabulated data
Crinoidea	115	620; 623	Appeltans et al. (2012), Rouse et al. (2013)
Asteroidea	343	1,890; 1,922	Appeltans et al. (2012), Mah and Blake (2012)
Ophiuroidea	270	2,064; 2,064	Appeltans et al. (2012), Stohr et al. (2012)
Holothuroidea	200	1,250; 1,683	Smiley et al. (1991), Appeltans et al. (2012)
Echinoidea	258	999	Kroh and Mooi (2011), Appeltans et al. (2012)

The Fossil Record and the Origin of Recent Forms

More than 13,000 echinoderm species have been recognized in the fossil record with their first appearance dated to the Cambrian (Fig. 1.2). Several body sub-plans can be distinguished: pentaradiate forms (stromatocystitids and gogiids), asymmetric forms (ceratocystitid stylophorans), bilateral forms (ctenocystoids), and spiral forms (helicoplacoids) (Smith 2005). These originated in a short period of time, perhaps as short as 10–15 My, in the waters off both Gondwana and Laurentia (Smith et al. 2013). Using molecular clock estimates, Pisani and colleagues (2012) place the origin of Echinodermata (the time of the divergence between Echinodermata and their proposed sister taxon, Hemichordata) in the late Precambrian, around 570 My ago. Given that stereom skeletal elements appear in the fossil record around 525 My ago, we should assume a diversification period of some tens of My before the articulated forms were established. It is important to note that the earliest record of a stereom almost coincides with the first articulated specimens (Zamora et al. 2013).

Fossil species have been incorporated into modern phylogenetic analyses to generate a more complete picture of echinoderm evolution using different methodologies. The groundbreaking study of Smith (1984) proposes that Echinodermata comprise two major monophyletic assemblages: the eleutherozoans and the pelmatozoans (these ones represented by forms with stalks and calyces). Sumrall's cladistic analysis on a similar data set suggests an alternative arrangement (Sumrall 1996). He was the first to consider carpoids (homalozoans) as a monophyletic group and derived from modern echinoderm clades. At the same time it was considered that the variety of symmetries existing in the Cambrian is due to paedomorphic reductions from a pseudo-fivefold symmetric ancestor (Sumrall and Wray 2007). More recently, David and Mooi (1998) and David and colleagues (2000) have suggested another alternative topology, introducing blastozoans, a subphylum that includes all brachiole-bearing forms (i.e., eocrinoids). Importantly,

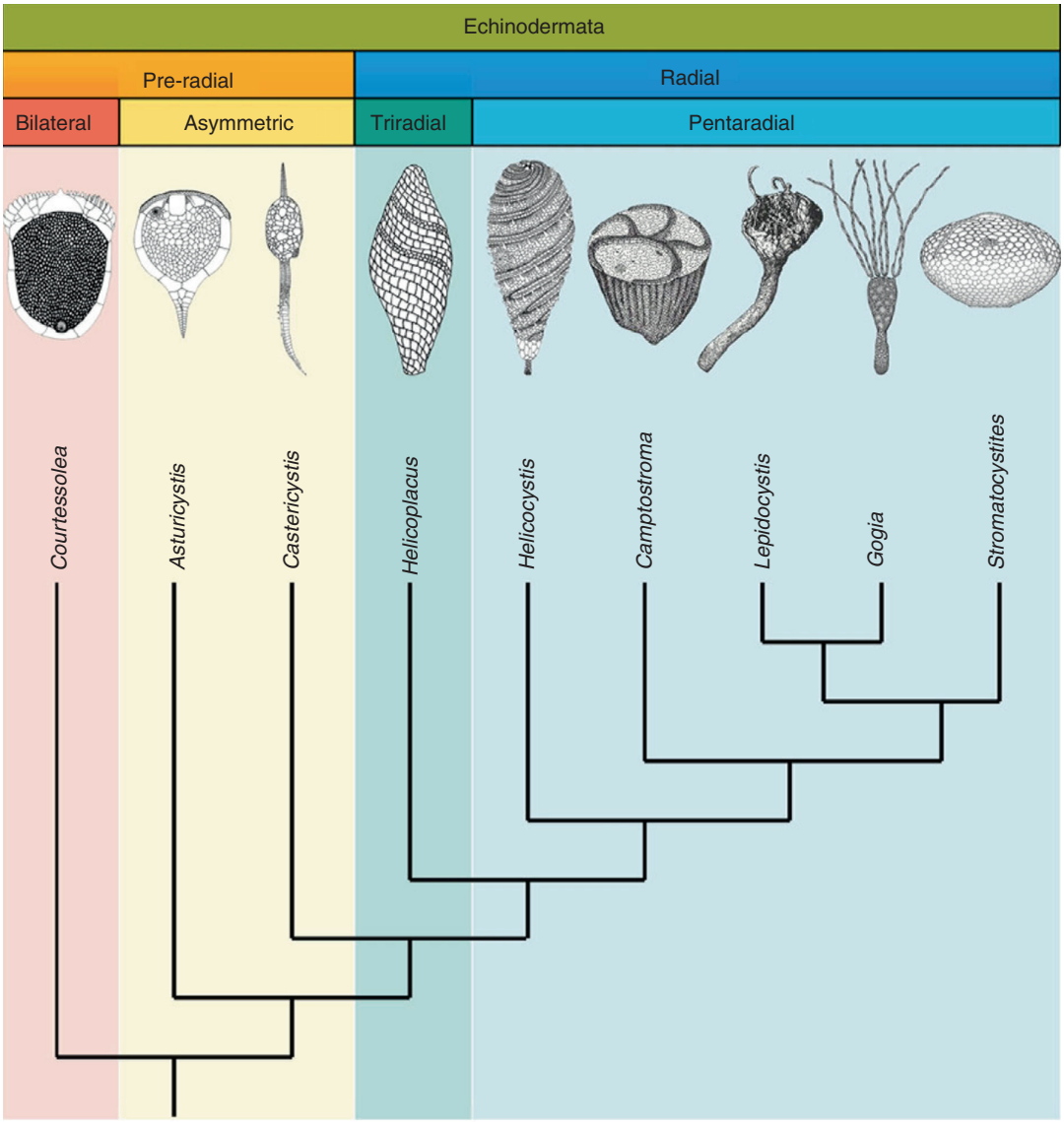


Fig. 1.2 A phylogenetic tree showing various Cambrian echinoderms, including the early bilateral representative *Courtessolea* and the most primitive pentaradial form *Helicocystis* (Figure taken from Smith and Zamora (2013)©)

they specifically propose that edrioasteroids would represent good proxies for the earliest echinoderms. A recent hypothesis suggests that the bilateral echinoderm Ctenoimbricata would represent the plesiomorphic condition for echinoderms (Zamora et al. 2012). This is based on the ontogeny and sister group relationships of modern echinoderms that suggest a bilateral species at the base of the echinoderm tree. Ctenocystoids would represent a next step, some of them having retained the primitive condition of the group while others became slightly asymmetric. In this scenario, the cinctans and solutes were more derived forms and represent the asymmetric condition before the appearance of radial forms. Radial echinoderms started with the helicoidal three ambulacra-bearing helicoplacoids. Pentaradial echinoderms also appeared in the Cambrian represented by edrioasteroids and some blastozoan groups.

Among the Paleozoic fossils, four groups of non-pentameric forms, the "homalozoans," have been at the center of intense, sometimes bitter, debate concerning the origin of a phylum related to the Echinodermata, the Chordata. The position and nature of some echinoderm structures, such as mouth, anus, or the ambulacra, have been used by many authors as arguments to suggest that homalozoans were stem taxa to all the chordates (Jefferies 1968) or early echinoderms (Ubaghs 1975; Parsley 1991). Most modern authors tend to align themselves with this latter position (Ruta 1999; Smith 2008).

However, as it happens for other fossil groups, the debates still revolve around the placement of a few key fossil groups, for instance, the carpoids within the subphylum Homalozoa. While some authors regard some of these groups as primitive, others consider them to be secondarily derived.

All extant echinoderms are derived from a few taxa that survived the Permo-Triassic extinction event. This has been clearly established for the crinoids, asteroids, and echinoids. However, all of these originated in the early Ordovician. Molecular clock analysis has allowed to estimate the times of divergence for the different classes, ranging from 509 My for the divergence of crinoids to 480 My for the eleutherozoan echinoderms (Pisani et al. 2012). The phylogenetic relationships among all extant echinoderm classes have been the subject of debate for many years. While there is consensus concerning the position of Crinoidea as the sister group to the remaining echinoderms (Eleutherozoa), there are clearly divergent opinions regarding the interrelationships of the remaining taxa. While some molecular analyses have suggested a clade that includes ophiuroids + echinoids + holothuroids (Littlewood et al. 1997; Pisani et al. 2012), there is an alternative view, supported mostly by comparative morphologists and paleontologists, which assumes a close association between asteroids and ophiuroids (Mooi and David 2000; Janies 2001). These two hypotheses are known as the "Cryptosyringida" and "Asterozoa-Echinozoa" hypothesis, respectively (Fig. 1.3).

More, and probably different, data sets are needed to resolve disputes regarding the relationships. It has become clear that the results that lead to the establishment of specific relationships are highly dependent on the choice of parameters and methods (Janies et al. 2011). Interestingly, a very recent multigene analysis (219 genes from all echinoderm classes) using Bayesian methodologies and dealing with some older methodological biases seems to clearly support Asterozoa (Telford et al. 2014). The clarification of the internal phylogeny of Echinodermata is of key relevance, since it will provide important insights into the evolutionary history of both the adult and the larval forms.

Life History Diversity, Larval Forms, and Evolution of Development

Most echinoderms spawn freely with fertilization occurring in the water column. Development proceeds through a dispersive larva, although a few species brood their embryos. Species that have small eggs (approx. <150 μm diameter) develop through feeding (planktotrophic) larvae. In contrast, species with large eggs (approx. >300 μm diameter) have nonfeeding (lecithotrophic) larvae fully provisioned by the egg (Raff and Byrne 2006). The feeding planktotrophic larva is considered to be a plesiomorphic character for modern echinoderms (Strathmann 1985; Smith 1997; McEdward and Miner 2001; Raff and Byrne 2006). Possession of a larval phase is suggested to have arisen through intercalation between the gastrula and juvenile life phases in an ancestral form (Sly et al. 2003). The feeding bipinnaria larvae of asteroids and the auricularia larvae of holothuroids are very similar to the tornaria larva of the Hemichordata (see Chapter 2). These larval forms – the so-called dipleurula-type larvae – are considered to represent the basal-type larva for the Ambulacraria (Peterson et al. 2000b; Raff and Byrne 2006).

Planktotrophic larvae feed on phytoplankton and the ciliary bands that loop around the body are used for capturing food and for locomotion (Strathmann 1985). Evolution of a large egg freed larvae from the necessity to feed, resulting in the reduction and loss of superfluous feeding structures (Raff and Byrne 2006). As a result, lecithotrophic echinoderm larvae lack a functional

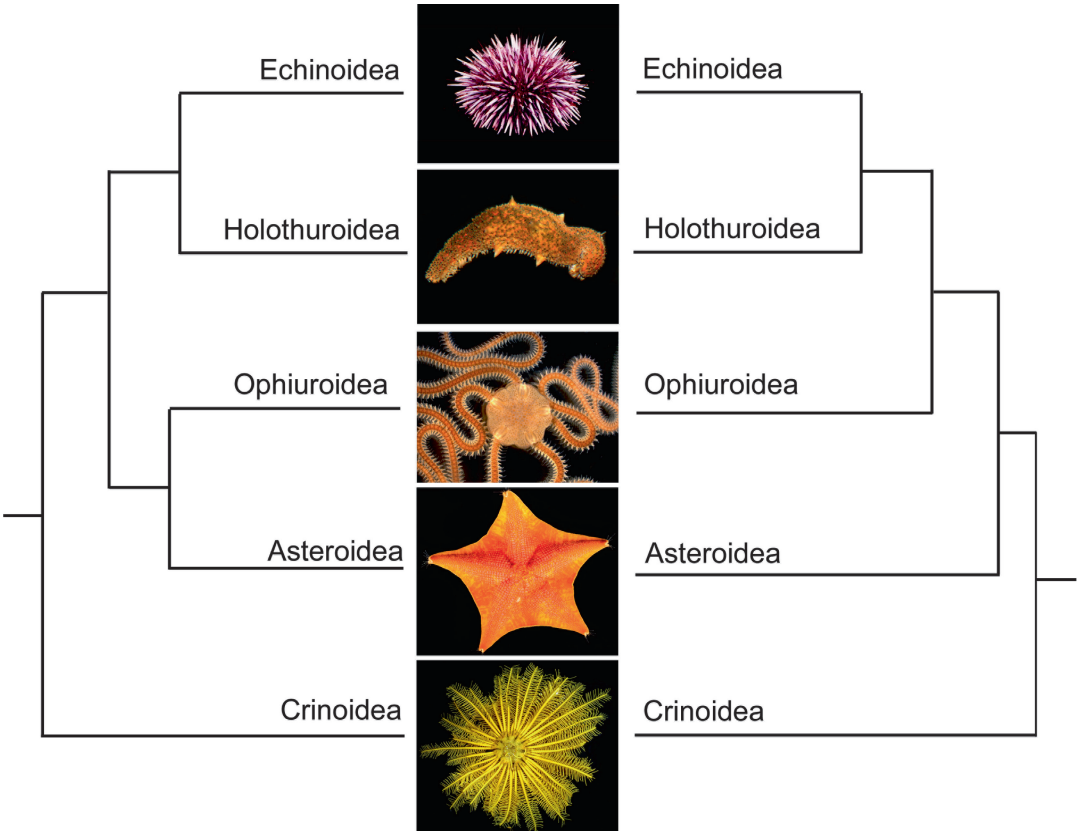


Fig. 1.3 Alternative hypotheses proposed for the relationships among extant echinoderm classes. The *left side* of the figure corresponds to the Asterozoa-Echinozoa hypothesis and the *right side* to the Cryptosyringida hypothesis

gut and have a simplified pattern of ciliation and may be planktonic or benthic. Lecithotrophy is considered to be the derived larval form for modern echinoderms and appears to have arisen independently and frequently in many echinoderm clades. Moreover, once lecithotrophic development evolved, subsequent radiation may have generated new species with this life history mode (Jeffery et al. 2003; Hart et al. 2004). The presence of lecithotrophic larvae with nonfunctional feeding structures also supports the hypothesis that these larvae arose from an ancestral adult form with a feeding larva (Raff and Byrne 2006). After 500 million years of larval evolution, approximately 68 % of echinoderms with known development have the supposedly derived, lecithotrophic larval type (Uthicke et al. 2009).

Rapid evolution of development, as seen in *Heliocidaris* sea urchins and asterinid sea stars, has resulted in diverse larval phenotypes. The two *Heliocidaris* species, one with a feeding (*H. tuberculata*) and one with a nonfeeding (*H. erythrogramma*) larva, are used as a model comparative system to investigate the developmental and genetic mechanisms underlying the evolutionary switch to a lecithotrophic larva (Wray 1996; Raff and Byrne 2006). The full range of larval types in the Echinodermata is evident in the asterinids (Byrne 2006). These asteroids include taxa with feeding (e.g., *Patiria*, *Patiriella*) and nonfeeding (e.g., *Meridiastra*) planktonic larvae, species with strange-looking nonfeeding benthic larvae (*Parvulastra*, *Asterina*) that maintain a tenacious hold on the seafloor, and species with larvae that swim in the gonad followed by metamorphosis and birth as nearly sexually mature asteroids.

Generally, the zygotes of species with small eggs give rise to two types of feeding larvae: the pluteus-like larvae of sea urchins and brittle stars and the auricularia-like larvae of sea cucumbers

and sea stars (Hyman 1955; Raff and Byrne 2006). The Echinoidea, Ophiuroidea, Holothuroidea, and Asteroidea also include species with various types of nonfeeding (lecithotrophic) larvae. Crinoidea (sea lilies and feather stars) are the only echinoderm class that does not have a feeding larva. Their embryos develop typically into a secondarily derived nonfeeding larva, the barrel-shaped doliolaria. Interestingly, one species has an auricularia-like ciliary band indicating an ancestral form with feeding larvae (Nakano et al. 2003). Figure 1.4 displays representative larval types for each echinoderm class, along with their adult forms, arranged according to one of the alternative phylogenetic arrangements currently suggested for this phylum (see Fig. 1.3 for the alternatives). The great diversity of larval forms in echinoderms with feeding and nonfeeding modes are illustrated for each class (Balser 2002; Byrne and Selvakumaraswamy 2002; Emlet et al. 2002; McEdward et al. 2002; Sewell and McEuen 2002).

At the end of the planktonic phase, larvae settle and the juvenile pentaradial form arises through a series of marked changes during metamorphosis. The adult rudiment arises on the left side of the larva. The origin of adult tissues and organs is complex and in many cases unknown. The details of the developmental process involved in the genesis of the different structures will be discussed in other sections of this chapter.

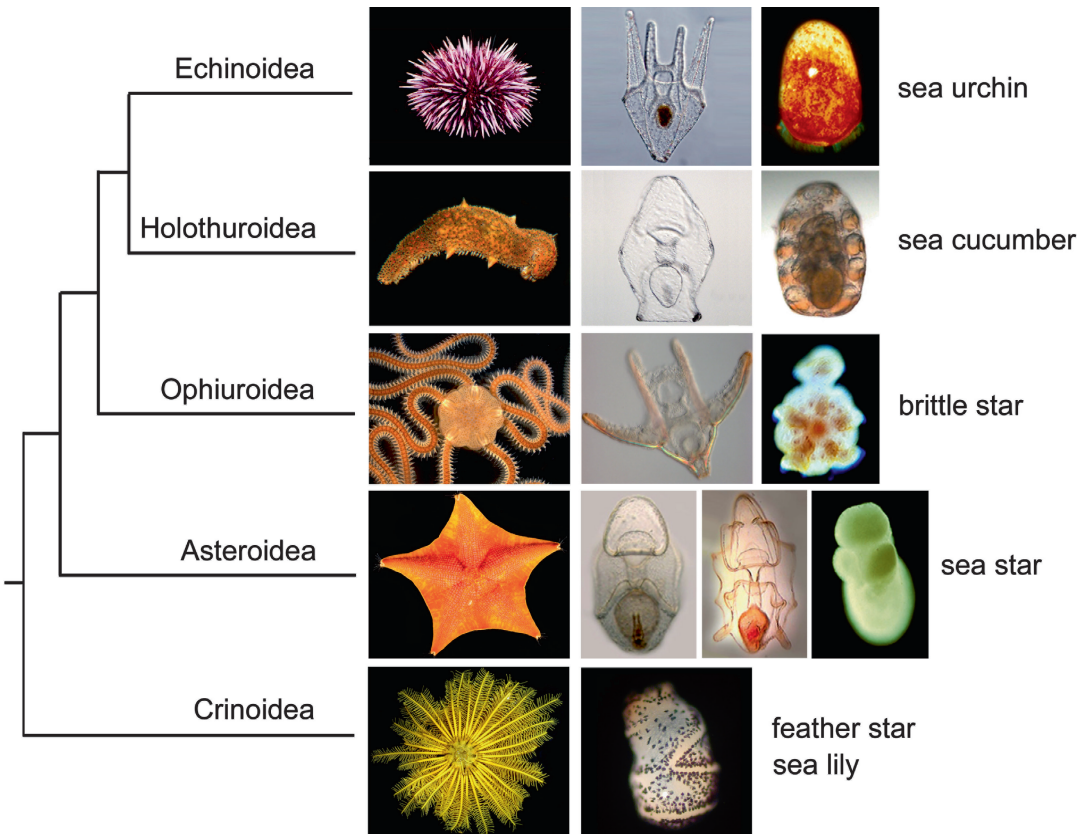


Fig. 1.4 One scenario of echinoderm interrelationships, after Janies (2001), used to illustrate adults and larvae. *From left*, each column displays, for each class, representatives of adult phenotypes (for species names and image credits see Fig. 1.1), the most representative type of planktonic larvae and adult common names. The larvae are *from left to right*: *Strongylocentrotus purpuratus* echinopluteus and *Heliocidaris erythrogramma* reduced pluteus for Echinoidea; *Parastichopus parvimensis* auricularia (Courtesy of Veronica Hinman) and *Holothuria scabra* doliolaria for Holothuroidea; *Amphiura filiformis* ophiopluteus (Courtesy of David Dylus and Paola Oliveri) and *Clarcoma pulchra* vitellaria (Courtesy of Paula Cisternas) for Ophiuroidea; *Meridiastra calcar* bipinnaria, brachiolaria, and vitellaria for Asteroidea; *Metacrinus rotundus* doliolaria (Courtesy of Hiroaki Nakano) for Crinoidea

Echinoderm Genomes: A Window into the Regulatory Landscape

Our understanding of the development of animals and some evolutionary trends within taxa is now being enhanced by our knowledge of genomes and this is also true for the Echinodermata (Kondo and Akasaka 2012). The ongoing generation of genomic data from different animal systems is providing unprecedented access to the mechanisms that control morphogenesis and its changes over evolutionary time.

After a first wave of sequencing efforts, concentrated on the so-called "model" organisms (*Drosophila melanogaster*, *Caenorhabditis elegans*, *Mus musculus*), the focus has shifted to other systems, including marine invertebrates. The sequencing of the genome of the sea urchin *Strongylocentrotus purpuratus* (814 megabases) was pioneering work and allowed the comprehensive characterization of genes in a species with a long tradition as a model system for developmental biology and a key reference for investigation of the genetic control of embryogenesis; see (Davidson 2006). The sequencing and annotation of the sea urchin genome, carried out by an international team of scientists (Sea Urchin Genome Consortium), allowed gene families to be characterized and, by comparison with other taxa, their evolutionary dynamics to be traced within the Bilateria and Deuterostomia. Some unexpected findings such as the expanded innate immunity repertoire or the huge numbers of genes devoted to sensory systems (including vision and hearing) highlight once more the importance of having access to complete genome sequences if we are to understand developmental and evolutionary processes.

Important as knowledge of genome sequences is, the best way to follow development – and to infer evolution (see Domazet-Loso and Tautz 2010) – is through the characterization of the transcriptomes and proteomes of different species. The former provides detailed information on global changes of transcription in time and/or space and the latter on similar changes but at the level of proteins. Detailed transcriptome analyses have been performed on some echinoderms, but in no case do they match the detailed characterization of transcript variations that have occurred during the development of *Strongylocentrotus purpuratus. S. purpuratus* transcriptomes have been analyzed at 22 different developmental times (Materna et al. 2010; Tu et al. 2012). The extent of the analysis has also allowed the definition of structural parameters for all protein-coding genes (such as intron/exon sizes, intergenic distances, numbers of introns/exons per gene, etc.). These data are incorporated into accessible databases (e.g., EchinoBase: http://mandolin.caltech.edu/Echinobase/). Other echinoderms for which transcriptome data have been generated are the echinoid *Heliocidaris erythrogramma* (mixed developmental stages) (Wygoda et al. 2014), the holothurians *Holothuria glaberrima* (intestinal regeneration) (Rojas-Cartagena et al. 2007; Du et al. 2012) and *Apostichopus japonicus* (mixed developmental stages (Du et al. 2012) or adult regenerating tissues (Sun et al. 2011)), as well as the ophiuroids *Ophiocoma wendtii* (gastrula) (Vaughn et al. 2012) and *Amphiura filiformis* (regenerating arms) (Burns et al. 2012). Many other species are currently being sequenced and analyzed; some of the results are accessible through different websites. Methodologies and the depth of sequence information vary between studies.

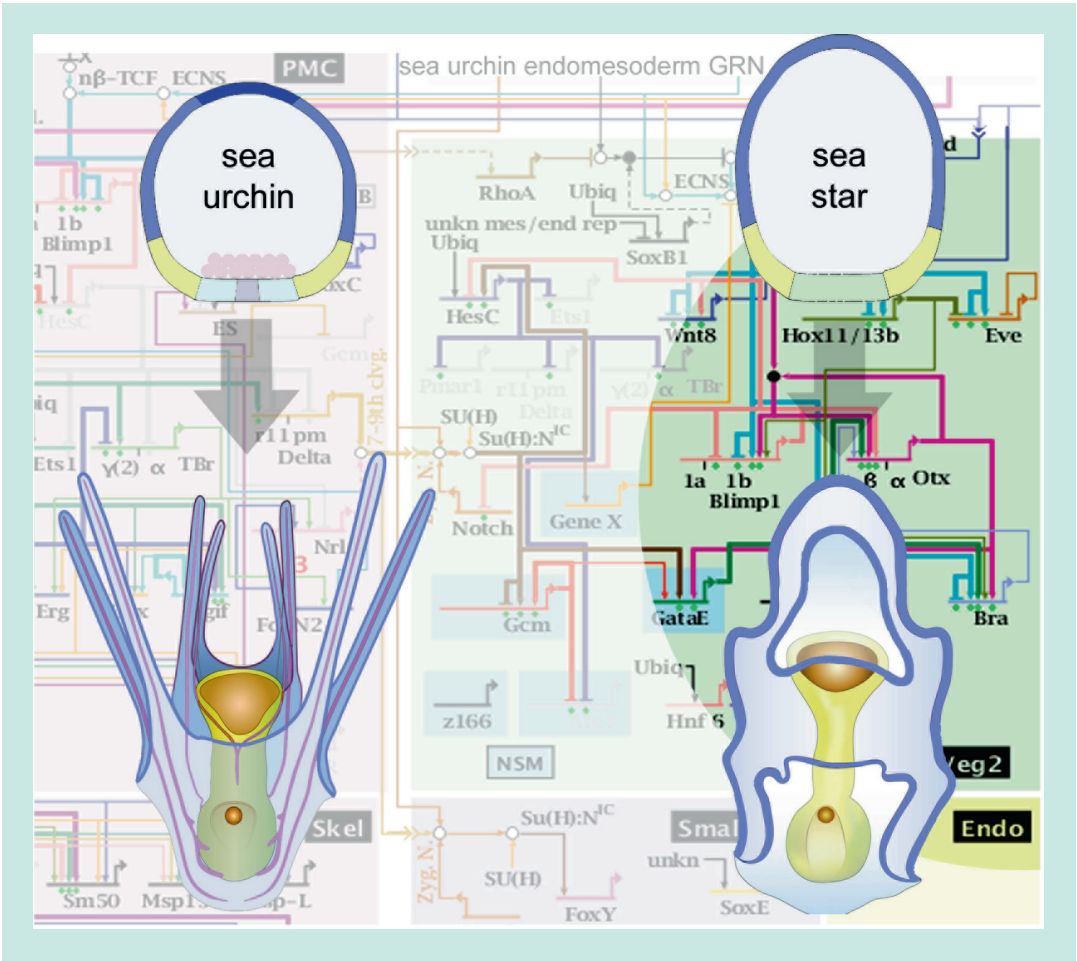
Most recently, other echinoderm genomes have been sequenced, most of which are from echinoids. We have complete genomic and extensive transcriptomic data for the first asteroid species, *Patiria miniata* (http://blast.hgsc.bcm.tmc.edu/blast.hgsc?organism=Pminiata). These data should prove especially useful for understanding echinoderm genome evolution and the changing patterns of gene expression associated with the diversification of the echinoderm groups. Other echinoid genomes currently being sequenced are *Paracentrotus lividus* (European consortium), *Lytechinus variegatus*, *Eucidaris tribuloides*, *Strongylocentrotus franciscanus*, and *Strongylocentrotus fragilis* (Baylor College of Medicine Human Genome Sequencing Center, Houston, and Caltech, Pasadena, USA) and the two *Heliocidaris* species with feeding (*H. tuberculata*) and nonfeeding (*H. erythrogramma*) larvae. For *H. erythrogramma* a complete developmental transcriptome is available from early embryogenesis to the juvenile stages (Wygoda et al. 2014).

An alternative approach to understanding echinoderm developmental processes is the use

of high-throughput proteomic tools, which allows researchers to follow hundreds of proteins (and their post-transcriptional modifications) at once. These techniques have been introduced recently, and their full potential is realized in organisms for which the genomes are already sequenced, for instance, in *Strongylocentrotus purpuratus* (Mann et al. 2010; Adams et al. 2012). Some of the pioneering studies involved the use of different techniques. For instance, the radial nerve cord and coelomocyte protein complements of the asteroids have been characterized using combinations of 1 and 2D electrophoresis plus MALDI-TOF (*Marthasterias glacialis*) (Franco et al. 2011a, b); techniques that were incorporated to the study of phosphorylation patterns during neuronal regeneration (Franco et al. 2012). These are just a few examples of a growing number of comprehensive analyses of protein complements.

In the near future, new genome sequences, in combination with high-throughput transcriptomic and proteomic data, will change the way we see and analyze developmental processes in echinoderms. Moreover, as mentioned above, the comparison of patterns across taxa should also revolutionize the study of evolutionary change.

The use of echinoderms in developmental biology has a long and fertile tradition. In fact, it was through working with these alluring marine creatures that fundamental concepts were made and incorporated into our current knowledge on the function of cells and embryos. These include understanding the role of cyclins in the animal cell cycle, chromosomes as determinants of development, the plasticity of blastomere fates, and the presence of maternal messages in embryos. Our understanding of the molecular control of development, the structure of the gene regulatory apparatus, and recent advances in gene regulatory networks (GRN) as control factors of animal development also stem from the use of echinoderm model systems. Echinoderms present biologists many practical features, including ready access to fertile gametes, the transparency of their embryos, and their relative ease of manipulation in the laboratory. Coupled with the recent sequencing of the genomes of several members of the phylum, it is clear that use of this group of animals as model organisms will continue to be at the center of our advances in understanding, not only of the intricate processes controlling the development of individual animals but also of the fascinating mechanisms that underlie the diversification of body plans over evolutionary time. Our future endeavors will also benefit from the long tradition of observational studies of echinoderms in ecology and in the fossil record and from in-depth studies of the evolution of their life stories. The integration of this traditional research with more modern approaches based on genomic regulatory systems should prove especially fruitful in providing us with a better understanding of specific micro- and macro-evolutionary processes. Among the echinoderms used in developmental biology, the sea urchin *Strongylocentrotus purpuratus* deserves special mention. This species, from North America's west coast, was instrumental to the incorporation of molecular techniques to the study of animal development. From the original characterization of the dynamic changes of transcription in embryogenesis to recent analyses of gene regulatory networks, *S. purpuratus* has been an important model in our modern understanding of developmental processes. The ease of obtaining billions of gametes for synchronous embryo culture, the transparency of the embryo, and the ability to introduce foreign DNA or RNA into the embryos have made of this urchin an ideal model for the study of developmental mechanisms and their molecular control. Given the rich history in research with echinoderms and the recent incorporation of a wide array of new technologies, echinoderms will undoubtedly continue to be center stage within the EvoDevo field.



EMBRYONIC AND LARVAL DEVELOPMENT

While the implications of these alternative phylogenies on the evolution of larval types will be discussed at the end of this section, we will focus first on embryogenesis, i.e., the development from egg up to larva, for each of the five echinoderm classes, highlighting, where possible, commonalities and differences.

Since the classical studies of Derbès (1847) on the formation of the archenteron, the sea urchin embryo has served as a model system for developmental biology. Sea urchin gastrulation is considered as the archetypal model for a deuterostome morphogenetic process (McClay et al. 2004). Starting with the discovery of pronuclear fusion by Fol (1877) and Boveri's experiments on the developmental fate of polyspermic eggs (Boveri 1902), the sea urchin embryo has provided a powerful tool for the study of the role of genome activities during development (reviewed in Davidson et al. 1998). In particular, the process of specification of the endomesodermal territories is extraordinarily well known in the sea urchin *Strongylocentrotus purpuratus* and has led to the most exhaustive characterization of a gene regulatory network (GRN) for any developmental system. As a consequence, there is an extensive literature on sea urchin embryos compared to what has been published for other echinoderms, and thus, the organization of this section reflects this knowledge bias. We need to point out here that the study of regulatory mechanisms in

echinoderms, particularly in sea urchins, has been facilitated by the routine use of knockdown methodologies, particularly those using morpholino-modified oligonucleotides. This, with the regular use of transgenesis, shows the sharp contrast between the gene analysis in echinoderms and those performed in most other phyla.

Development of Echinoidea (Sea Urchins)

Many sea urchin species have been used to characterize the basic processes involved in their embryonic development. Starting with the Mediterranean *Paracentrotus lividus*, which appeared on the scientific scene associated with the abovementioned early studies and the spectacular blastomere recombination experiments of Hörstadius (1939, 1973), important insights have been obtained using the Atlantic *Lytechinus variegatus*, the Western Pacific *Hemicentrotus pulcherrimus*, and the Eastern Pacific *Strongylocentrotus purpuratus*, the latter being the first echinoderm species with a sequenced genome (Sodergren et al. 2006) and for which the first GRN that controls the specification of an embryo was established (Davidson et al. 2002). Figure 1.5 displays adult specimens of all these species. The following description of sea urchin embryonic development represents a summary of the knowledge obtained by studying these species and, thus, provides an overview for sea urchin development, keeping in mind that differences exist among the species.

The eggs of sea urchins with feeding larvae range from 80 to 180 μm in diameter. The meiotic divisions associated with oogenesis are completed while the eggs are still in the ovary. The egg has a small, clear, eccentrically located pronucleus. This is relatively homogeneous and contains uniformly distributed yolk granules and numerous small lipid vesicles and other organelles (Byrne et al. 1999). Together, these granules and the lipids supply the embryo and early larva with the energy sources and precursor molecules needed prior to feeding (Scott and Lennarz 1989). Two envelopes surround the sea urchin egg: the inner vitelline envelope and the outer jelly coat (Glabe and Vacquier 1977).

Fertilization involves two fusion events: gamete fusion, the fusion of the sperm and egg plasma membranes, and pronuclear fusion, the fusion of the male and female haploid pronuclei. As the surfaces of the gametes approach each other, a specific interaction takes place between the sperm protein bindin (Vacquier and Moy 1977) and a receptor located on the egg surface (Giusti et al. 1997; Stears and Lennarz 1997). The sperm-egg binding reaction causes the exocytosis of the sperm's acrosomal vesicle, with proteolytic enzymes being released that allows the sperm cell to penetrate the jelly coat and establish contact with the vitelline envelope (Dan and Hagiwara 1967; Franklin 1970; Levine et al. 1978). At this point, the first fusion event of fertilization, sperm-egg plasma membrane fusion, or gamete fusion takes place, facilitating that the sperm pronucleus moves towards the egg pronucleus. Sperm-egg fusion triggers a complex series

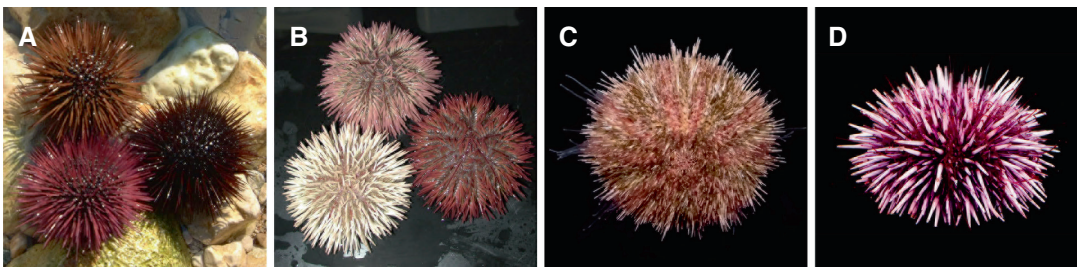


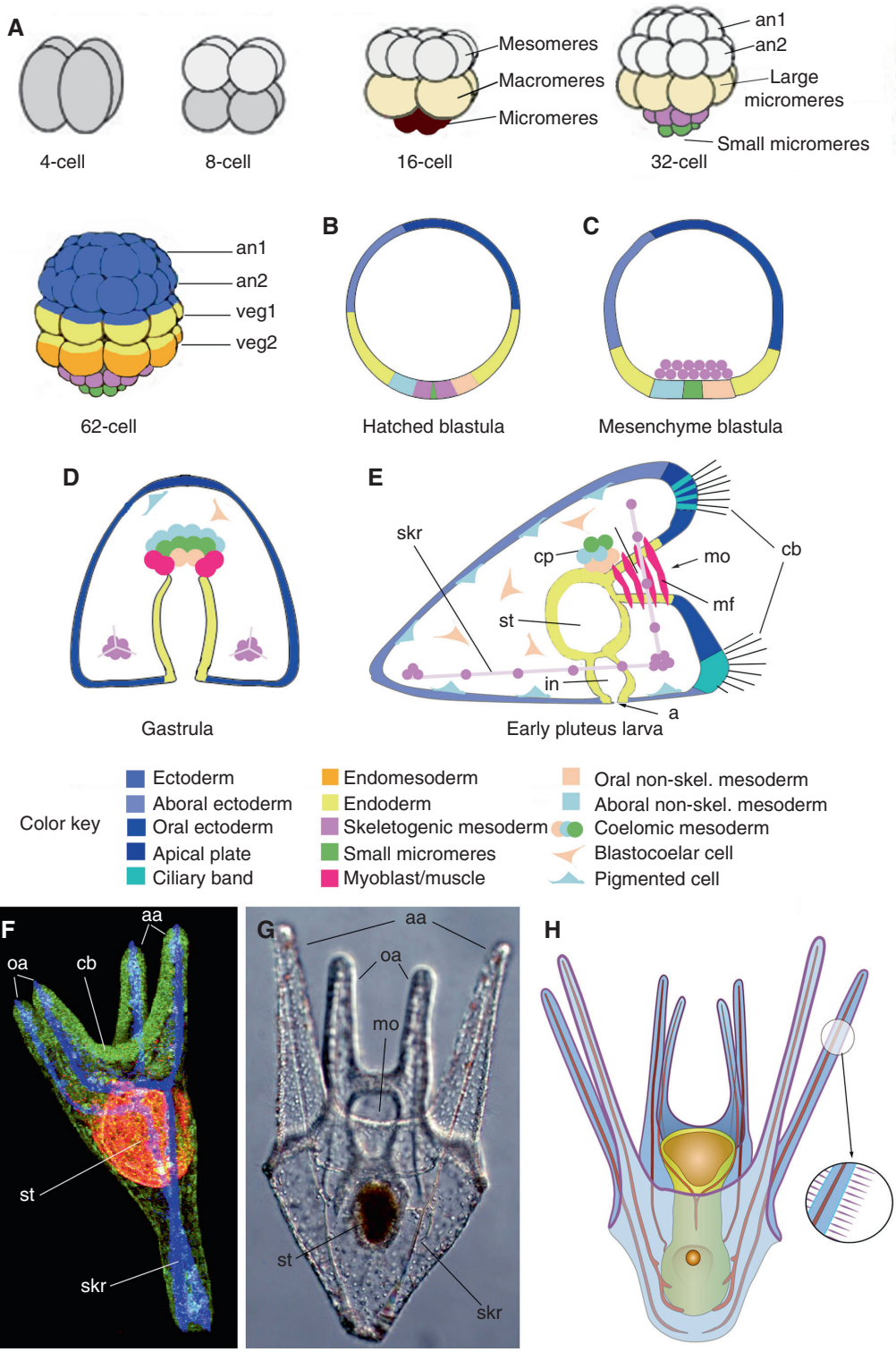
Fig. 1.5 Sea urchin species most commonly used in developmental biology. *From left to right*, *Paracentrotus lividus* (**A**, Courtesy of Christian Gache), *Lytechinus variegatus* (**B**, Courtesy of David McClay), *Hemicentrotus pulcherrimus* (**C**, Courtesy of Koji Akasaka), and *Strongylocentrotus purpuratus* (**D**, Courtesy of Mattias Ormestad). Adult specimen sizes range from about 50 to 100 mm in diameter for all 4 species

of responses. Among the most important are processes that prevent polyspermy. These start about 20 s after sperm attachment and are complete already by the end of the first minute of fertilization. Two complementary processes prevent polyspermy in sea urchins: a fast reaction, accomplished by a transient depolarization of the egg's plasma membrane (Jaffe 1976; Schuel and Schuel 1981), and a slower reaction, involving the more permanent production of a physical barrier caused by the exocytosis of cortical granules (Just 1919). Cortical granules of sea urchins contain many different components necessary to accomplish their varied tasks. Proteases dissolve the connection between the vitelline envelope and the cell membrane; they clip off the bound receptor and any sperm attached to it (Vacquier et al. 1973; Glabe and Vacquier 1978). Mucopolysaccharides produce an osmotic gradient that causes water to enter the space between the plasma membrane and the vitelline envelope, causing the envelope to expand and become the fertilization envelope (Hall 1978). A peroxidase enzyme hardens the fertilization envelope by cross-linking tyrosine residues on adjacent proteins (Foerder and Shapiro 1977; Mozingo and Chandler 1991). Finally, the cortical granules release a sticky protein, hyaline, which forms a tough extracellular matrix around the embryo (Hylander and Summers 1982). This hyaline layer holds the cells of the early embryo together until they develop cell junctions at the blastula stage. The second fusion event in fertilization, pronuclear fusion, usually occurs within 30–45 min after gamete fusion; through it the two pronuclei merge and a diploid zygote nucleus is formed.

Figure 1.6 displays the development of the sea urchin embryo from the 4-cell to the pluteus larva stage. Cleavage of sea urchin embryos is holoblastic, radial, and in the majority of stages equal. The exception is the fourth cleavage, which is unequal and thus a unique feature of echinoids with small eggs and feeding larvae. The first and second cleavages are both longitudinal, intersecting the animal and vegetal poles. These divisions lie at right angles to one another, dividing the embryo into four cells of equal size. The third cleavage is equatorial, perpendicular to the first two cleavage planes. This cleavage separates the animal and vegetal hemispheres from one another, giving rise to the eight-cell stage. Because all the cells of the embryo in each of the first three cleavages are equal in size, cleavage up to this point is said to be equal. The fourth cleavage, however, is very different from the first three. The upper four cells divide meridionally, forming equal-sized cells called mesomeres. The lower four cells divide unequally and horizontally to produce four larger macromeres and below them four smaller cells called micromeres, located at the vegetal pole of the embryo (Summers et al. 1993). At the fifth cleavage the eight mesomeres divide equally and horizontally, forming two tiers of cells in the animal hemisphere (an1 and an2), one staggered above the other. The four macromeres divide meridionally, forming a tier of eight cells, while the micromeres divide unequally once more, generating four large micromeres and four small micromeres (Okazaki 1975; Pehrson and Cohen 1986; Cameron and Davidson 1991). At sixth cleavage all the cells divide horizontally, producing the 60-cell stage embryo. At this point the subdivision of the embryo, from the animal to the vegetal pole, is as follows: 16 an1, (two layers of eight cells each), 16 an2 (two layers of eight cells each), eight veg1 (vegetal tier one), eight veg2 (vegetal tier one), and 12 micromeres (eight large micromeres and four small micromeres) (Fig. 1.6A).

The blastula stage of sea urchin development begins at the 128-cell stage. Cleavage continues, producing progressively smaller and smaller cells. The cells form a hollow sphere surrounding a central cavity or blastocoel and they become organized as a true epithelium, with permanent cell junctions and a complex extracellular matrix on both the interior and exterior surfaces.

The formation of the blastocoel is accomplished by the adhesion of the blastomeres to the hyaline layer and by an influx of water that results in an expansion of the internal cavity (Dan 1960; Wolpert and Gustafson 1961; Ettensohn and Ingersoll 1992). The cells at the vegetal pole of the blastula begin to thicken, forming a vegetal


A
4-cell
8-cell
Mesomeres
Macromeres
Micromeres
16-cell
an1
an2
Large micromeres
Small micromeres
32-cell
an1
an2
veg1
veg2
62-cell
B
Hatched blastula
C
Mesenchyme blastula
D
Gastrula
E
skr
cp
mo
cb
mf
st
in
a
Early pluteus larva
Color key
Ectoderm
Aboral ectoderm
Oral ectoderm
Apical plate
Ciliary band
Endomesoderm
Endoderm
Skeletogenic mesoderm
Small micromeres
Myoblast/muscle
Oral non-skel. mesoderm
Aboral non-skel. mesoderm
Coelomic mesoderm
Blastocoelar cell
Pigmented cell
F
aa
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G
aa
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plate. A small tuft of long cilia forms at the animal pole of the blastula. This allows the embryo to start rotating inside the fertilization membrane. At this point an enzymatic complex is secreted by the cells of the animal half, by which the blastula hatches and starts to swim freely (Lepage et al. 1992; Reynolds et al. 1992; Ghiglione et al. 1994).

Starting from the late cleavage and blastula stages, the sea urchin embryo can be considered as composed of "territories." These territories are distinguished by specific, differential patterns of gene expression, individual cell lineage histories, and cell fates. Five major embryonic territories can be distinguished by the 60-cell stage: the small micromere, the skeletogenic mesenchyme, the vegetal plate, the aboral ectoderm, and the oral ectoderm territories (Fig. 1.6A; Davidson 1989, 1990; Cameron and Davidson 1991; Davidson et al. 1998).

The four small micromere founder cells arise at the unequal fifth cleavage; they divide only once more during embryogenesis and contribute to the coelomic pouch and adult rudiment (Juliano et al. 2010). The skeletogenic cells are the sister cells of the small micromeres and they give rise to the skeleton in the larva.

The vegetal plate territory generates the archenteron during gastrulation and all mesodermal elements. The aboral ectoderm produces a squamous epithelium that forms the wall of the late embryo and the larva, except for the oral and ciliated band domains. The oral ectoderm territory produces a variety of cell types and structures: the mouth, the oral hood, the ciliated bands, and most or all components of the larval nervous system.

Once cells have acquired unique identities and begin to express different sets of genes, the stage is set for morphogenesis and differentiation. Morphogenesis begins shortly after cleavage in echinoderms, quickly establishing the three primary germ layers. Morphogenetic events have been extensively studied in sea urchins because of the easiness with which it is possible to experimentally manipulate embryos in various informative ways. The advanced blastula consists of a single layer of about 500 cells that has the shape of a hollow ball, flattened and thickened at the vegetal side (Fig. 1.6B).

The first overt morphogenetic event is the ingression of a subset of mesenchyme cells from the vegetal pole region of this late blastula (Fig. 1.6C). The primary mesenchyme cells (PMCs), which are derived from the large micromeres and are located approximately in the center of the vegetal plate region (Burke et al. 1991), begin to change. They start extending and contracting long filopodia from their inner surface. Then, they lose their affinity for the apical lamina and for their epithelial neighbors, gaining an affinity for the extracellular matrix and the basal lamina that lines the blastocoel (Fink and McClay 1985; Amemiya 1989). This cell movement is termed ingression. Ingressing cells are

Fig. 1.6 Sea urchin development. (**A**) Cleavage stages seen along the animal (*top*)-vegetal (*bottom*) axis. At the 16-cell stage there are four micromeres (*brown*) at the vegetal pole, four central macromeres (*light yellow*), and eight mesomeres (*gray*) at the animal pole. The colors indicate when the cells begin to be specified towards ectoderm, endoderm, and mesoderm (see color key). (**B**) Hatched blastula stage, midsagittal section. The ectoderm is already subdivided (as indicated by different shades of *blue*) and the non-skeletogenic mesoderm (oral and aboral) has separated from the endoderm. (**C**) Mesenchyme blastula stage, midsagittal section. Primary mesenchyme cells have ingressed into the blastocoel while small micromeres stay behind. (**D**) Midsagittal section of a mid-gastrula stage, showing the gut invaginating, the skeletogenic cells beginning to synthesize the skeleton, and non-skeletogenic mesoderm at the tip of the archenteron subdividing into domains occupying different positions along the oral/aboral and animal/vegetal axes (different cell types are indicated following the color key). (**E**) Pluteus larva, lateral view, showing the definite structures and cell types generated during embryogenesis. (**F**) *Paracentrotus lividus* pluteus larva stained to show the gut (*red*), the skeleton (*blue*), and the ectoderm (*green*) (Courtesy of David McClay). Length of larva, from posterior end to anterior tip = 120 μm. (**G**) *Strongylocentrotus purpuratus* at the four-arm stage larva. Length of larva = 200 μm. (**H**) Scheme of the eight-arm pluteus stage larva (Courtesy of Santiago Valero-Medranda) highlighting internal skeleton (*brown*) and digestive system (*yellow/orange*). The inset shows details of the ciliary band on one larval arm (*purple*). Abbreviations: *a* anus, *aa* anal arm, *an* animal, *cb* ciliary band, *cp* coelomic pouch, *es* esophagus, *in* intestine, *mf* muscle fiber, *mo* mouth, *oa* oral arm, *skr* skeletal rod, *st* stomach, *veg* vegetal

bottle-shaped with their basal end protruding into the blastocoel and their apical end narrowed into the form of a thin strand. The embryo at this early stage of gastrulation is referred to as a mesenchyme blastula (Fig. 1.6C).

Once inside the blastocoel, PMCs migrate seemingly at random for a brief period, actively making and breaking filopodial connections to the wall of the blastocoel. These filopodia are not thought to function in locomotion; rather they appear to explore and sense the blastocoel wall and may be responsible for receiving dorsoventral and animal-vegetal patterning cues from the ectoderm (Malinda et al. 1995). Eventually, PMCs congregate in the vegetal half of the embryo, in a ring pattern, with two major aggregates of cells (the ventrolateral clusters). Here, PMCs become round, retract their cilia, and fuse into syncytial strands (Hodor and Ettensohn 1998), which will form the axis of the calcium carbonate spicules of the larval skeleton (for a recent review, see McIntyre et al. 2014).

As the ring of primary mesenchyme cells leaves the vegetal region of the blastula, the remaining cells at the vegetal plate move to fill in the gaps, fold inwards, and become elongated in a process called "invagination." This process has been conventionally divided into two distinct temporal phases, primary and secondary invagination (Dan and Okazaki 1956; Kinnander and Gustafson 1960). Within a few hours, the thickened vegetal plate bends inwardly. As shown by serial reconstructions of *Lytechinus pictus* embryos, relatively few cells (about 100) take part in this first step (Ettensohn 1984). At the time of invagination, the vegetal plate cells (and only these cells) secrete a chondroitin sulfate proteoglycan into the inner lamina of the hyaline layer, located directly beneath them. This hygroscopic molecule swells the inner lamina, but not the outer lamina, causing the vegetal region of the hyaline layer to buckle (Lane et al. 1993). Slightly later, a second force arising from the movements of epithelial cells adjacent to the vegetal plate facilitates this invagination by pushing the buckled layer. The invaginated region is called the archenteron (primitive gut), and the opening of the archenteron at the vegetal region is called the blastopore. Sea urchins are deuterostomes and thus the blastopore, later in development, will form the anus of the larva. By the end of this primary invagination, the archenteron, which is roughly cylindrical in shape, has extended between one-fourth and one-half of its total length across the blastocoel. When the primary invagination is completed, the length of the gut rudiment scarcely changes during a couple of hours. Meanwhile, secondary mesenchyme cells (SMCs) become visible at the tip of the gut rudiment. These cells are also called non-skeletogenic mesoderm (NSM) and are the descendants of the veg2 blastomeres formed at the sixth cleavage (Horstadius 1973; Cameron et al. 1991). SMCs begin to extend long, thin filopodia into the blastocoel and towards the area of the animal pole, exploring putative attachment sites, while they remain attached to the gut rudiment (Hardin 1988; Hardin and McClay 1990). After a brief pause, the second phase of archenteron formation begins. During this time, the archenteron extends dramatically, sometimes triplicating its length. The embryo now has reached the mid-gastrula stage (Fig. 1.6D). In this process of extension, the wide, short gut rudiment is transformed into a long, thin tube. It has been proposed that contraction of the filopodia interconnecting the archenteron tip and the apical plate pulls the gut rudiment upward (Takata and Kominami 2004). At this point, the existence of tension in SMC filopodia is evident. Further, elongation of the archenteron is blocked when the pseudopodia are broken by expanding the blastocoel (Dan and Okazaki 1956) or with the use of a laser beam (Hardin 1988). Together with the help of forces exerted by SMC filopodia, cellular rearrangements lead to the formation of a slender archenteron. These cells of the archenteron rearrange themselves by migrating over one another and, at the same time, they flatten (Ettensohn 1985; Hardin and Cheng 1986). This phenomenon, wherein cells intercalate to narrow the tissue and at the same time move it forwards, is called convergent extension. Cell division continues to produce more endodermal and secondary mesenchyme cells while the archenteron extends (Martins et al. 1998).

As the archenteron elongates, secondary mesenchyme cells delaminate from its tip and disperse

within the blastocoel, where they proliferate to form four types of non-skeletogenic mesoderm (NSM) cells (Ettensohn and Ruffins 1993): pigment cells (Gibson and Burke 1985, 1987), blastocoelar cells (Tamboline and Burke 1992), coelomic pouch cells, and circumesophageal muscle cells (Ishimoda-Takagi et al. 1984; Burke and Alvarez 1988; Andrikou et al. 2013). These cell types are specified long before delaminating from the tip of the archenteron where they are arranged spatially to occupy different positions along the animal/vegetal and oral/aboral axis (see different color cells in Fig. 1.6D, E; for the specification state of these NSM cells at the tip of the archenteron see Luo and Su 2012 and Andrikou et al. 2013).

Soon after elongation starts in *S. purpuratus* embryos, the archenteron bends ventrally, towards the prospective oral region, while in *L. variegatus* embryo this event occurs later on, as the tip of the archenteron approaches the animal pole of the blastocoel. The oral epithelium and cells at the tip of the archenteron make contact, and an opening is produced in the epithelia, which will become the larval mouth. The blastopore will develop into the anal opening of the digestive tract. Just before the archenteron makes contact with the prospective oral field, another important morphogenetic movement, coelom formation, begins. This is the time when myoblasts from each coelomic pouch extend pseudopodia towards the outer surface of the esophagus, eventually forming muscle fibers. After full elongation of the archenteron, constrictions subdivide the endoderm into foregut, midgut, and hindgut, and this regionalization not only becomes evident morphologically but also is clearly reflected in patterns of region-specific gene expression (Cole et al. 2009; Annunziata and Arnone 2014; Annunziata et al. 2014). During this period, termed prism stage, the embryo takes on the shape of a rounded, truncated pyramid. The side of the embryo where the mouth will open (stomodeum) becomes flattened, forming the oral surface of the developing larva. The blastopore side of the embryo also becomes flattened and forms the anal surface of the developing larva. A ciliary band develops around the stomodeum. Ciliary band cells are interspersed with neurons that begin to differentiate at this stage to, eventually, form the complex neuronal network typical of the pluteus larva (for a review of the sea urchin larva nervous system, see Burke et al. 2006). Also at this stage, the apical organ, where serotonergic and other type of neurons that remain to be characterized will develop, becomes morphologically evident as a disk of thick ciliated epithelium at the animal pole of the embryo (indicated as a dark blue region in Fig. 1.6B–E; see Byrne et al. 2007).

As development proceeds, the embryo elongates slightly along the dorsoventral axis and two arms, the oral arms, appear and extend outwards from the oral lobe. Two additional arms, the anal arms, appear and extend outwards at the junction of the oral and anal surfaces. The embryo has reached the pluteus stage (Fig. 1.6E). The triradiate spicules develop into skeletal rods that extend through the body and inside the arms. The myoblasts have fused to form circumesophageal muscle fibers and the coelomic pouches are fully shaped. From a portion of the left coelomic pouch, a duct-like structure, the hydroporic canal, extend to the aboral ectoderm where the hydropore forms, thus showing the first morphological signature of left-right asymmetry of the pluteus larva (Luo and Su 2012).

Because of the morphogenetic changes of the larva, the developing digestive tract is bent into a J-shape structure. The stomach enlarges and fills a large part of the body of the pluteus while the arms elongate. When completely formed, the anal arms are longer than the oral ones. A pluteus larva at this stage of development is referred to as the four-armed pluteus larva (Fig. 1.6F, G). Sequential elongation of additional arms (up to eight; Fig. 1.6H) and important modifications of the mesoderm occur during the various planktonic larval stages (see Smith et al. 2008b for progression of *Strongylocentrotus purpuratus* larval stages). A period of extensive feeding and continued larval development is required before metamorphosis to a miniature sea urchin juvenile occurs (see below).

A vast diversity of echinoids develops through nonfeeding larvae (an example is shown in Fig. 1.7). Details of embryology and larval development in these echinoids are available for several species (Raff 1992; Morris 1995; Emlet et al. 2002). Some species such as *Holopneustes*

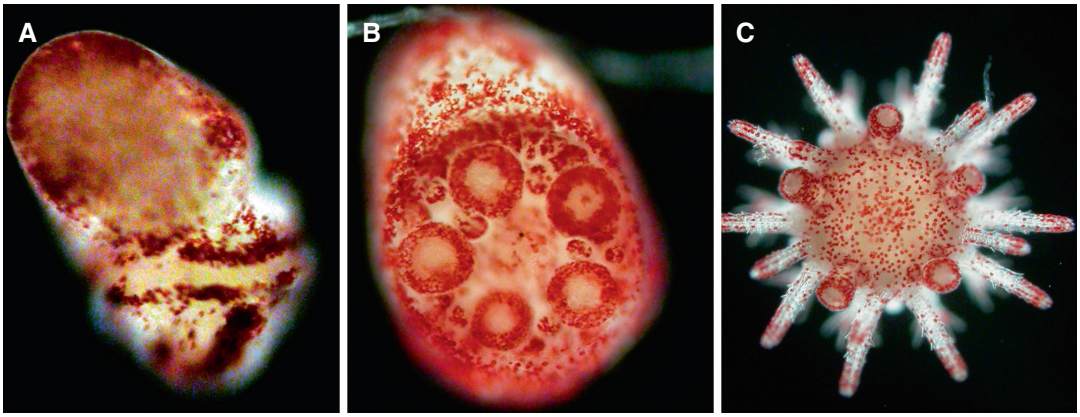


Fig. 1.7 Developmental stages of *Heliocidaris erythrogramma*, a species with nonfeeding larvae. (**A**) Seventy-two-hour-old reduced pluteus. (**B**) Ninety-six-hour-old metamorphosing larva. (**C**) Seven-day-old juvenile. Length of larvae in (**A**) and (**B**) is 400 μm; diameter of juvenile in (**C**) is 500 μm. (courtesy of Paula Cisternas)

purpurescens completely lack any pluteal features (Morris 1995), while *Heliocidaris erythrogramma* has a vestigial pluteal arm skeleton; here, band segments are interpreted as expressions of epaulets (specialized ciliated swimming structures) rather than the feeding ciliated band of the pluteus (Emlet 1995). *Phylacanthus imperialis* has a yolky nonfeeding pluteus with a reduced number of arms (Olson et al. 1993).

Many studies in the last decade have been performed to elucidate the molecular basis of territory specification in the sea urchin embryo (see diagram of basic tenets in Fig. 1.6). These studies have demonstrated the interplay between signaling events and gene regulatory interactions which underlie the specification and patterning of the sea urchin larval nervous system in species with feeding larvae (for review, see Angerer et al. 2011); the specification of the embryo left-right axis (Molina et al. 2013); the specification, formation, and patterning of the larval skeleton (for review, see McIntyre et al. 2014); and, possibly at an even deeper level of detail, the specification of the endomesoderm and its derived structures. Because the regulation of morphogenesis of the gastrointestinal system is a key innovation in metazoan evolution, endoderm specification is described in detail here, both for sea urchin and for other echinoderm embryos.

Endodermal and mesodermal cell types often share a common cell lineage in bilaterian animals, forming the so-called endomesoderm, and sea urchins are no exception. The endomesoderm precursor cells initially have the potential to develop either as mesodermal or endodermal cells until their cell fates become spatially segregated by the exclusive activation of different specification programs activated in different subsets of them.

The endomesoderm lineages emerge from the vegetal plate and form four distinct embryonic lineages: small micromeres, skeletogenic mesoderm, non-skeletogenic mesoderm, endoderm. The fourth cleavage, as already mentioned (see also Fig. 1.6A), is uneven and results in small and large tiers of cells, the micromeres and macromeres, respectively. At fifth cleavage, the micromeres divide further, giving rise to small and large micromeres. The small micromeres, which reside at the polar center of the vegetal plate where they will divide only once more during the blastula stage, remain as "set aside cells" at the tip of the archenteron during gastrulation. At a later larval stage, these cells move into the coelomic pouches, where they seem to contribute to the formation of the adult rudiment (Cameron and Davidson 1991; Juliano et al. 2010). The sister cells of the small micromeres, the large micromeres or skeletogenic mesenchyme cells, give rise to the skeletogenic mesoderm which will eventually form the skeleton of the pluteus larva.

The macromere descendants will give rise to non-skeletogenic mesoderm, endoderm, and

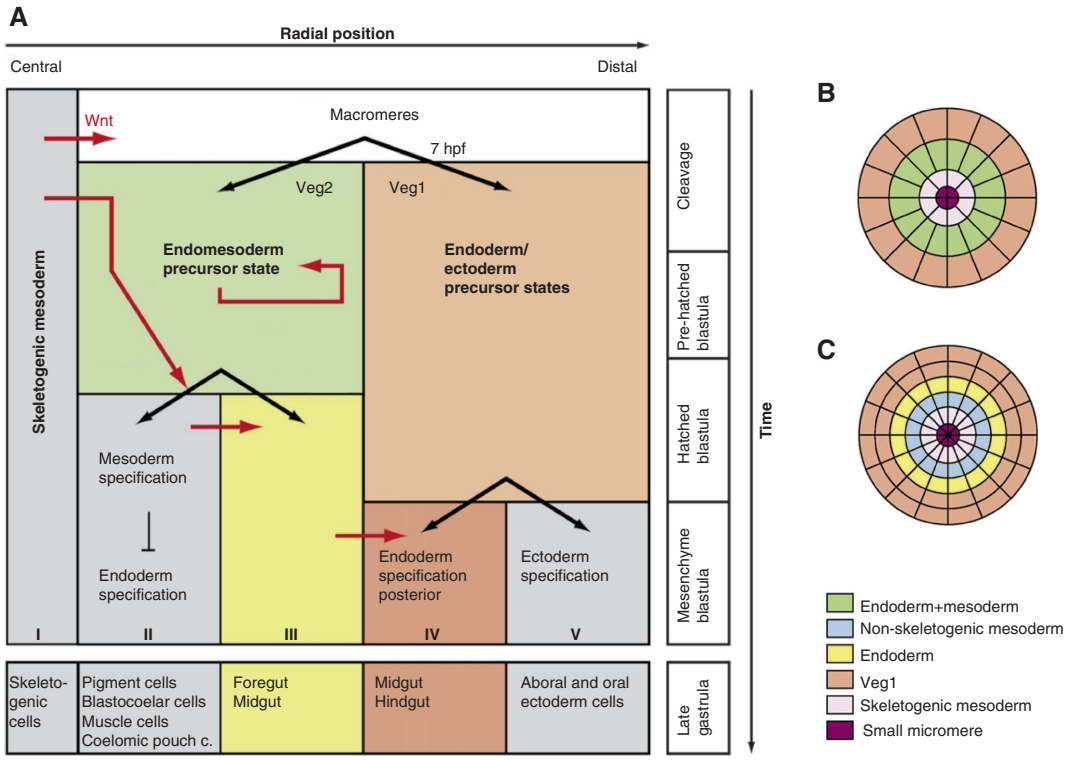


Fig. 1.8 Sea urchin endomesoderm specification. (**A**) Diagram showing the flow of information during the process of endoderm specification. Different embryonic territories are color-coded. Signaling processes occurring between different territories are marked with *arrows*. Next to the *arrows*, the temporal frames in which these interactions happen are given. The horizontal axis represents the spatial organization of the different territories, from central (*left side*) to distal domains (*right side*). The temporal arrangement of embryonic stages is represented along the vertical axis. (**B**, **C**) Schematic representation of embryonic domains seen from a vegetal view. Different colors label rings of cells with similar embryonic fates. (**B**) Territorial fates of cells at 7th cleavage. (**C**) Cellular fates at 8th cleavage. The color codes are indicated in the *bottom right* legend (Adapted and modified from Peter and Davidson (2010))

some ectodermal cells, with very complex molecular events driving the specification of each of these germ layers. The first segregation event leads to the veg1 and veg2 lineages at the sixth cleavage stage. The veg2 layer of cells will give rise to the non-skeletogenic mesoderm and the endoderm, whereas from the veg1 parts of the endoderm and the ectoderm will be formed (Fig. 1.8A). When they are born, the circular eight-cell veg2 tier abuts the polar micromere-derived cells and the eight-cell veg1 tier overlies the veg2 tier. In these embryos the veg2 lineage consists of two concentric rings of cells, the inner ring destined to become mesoderm and the outer ring destined to become oral endoderm.

At the blastula stage, the cells of the four lineages which form four concentric domains within the vegetal plate can be distinguished. At the center are the small micromere descendants, surrounding them are the skeletogenic cells, and abutting them are the veg2 and, more peripherally, the veg1 rings of cells (Davidson et al. 2002; Peter and Davidson 2010). The tier of cells closer to the micromere descendants becomes the non-skeletogenic mesoderm and will, eventually, give rise to three distinct mesodermal lineages: pigment cells, blastocoelar cells, and muscle cells. These mesodermal cells are also called secondary mesenchyme cells (SMCs) (Fig. 1.8B, C; Cameron et al. 1991).

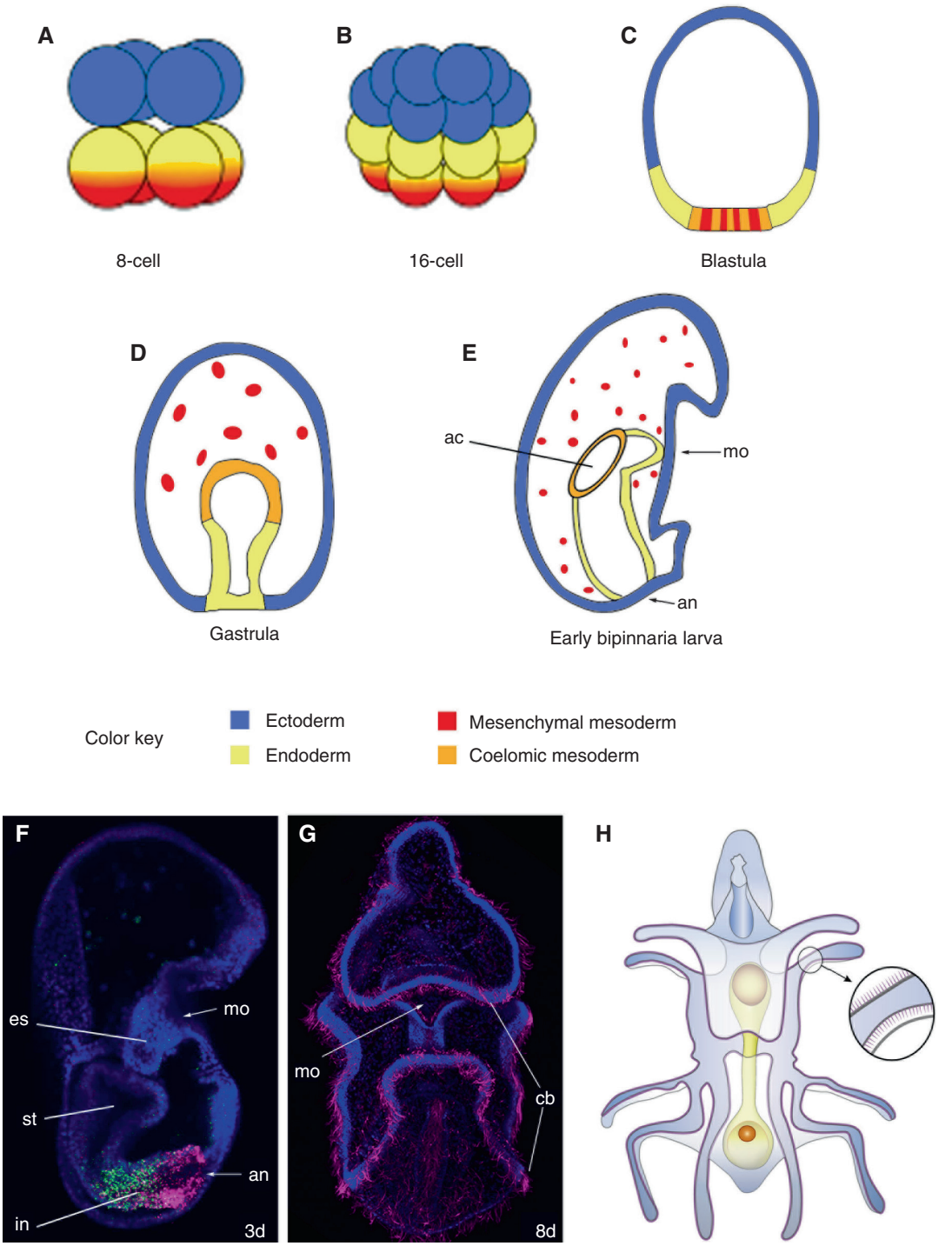
The next event, at around 20 h post fertilization (at 15 °C) in *S. purpuratus*, is the ingression of the 16 descendants of the large micromeres into the blastocoel, which will fuse later on and form the skeleton. These cells are called primary mesenchyme cells (PMCs), because they are the first ones to ingress into the blastocoel (Burke et al. 1991). As the PMCs ingress, the SMC precursors, which encircle the PMCs, move to occupy the space vacated by these ingressing cells. The movements displace the SMC precursors towards the center of the vegetal plate (Fig. 1.6C). During gastrulation, together with the small micromeres, these cells will be part of the tip of the archenteron (Fig. 1.6D).

According to a detailed fate map study, performed in the species *Lytechinus variegatus* (Ruffins and Ettensohn 1996) and in part confirmed by gene expression studies in both *Lytechinus variegatus* and *Strongylocentrotus purpuratus*, the SMC precursors are partially segregated and differentially distributed in the vegetal plate of the mesenchyme blastula stage embryo. This suggests that developmental decisions regarding the specification of SMC precursors are being made during the interval between the stages of the hatched blastula and the late mesenchyme blastula. The pigment cell and the blastocoelar cell precursors show an asymmetric distribution within the vegetal plate, with the first to be found usually facing the future aboral ectoderm and the second facing the future oral ectoderm. When it comes to the muscle cell progenitors, a less clear distribution is observed, mostly due to the failure of scoring myoblasts independently from nearby foregut cells (Ruffins and Ettensohn 1996). Recent studies suggest that myoblast precursors are indeed specified later on, soon after having undergone epithelial mesenchyme transition at the very early gastrula stage (Andrikou et al. 2013).

Development of Asteroidea (Sea Stars)

Although sea stars are not as extensively studied as the sea urchins, the embryo of the sea star *Patiria miniata*, a species with a feeding larva, has been investigated over the last decade for analysis of gene expression during embryogenesis (Hinman et al. 2003a; Hinman and Davidson 2007; McCauley et al. 2010). Given that its genome is currently being sequenced, *Patiria* may now be considered a sea star developmental model organism. Here, a review on the development of *Patiria miniata* is provided based on Hinman et al. (2003b). Figure 1.9 shows the developmental progression of *P. miniata* from oocyte to bipinnaria and brachiolaria larvae. As is typical for echinoderms, cleavage is equal (although in *P. miniata* it is not strictly stereotypic) and the 16-cell embryo generally consists of equal-sized blastomeres (Fig. 1.9B). Also like sea urchins, sea star early

Fig. 1.9 Development of Asteroidea. (**A**, **B**) Early cleavage stages, animal pole towards the *top*. As in sea urchins, vegetal blastomeres give rise to endomesoderm (*yellow* and *red*), while the animal blastomeres become ectoderm (*blue*). Cleavage is equal in sea stars, as typical of most echinoderms, and micromeres are not formed. (**C**) Blastula, lateral view. A thickening at the vegetal pole, the vegetal plate, is noticeable. Unlike sea urchins, no mesoderm has ingressed before gastrulation starts. (**D**) Mid-gastrula, mesenchyme cells (*red*) migrate from the top of the archenteron. (**E**) Lateral view of an early bipinnaria larva; oral surface is to the *right*. The archenteron curves towards the involuting ectoderm of the oral plate, the anterior coeloms (*orange*) extend vegetally. (**F**) *Patiria miniata* bipinnaria larva, lateral view after 3 days of development. Regionalization of the digestive tube is evident from both morphology and ParaHox gene expression patterns: *PmLox* expression (*green*) marks the anterior part and *PmCdx* (*magenta*) the posterior part of the intestine. Length of larva = 300 μm. (**G**) Fluorescence immunostaining with an antibody against acetylated tubulin (*magenta)* which reveals the distribution of cilia in the 8-day-old bipinnaria larva, oral view. Length of larva = 400 μm. (**H**) Schematic depiction of a brachiolaria larva (Courtesy of Santiago Valero-Medranda), highlighting the digestive system (*yellow*/*orange*) and ciliary bands (*purple*; see inset). Abbreviations: *ac* anterior coelom, *an* anus, *cb* ciliary band, *in* intestine, *mo* mouth, *es* esophagus, *st* stomach


A
B
C
8-cell
16-cell
Blastula
D
E
ac
mo
an
Gastrula
Early bipinnaria larva
Color key
Ectoderm
Endoderm
Mesenchymal mesoderm
Coelomic mesoderm
F
es
mo
st
an
in
3d
G
mo
cb
8d
H

embryos can be seen as divided into an1, an2, veg1, and veg2 cell lineages. Because cleavage is equal, sea star embryos do not form micromeres. In the fully formed blastula, the ectoderm is covered with cilia and the embryos start to rotate within the fertilization envelope, about 1 h before hatching, which is at around 26 h at 15 °C. Prior to gastrulation, a thickened vegetal plate appears (Fig. 1.9C). Similar to sea urchins, this is the region from which all endodermal- and mesodermal-derived structures will develop. Remarkably, gene orthologs of many of the regulatory genes expressed in the sea urchin endomesodermal territories are also expressed in the presumptive endoderm and mesoderm of sea stars (Hinman et al. 2003a; Hinman and Davidson 2007; McCauley et al. 2010). However, because sea star larvae do not form a skeleton, the genes that control skeletogenic mesoderm formation in sea urchin larvae are found to be absent, or expressed very differently, in sea star larvae. See Table 1.2 for details.

Gastrulation occurs via sequential invagination from the inner- to outermost cells in the vegetal plate. Cell labeling experiments in *Asterina pectinifera* indicate that the early part of the invaginating archenteron, which derives from the veg2 lineage, contributes to the formation of the rounded top of the archenteron in mid to late gastrulae and also to the anterior coeloms plus the esophagus of the bipinnaria larva. Later invaginating veg2 cells will contribute to the formation of the stomach, while the hindgut derives, in part, from the still later invagination of the veg1 cells (Kuraishi and Osanai 1992). Mesenchyme cells migrate from the top of the archenteron during gastrulation, but unlike in sea urchins, many presumptive mesoderm cells remain associated with the archenteron for a longer period (Fig. 1.9D), developing later on into prominent anterior coeloms on either side of the bipinnaria larval esophagus (Fig. 1.9E; Byrne and Barker 1991). While several blastocoelar cells are generated during gastrulation and remain as scattered cells into the blastocoel at later stages, pigment cells do not form in sea star embryos, which thus develop into completely transparent larvae.

In the late bipinnaria larva, the mouth is fully formed and the gut tube is clearly divided into esophagus, stomach, and intestine, which opens posteriorly through the anus (Fig. 1.9F). In *Patiria miniata*, similarly to *Strongylocentrotus purpuratus*, patterning of the gut tube is evident before any morphological signs are evident: for instance, two ParaHox genes, *PmLox* and *PmCdx,* are expressed in staggered domains of the early intestine, with only partial overlap (Annunziata et al. 2013; see Table 1.2 for comparison). By the late bipinnaria larval stage, ciliated cells distributed over the ectoderm at earlier stages have coalesced into two distinct bands, one that loops above the mouth and one below it, the latter extending from the ventral surface to the anterior, dorsal margins of the ectoderm. As in all echinoderm larvae, cilia can be visualized using an antibody against acetylated α-tubulin (Fig. 1.9G). It is interesting to note that similarly to echinopluteus larvae, bipinnaria larvae have an apical concentration of serotonergic neurons (Byrne et al. 2007). Neurons lie beneath the two loops of the ciliated epithelium and innervate the bands (Nakajima et al. 2004). These neurons coordinate the action of the cilia to enable the larvae to swim and feed in response to the environmental cues provided in the water column. Recently, the specification process and the gene regulatory network that describes the distribution of ciliary band-associated neurons in the sea star bipinnaria larva have been described (Yankura et al. 2013). This process involves genes such as *soxB1*, *soxC*, *nk2.1*, and *six3*, as well as the involvement of Delta-Notch signaling, which can be regarded as common features of nephrozoan neurogenesis (Burke et al. 2014; see also Table 1.2).

Taken together, both asteroid and echinoid feeding larvae form morphologically similar digestive tracts. While endomesoderm is derived from the vegetal pole of both sea star and sea urchin embryos, the formation of mesoderm differs remarkably in these two echinoderm representatives: sea urchins have at least two mesodermal cell types, pigment cells and micromere-derived skeletogenic mesoderm, which are absent in the larval sea star. However, the major difference between sea star and sea urchin feeding larvae is that the latter produces

Table 1.2 Main domains of expression of genes in echinoderm development based on data from in situ hybridization with a focus on transcription factors involved in axial patterning, endomesoderm specification, and neurogenesis

Gene	Class				
	Echinoidea	Asteroidea	Holothuroidea	Ophiuroidea	Crinoidea
hox3	Oral mesoderm of juvenile: dental sacs, spines and beneath epineural folds (Arenas-Mena et al. 1998; Morris and Byrne 2005)	N.A.	N.A.	N.A.	N.A.
hox4	N.P.	Anterior coelom, right and left coelom, hydrocoel; Juvenile coelomic epithelium (Cisternas and Byrne 2009)	N.A.	N.A.	N.A.
hox5	Juvenile rudiment epithelium of epineural folds, primary podia (Morris and Byrne 2005; 2014)	N.A.	N.A.	N.A.	Endomesoderm of auricularia larva (Hara et al. 2006)
hox7	Somatocoel and epineural canal of presumptive juvenile (Peterson et al. 2000a)	N.A.	N.A.	N.A.	Endomesoderm of auricularia larva (Hara et al. 2006)
hox8, hox9/10	Somatocoel of presumptive juvenile (Arenas-Mena et al. 2000)	N.A.	N.A.	N.A.	Endomesoderm of auricularia larva (Hara et al. 2006)
hox11/13a	Somatocoel of presumptive juvenile (Arenas-Mena et al. 2000); Vestibule roof epithelium (Morris and Byrne 2005)	N.A.	N.A.	N.A.	N.A.
hox11/13b	Most posterior embryonic endoderm (Arenas-Mena et al. 2006); anterior endoderm and mesoderm of late larva and somatocoel, anus, and spines of presumptive juvenile (Arenas-Mena et al. 2000)	Most posterior embryonic endoderm (Hinman et al. 2003a)	N.A.	N.A.	N.A.

(continued)

Table 1.2 (continued)

Gene	Class				
	Echinoidea	Asteroidea	Holothuroidea	Ophiuroidea	Crinoidea
gsx	Embryonic ectoderm (scattered cells) (Arnone et al. 2006)	Not expressed during embryonic development (Annunziata et al. 2013)	N.A.	N.A.	N.A.
xlox	Posterior embryonic endoderm (Arnone et al. 2006)	Posterior embryonic endoderm (Annunziata et al. 2013)	N.A.	N.A.	N.A.
cdx	Most posterior embryonic endoderm (Arnone et al. 2006)	Most posterior embryonic endoderm (Annunziata et al. 2013)	N.A.	N.A.	N.A.
otx	Embryonic endoderm and ciliary cells(Gan et al. 1995; Yuh et al. 2002; Nielsen et al. 2003); juvenile nerve ring and podia (Morris et al. 2004; Morris and Byrne 2005)	Embryonic and larval endoderm, coeloms, and ciliary cells (Shoguchi et al. 2000; Elia et al. 2010)	Embryonic endoderm and ciliary cells (Hinman et al. 2003b)	Embryonic ectoderm, gut, and ciliary cells (D.V. Dylus and P. Oliveri, 2014 unpublished); blastema and regenerating arm (A. Czarkwiani and P. Oliveri, 2014 unpublished)	Endoderm (enteric sac) of the auricularia larva (Omori et al. 2011)
gata1/2/3 (gatac)	Embryonic non-skeletogenic mesoderm (Davidson et al. 2002; Solek et al. 2013)	Embryonic mesoderm (Hinman et al. 2003a)	Embryonic non-skeletogenic mesoderm (McCauley et al. 2012)	Embryonic non-skeletogenic mesoderm (D.V. Dylus and P. Oliveri, 2014 unpublished); blastema of regenerating arm (Czarkwiani et al. 2013)	N.A.
gata4/5/6 (gatae)	Embryonic endomesoderm (Lee and Davidson 2004)	Embryonic endomesoderm (Hinman et al. 2003a)	Embryonic endomesoderm (McCauley et al. 2012)	Embryonic endoderm and non-skeletogenic mesoderm (D.V Dylus and P. Oliveri, 2014 unpublished)	N.A.
foxa	Embryonic endoderm (David et al. 1999; Oliveri et al. 2006)	Embryonic endoderm (Hinman et al. 2003a)	Embryonic endoderm (McCauley et al. 2012)	Embryonic endoderm (D.V Dylus and P. Oliveri, 2014 unpublished)	N.A.

tbr	Embryonic skeletogenic mesoderm (Croce et al. 2001)	Embryonic mesoderm (Hinman et al. 2003a)	Embryonic non-skeletogenic mesoderm (McCauley et al. 2012)	Embryonic endomesoderm (Dylus and Oliveri, unpublished)	N.A.
ets1/2	Embryonic mesoderm (Kurokawa et al. 1999; Rizzo et al. 2006)	Embryonic mesoderm (Hinman et al. 2003a)	Embryonic mesoderm (McCauley et al. 2012)	Embryonic endomesoderm (Dylus and Oliveri, unpublished); blastema of regenerating arm (Czarkwiani et al. 2013)	N.A.
pax6	Embryonic coelomic mesoderm (Yankura et al. 2010); Juvenile base of podia (Ullrich-Luter et al. 2011)	Embryonic apical ectoderm and coelomic mesoderm (Yankura et al. 2010)	N.A.	N.A.	Mesoderm (posterior axiocoel) of the auricularia larva (Omori et al. 2011)
six3	Embryonic animal pole ectoderm (Poustka et al. 2007; Wei et al. 2009)	Embryonic apical ectoderm (Yankura et al. 2010)	N.A.	N.A.	Mesoderm (anterior axiocoel) of the auricularia larva (Omori et al. 2011)
soxC	Embryonic animal pole and oral neurogenic ectoderm (Howard-Ashby et al. 2006; Poustka et al. 2007)	Embryonic neurogenic ectoderm (Yankura et al. 2013)	N.A.	N.A.	N.A.
nk2.1	Embryonic animal pole ectoderm (Takacs et al. 2004)	Embryonic animal pole ectoderm (Yankura et al. 2013)	N.A.	N.A.	N.A.
eng	Embryonic mesoderm, ciliary band; juvenile podia (Nielsen et al. 2003)	Larval coeloms, hydrocoel; juvenile nerve ring, radial nerve chord, and podia (Byrne et al. 2005)	N.A.	N.A.	N.A.

N.A. data not available, *N.P.* gene not present

a skeleton during embryogenesis, on which larval shape depends, whereas asteroid embryos and larvae entirely lack this structure.

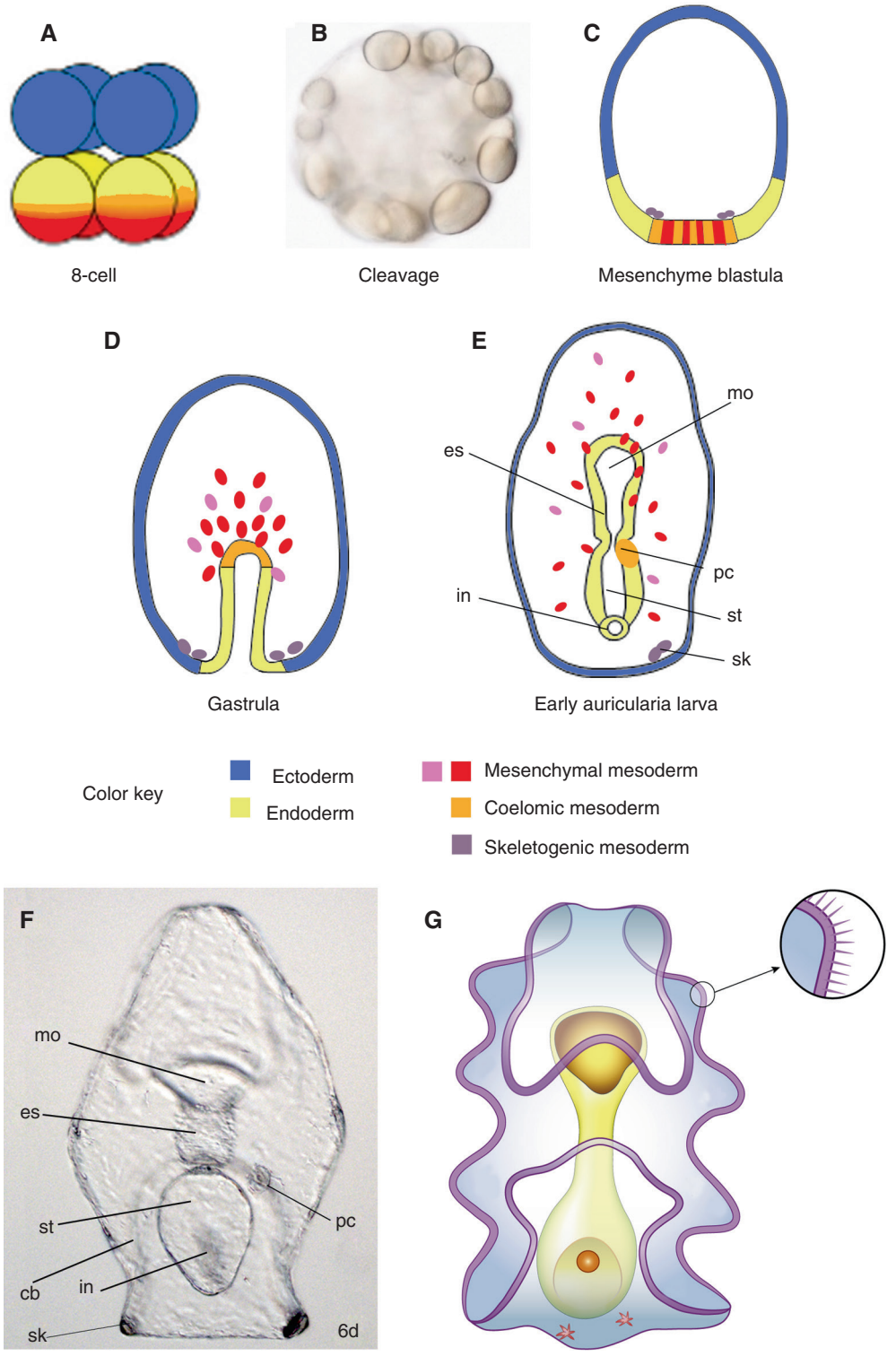
A vast diversity of asteroids develop through nonfeeding larvae. Details of embryology, larval development and larval plus juvenile nervous system formation in these asteroids are also available for several species (Byrne 1996, McEdward et al. 2002 and Elia et al. 2009). Asteroids with nonfeeding larvae completely lack the bipinnaria stage and are generally divided into the barrel-shaped larvae as seen in *Astropecten* species or the yolky brachiolaria larvae of some asterinid species (e.g., *Meridiastra calcar*) (Byrne 1996; McEdward et al. 2002). Some of the strangest larvae are the benthic brachiolaria of *Leptasterias hexactis* and *Parvulastra exigua* where the brachiolarial arms appear as three feet-like structures that maintain a tenacious attachment to the substratum (Byrne 1996; McEdward et al. 2002).

Development of Holothuroidea (Sea Cucumbers)

Several sea cucumber species have been the subject of embryological studies (reviewed in Hyman 1955; Smiley et al. 1991), in particular species of the Stichopodidae (e.g., *Stichopus*, *Apostichopus*, and *Parastichopus* species) (Holland 1981; Smiley 1986; Shoguchi et al. 2000). A comprehensive gene expression analysis during development in *Parastichopus parvimensis* is available (McCauley et al. 2012), rendering this species a reference model for the development of holothurians. Thus, development of this species is reviewed here.

Cleavage of *Parastichopus parvimensis* is equal and little cell-cell adhesion is seen between the blastomeres (Fig. 1.10A, B). Divisions are not synchronous. Blastulae are formed by 16 h (at 15 °C) after fertilization and hatch from the fertilization envelope at around 26 h. Prior to gastrulation, the embryos elongate along the animal-vegetal axis, with a thickening observed at the vegetal pole, which is termed the vegetal plate. The shape of the sea cucumber blastula closely resembles the one in sea stars, but unlike the latter, mesenchyme cells ingress from the vegetal plate before invagination of the archenteron occurs (Fig. 1.10C). During gastrulation, while most mesenchyme remains associated with the tip of the archenteron, a few cells migrate to take up positions near the blastopore. At the mid-gastrula stage, around 48 h of development, the gut has elongated, the mesenchyme has begun to migrate, and additional mesenchymal cells delaminate from the tip of the archenteron (Fig. 1.10D). At this stage, three distinct populations of mesenchyme cells can be identified by their specific regulatory signatures (McCauley et al. 2012): a skeletogenic mesenchyme cell type, which, as in sea urchins, uniquely expresses the gene *alx1* (a gene expressed in all mesodermal precursors of the sea star embryo) and two types of blastocoel cells which differ from each other by the expression of *gcm*, a gene which is exclusively expressed in pigmented cell precursors in the sea urchin embryo.

Fig. 1.10 Sea cucumber development. (**A**) As in sea urchins and sea stars, the vegetal blastomeres give rise to endomesoderm (*yellow* and *red*) in holothurians, while the animal blastomeres are destined to become ectoderm (*blue*). (**B**) Cleavage is equal in *Parastichopus parvimensis* and little cell-cell adhesion is seen between blastomeres. (**C**) Mesenchyme cells ingress into the blastocoel before gastrulation begins. In the vegetal plate of the mesenchyme blastula, presumptive endoderm (*yellow*) and mesoderm (*red* and *orange*) territories are already segregated. (**D**) At the mid-gastrula stage (around 48 h post fertilization at 15 °C in *P. parvimensis*), the mesenchyme has begun to migrate, with additional mesenchymal cells ingressing from the archenteron. Different colors indicate the different mesodermal cell types: skeletogenic cells (*purple*) and blastocoelar cells, expressing (*red*) or not (*pink*), the *gcm* gene. Early 3-day (**E**) and 6-day (**F**, *P. parvimensis,* courtesy of Veronica Hinman) auricularia larvae display regionalized tripartite digestive tracts. Length of larva in (**F**) = 400 μm. A posterior coelom is evident near to the *left* side of the midgut, but no obvious anterior coeloms are detected. A small skeletal spicule is evident in the posterior part of the larva. (**G**) Schematic representation of an apodid auricularia larva (Courtesy of Santiago Valero-Medranda), highlighting the digestive system (*yellow*/*orange*), ossicles (*brown*), and ciliary band (*purple*; see inset for details). Abbreviations: *cb* ciliary band, *in* intestine, *mo* mouth, *es* esophagus, *pc* posterior coelom, *sk* skeletal spicule, *st* stomach


A
B
C
8-cell
Cleavage
Mesenchyme blastula
D
E
mo
es
pc
in
st
sk
Gastrula
Early auricularia larva
Color key
Ectoderm
Endoderm
Mesenchymal mesoderm
Coelomic mesoderm
Skeletogenic mesoderm
F
mo
es
st
pc
cb
in
sk
6d
G

By 72 h of development at 15 °C, the mouth has formed and the embryo reaches the early auricularia larval stage (Fig. 1.10E). The archenteron has differentiated into morphologically distinct fore-, mid-, and hindgut regions which later give rise to the esophagus, stomach, and intestine, respectively, as seen in the 6-day auricularia larva (Fig. 1.10F). Starting at the early auricularia larva stage, presumptive muscle cells can be seen associated with the foregut and a thickened ciliary band is evident in the oral hood, looping above the anus. Also visible at these stages are the coelomic sacs: in particular and from the early auricularia larva stage, a posterior coelom is evident at the left side of the midgut. Auricularia larvae also display a hydroporic canal connecting the left coelomic sac with the dorsal surface of the larva, where the hydropore opens. In some species, such as *Stichopus tremulus*, the coelomic (distal) part of the archenteron sends tubular projections towards the dorsal surface to form the hydroporic canal as early as in the gastrula stage (Hyman 1955). The shape of the larval skeleton varies in auriculariae including the single posterior spicule such as seen in *Stichopus* and *Holothuria* and the wheel-shaped ossicles in apodid larvae (Sewell and McEuen 2002; Ramafofia et al. 2003; McCauley et al. 2012).

The auricularia larva further develops by incorporation of an elaborate ciliated band that extends around the body and projecting lobes (Fig. 1.10G). The lobes formed by the band can become very numerous, although they never develop into distinct larval arms as in the later bipinnaria larvae of asteroids or the plutei of echinoids and ophiuroids (compare larvae in Fig. 1.4). The auricularia superficially resembles the bipinnaria of asteroids, but the ciliary band in the former is organized as a continuous loop over the body, with a structure very similar to that in the tornaria larva of hemichordates (see Chapter 2), while in the bipinnaria of asteroids, it forms two unconnected loops, one smaller than the other (compare with Fig. 1.9G). The auricularia larva also displays in its anterior-most region an apical organ which contains two groups of serotonergic neurons associated with the right and left portions of the anterior ciliary band (Byrne et al. 2007). These neurons are flask-shaped and give rise to a serotonin-positive process.

A vast diversity of holothuroids develops through nonfeeding larvae. Details of their embryology and larval development are available for several species (reviewed in Smiley et al. 1991; Sewell and McEuen 2002). All dendrochirotid sea cucumbers have a barrel-shaped doliolaria larva with rings of cilia (Sewell and McEuen 2002).

Development of Ophiuroidea (Brittle Stars)

In ophiuroids with small eggs, the embryos develop into a pluteus larva (the ophiopluteus) that superficially resembles the echinopluteus larva of echinoids. Several other morphological aspects of these embryos, such as the early ingression of mesenchyme before gastrulation and the prismatic shape of the late gastrula displaying two lateral clusters of mesenchymal cells producing triradiate spicules, are similar to sea urchin embryos. However, cladistic analyses indicate that the pluteus larva may have arisen independently in ophiuroids and echinoids through a process of convergent evolution (Littlewood et al. 1997; Smith 1997). In fact, a closer look at the development of brittle star embryos suggests that there are probably more differences than similarities between these two echinoderm clades.

Artificial fertilization generally fails in ophiuroids and hence material for studying their early development must be obtained from natural spawning. This difficulty, together with the opacity of the embryos, explains the few available accounts of development in ophiuroids and why so little is known about the developmental processes and the mechanisms that underlie their regional specification. A recent study using fluorescent dyes and confocal imaging examined in great detail the early embryogenesis and cell fate specification in *Ophiopholis aculeata* (Primus 2005). This species is therefore used herein as an example of brittle star development. However, the following description also takes into account some general features of brittle star embryos (see Hyman 1955; Hendler 1991).

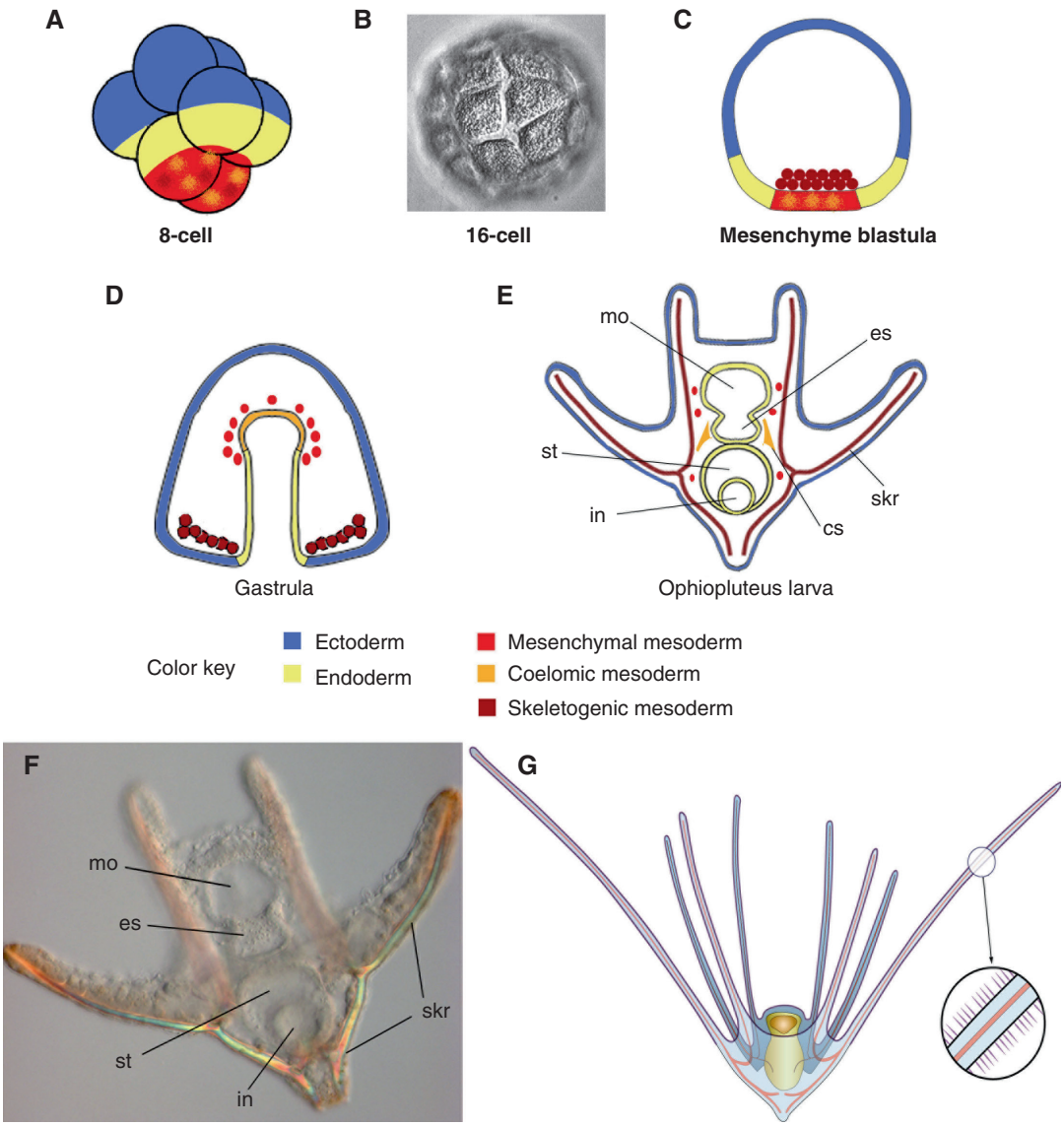


Fig. 1.11 Brittle star development. (**A**) Eight-cell stage showing unusual germ layer-specific contributions of each lineage (see *color code*). Vegetal pole is down. (**B**) *Ophiopholis aculeata* 16-cell stage showing the close association between blastomeres (Primus 2005). (**C**) Mesenchyme blastula stage showing early ingression of skeletogenic mesenchyme cells prior to gastrulation. (**D**) Late gastrula stage. Two lateral clusters of skeletogenic mesenchyme cells are present at the site where triradiate spicules will form. (**E, F**) Ophiopluteus larva showing tripartite gut and elongated arms supported by skeletal rods. The larva in (**F**) is a 4-day-old *Amphiura filiformis* larva (Courtesy of David Dylus and Paola Oliveri). Length of larva = 300 µm. (**G**) Schematic representation of the eight-arm ophiopluteus larva (Courtesy of Santiago Valero-Medranda) highlighting internal skeleton (*brown*) and digestive system (*yellow/orange*). The inset shows a detail of the ciliary band (*purple*). Abbreviations: *cs* coelomic sac, *es* esophagus, *in* intestine, *mo* mouth, *skr* skeletal rod, *st* stomach

Ophiopholis aculeata oocytes average 100–105 µm in diameter when shed. Polar bodies are produced between 30 and 60 min after spawning at 12 °C. The first three embryonic divisions in *O. aculeata* are equal (Fig. 1.11A). A fate map constructed using microinjected lineage tracers indicates that there is a major segregation of ectodermal from endomesodermal fates at first

cleavage, thus highlighting a first major difference between this embryo (and probably in general ophiuroid embryos) and the other echinoderms. Cleavage is equal in ophiuroids (Fig. 1.11B). Cell divisions are synchronous. The cell lineage of the *O. aculeata* embryo has been determined through the 64-cell stage. Cleavage in *O. aculeata* also differs from that of sea urchins with regard to the spatial arrangement of blastomeres in the early cleavage stages. Rather than being organized in orderly tiers, as is the case in sea urchins, early cleavage-stage embryos are typically arranged in a more compact manner (see close contact between blastomeres in Fig. 1.11B).

The *Ophiopholis aculeata* embryo forms a hollow blastula, the vegetal end of which flattens to form a vegetal plate where the blastopore will open, which ultimately becomes the anus of the larva. Similarly to sea urchins and sea cucumbers, mesenchyme cells ingress from the vegetal plate into the blastocoel prior to the onset of gastrulation (Fig. 1.11C). Following invagination, mesenchyme cells continue to be produced at the tip of the elongating archenteron, as in all the echinoderm classes examined so far. During gastrulation, numerous mesenchyme cells become localized in two lateral clusters and they will produce triradiate calcareous spicules that ultimately become the larval skeleton (Fig. 1.11D). Similarly to what was done with the sea urchin embryo, in experiments performed as early as at the time of Hans Driesch (Driesch 1892), the distribution of developmental potential in the early *O. aculeata* embryo was also examined by isolating different regions of the early embryo and following these isolates through larval development (Primus 2005). These analyses indicate that endomesodermal potential segregates unequally at the first, second, and third cleavages in *O. aculeata*. As a result, the unusual fate map reported in Fig. 1.11 was constructed; this highlights the differences in early development that exist between *O. aculeata* (and most likely other ophiuroids) and other Echinodermata. It is interesting to note that also the embryos of hemichordates with feeding larvae share the same early segregation of endomesodermal developmental potential observed in other echinoderm classes, thus making the early embryogenesis of ophiuroids an exceptional case within the Ambulacraria.

After gastrulation is completed, the tip of the archenteron differentiates as a thin-walled sac (Fig. 1.11D) from which two coelomic sacs are formed. The gastrula broadens its blastoporal surface and the ventral side becomes flattened. From the ventral surface, and near the animal pole, a stomodeal invagination is produced that, once fused with the archenteron, will establish the usual L-shaped digestive tract that soon will differentiate into esophagus, stomach, and intestine. By the fourth day of development, a pluteus larva with a tripartite gut and arms supported by calcareous spicules has formed (Fig. 1.11E, F). This larval morphology becomes more complex by further elongation of the primary four arms and the development of others, all supported by skeletal rods, and a well-defined ciliated band (Fig. 1.11G). As previously pointed out, ophioplutei superficially resemble echinoplutei (Fig. 1.4); thus, a similar nomenclature is used for their arms. However, the arms are not necessarily homologous between the two groups. Both generally have four pairs of arms, but there appears to be less variation in body form and number of larval arms in ophioplutei and the skeleton is generally less complex. Some additional morphological differences are seen in the larval body. While the bodies of ophioplutei are generally dorsoventrally flattened, those of echinoplutei are often laterally flattened. A striking difference between the ophioplutei and all other echinoderm larvae is that the ophioplutei do not present a clear apical concentration of serotonergic neurons, which here are distributed in two lateral ganglia with few cell bodies located within the ciliary band (Byrne et al. 2007).

A vast diversity of ophiuroids develops through nonfeeding larvae. The details of embryology and larval development in these brittle stars are available for several species (reviewed in Selvakumaraswamy and Byrne 2006). The nonfeeding larvae of ophiuroids are morphologically diverse, ranging from species with nonfeeding yolky ophioplutei with a reduced number of arms to vitellaria larvae with patches or rings of cilia (Selvakumaraswamy and Byrne 2006).

Development of Crinoidea (Sea Lilies and Feather Stars)

Crinoidea is the only echinoderm class that does not have any species with a feeding larva. Their early development, therefore, cannot be easily compared with the above descriptions. Crinoids include the feather stars and sea lilies. Feather stars lose their stalk during development, but sea lilies retain it throughout adulthood (Holland 1991).

Development of crinoids has been reported for several species (Holland 1991; Balser 2002; Nakano et al. 2003; Kohtsuka and Nakano 2005). The embryos and larvae of stalked crinoids (sea lilies), which are considered the most basal group of extant echinoderms (Foote 1999; Janies 2001), have been described only recently (Nakano et al. 2003), including several gene expression studies (Hara et al. 2006; Nakano et al. 2009; Omori et al. 2011). Due to the relevance of this group of animals for studies on the origin of the larval and adult body plan of echinoderms and all deuterostomes and because of the availability of these recent molecular studies, they have been chosen here as reference for crinoids development.

The sea lily *Metacrinus rotundus* develops through two successive larval stages: the first is a nonfeeding auricularia stage with ciliary bands similar to those present in the auricularia and bipinnaria larvae of holothurians and asteroids (the dipleurula-type larva of the Ambulacraria); the second is a barrel-shaped doliolaria larva containing circumferential ciliary bands (similar to the earliest larval stage of stalkless crinoids, the doliolaria of holothuroids, and the vitellaria of ophiuroids). Cleavage in *Metacrinus rotundus* is holoblastic, radial, and equal. By the 32-cell stage, a large pore forms in the vegetal area (arrowhead in Fig. 1.12B), possibly equivalent to the pore found at the vegetal side of feather star embryos (Holland 1991). By 24 h (at 15 °C), a gastrula results from invagination at this vegetal pole (Fig. 1.12C). During the next few hours the blastopore closes, while the embryo becomes uniformly ciliated and begins to rotate inside the fertilization envelope. The *M. rotundus* embryo hatches at the late gastrula stage (Fig. 1.12D). Unlike what is observed for the gastrulae of the feather star *Antedon* (reviewed in Hyman 1955), no mesenchymal cells are detected in the blastocoel of *M. rotundus* at the early gastrula stage.

A few hours after closure of the blastopore, a circular constriction in the middle of the archenteron appears, that can now be regarded as a closed sac. Several rearrangements of this archenteral sac occur, which ultimately give rise to three separate sacs: the anterior "axo-hydrocoel" (Hara et al. 2006), which is the first one to differentiate; the central "enteric sac"; and the posterior lobe, also called "presumptive somatocoel". At this point, the embryo has reached the early auricularia larval stage (Fig. 1.12E). A few putative mesenchymal cells are observed in the blastocoelar space, which contains the axo-hydrocoel, the middle part, and the posterior lobe. Thirty hours (at 15 °C) after blastopore closing, the presumptive somatocoel separates into left and right somatocoels, and the enteric sac elongates posteriorly, moving into a space between the left and right somatocoels. This larva, after 3 days of development, has reached the auricularia stage (Fig. 1.12F). The overall shape of this larva, possessing an anterior and a posterior ciliated band, is reminiscent of that of the sea cucumber auricularia and the starfish bipinnaria larvae. In fact, although the ventral side of this larva is indented by a vestibular invagination, in the roof of which is a mouth invagination (Fig. 1.12F), this is not connected with the rest of the gut. Similarly to what is observed in other echinoderm larvae, the left side of the axo-hydrocoel establishes communication with the exterior via a hydropore.

The expression patterns of genes known to have important roles in patterning metazoan embryos have been recently analyzed during *Metacrinus rotundus* development. These are the homologs of the Hox genes *hox5*, *hox7*, *hox8*, and *hox9/10* (Hara et al. 2006) as well as *six3*, *pax6*, and *otx* (Omori et al. 2011). All these genes appear to have a role in patterning the larval endomesoderm during early development in stalked crinoids (Fig. 1.12D–F; see Table 1.2 for comparison with other echinoderms).

The *Metacrinus rotundus* auricularia larva has a short life and within a few days undertakes

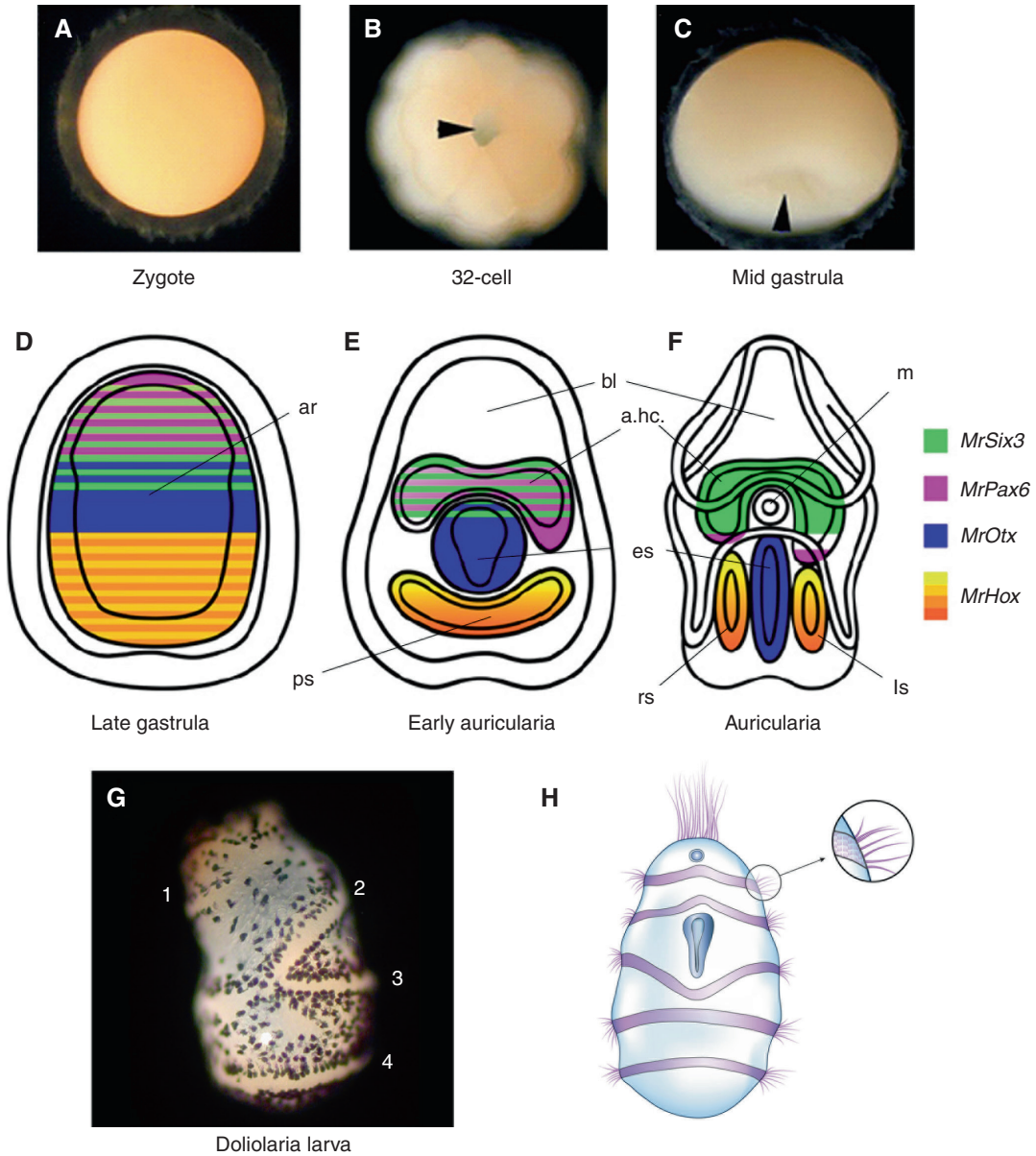


Fig. 1.12 Sea lily (*Metacrinus rotundus*, **A**–**G**) and feather star (*Antedon*, **H**) development. (**A**) Fertilized egg within a rough fertilization envelope. (**B**) Thirty-two-cell embryo with putative vegetal pore (*arrowhead*). (**C**) Approximate side view of a mid-gastrula (27.5 h post fertilization) showing the blastopore (*arrowhead*). (**D–F**) Development and expression of Hox genes (represented as *yellow*, *pale orange*, *orange*, and *dark orange* areas, corresponding to *MrHox5*, *MrHox7*, *MrHox8*, and *MrHox9/10*, respectively), *MrSix3* (*green*), *MrPax6* (*pink*), and *MrOtx* (*blue*) in the endomesoderm of *M. rotundus* from late gastrula to auricularia larva. (**G**) Ten-day *M. rotundus* doliolaria larva (Courtesy of Hiroaki Nakano) showing circumferential ciliary bands (1–4). Length of larva = 500 μm. (**H**) Schematic representation of a feather star (*Antedon*) doliolaria larva (Courtesy of Santiago Valero-Medranda), highlighting adhesive pit (*top blue circle*), vestibule (*blue oval*), and ciliary bands (*purple*; see inset for details). Abbreviations: *a.hc.* axo-hydrocoel, *ar* archenteron, *bl* blastocoel, *es* enteric sac, *ls* left somatocoel, *m* mouth, *ps* presumptive somatocoel, *rs* right somatocoel (Modified and adapted from Nakano et al. (2003) (**A**–**C**) and Omori et al. (2011) (**D**–**F**))

several morphogenetic transformations. The mouth invagination closes, the overall dimensions of the larvae shrink, and the ciliary bands become rearranged as the auricularia transforms into the doliolaria. Some parts of the bands break up, whereas others fuse, eventually forming four circumferential ciliary bands. The 10-day-old larva has reached the typical barrel shape of a doliolaria larva and the doliolaria plus vitellaria of sea cucumbers and brittle stars, respectively (Fig. 1.12G, H), similar to feather stars, (Holland 1991) and the dololaria and vilellaria of sea cucumbers and brittle stars, respectively (Byrne and Selvakumaraswamy 2002; Sewell and McEuen 2002).

Gene Regulatory Networks in Echinoderm Evolution and Development

The circuitry of endomesoderm specification in the sea urchin embryo has been studied in detail and has led to the elaboration of a complex gene regulatory network (GRN) model that displays how endomesoderm development progresses from fertilization until 30 h post fertilization (hpf) at 15 °C (in *Strongylocentrotus purpuratus*), when the tissue has already been segregated into definitive endoderm and mesoderm (Davidson et al. 2002; Ransick and Davidson 2006; Croce and McClay 2010; Peter and Davidson 2010, 2011; Lhomond et al. 2012; Materna and Davidson 2012). This is possibly the best GRN so far described which accounts for a complex developmental process, in space and time, and it is here used as an example of how this functional approach can be applied to gain a better understanding of the development of an entire embryo or parts of it (see Vol. 1, Chapter 2).

The endomesoderm in the sea urchin embryo *Strongylocentrotus purpuratus*, as mentioned above, derives at the sixth cleavage (about 7 hpf) in the vegetal half of the embryo from the veg2 lineage, whereas from the veg1 lineage only the most part of the oral endoderm and ectoderm will form (see also Figs. 1.6 and 1.8). Then, at 18hpf, the veg2 lineage consists of two concentric rings of cells, the inner ring (veg2L) destined to become mesoderm and the outer ring (veg2U) destined to become endoderm. Using a system-wide perturbation analysis approach, Davidson and collaborators have been able to provide a causal explanation for the dynamic process underlying the separation of the regulatory state leading to the different fates of the veg2 and veg1 lineages plus the further partitioning of the veg2 lineage in two distinct domains (rings), with their specific regulatory states. The dynamics of gene interactions happening in time and space within the endomesoderm is reflected in a complex GRN that describes the process in unprecedented detail (http://sugp.caltech.edu/endomes) (Fig. 1.13).

Within this GRN, three molecular components constitute the core machinery of endomesoderm segregation: the Delta/Notch pathway and the transcription factors *Sp-FoxA* and *Sp-Gcm*. The Delta/Notch pathway regulates non-skeletogenic mesoderm (NSM) specification (Sherwood and McClay 1999; Sweet et al. 2002). *Sp-Delta*, the ligand of the pathway, is first expressed in the skeletogenic mesoderm, the derivative of the large micromeres, at around 8–9hpf, where it has been demonstrated that it signals to the neighboring ring of veg2 endomesodermal cells, turning on *Sp-Gcm* transcription (Ransick and Davidson 2006). After the veg2 tier of cells segregates into an inner and outer tier, Sp-Delta signal is only received in the inner tier, adjacent to the skeletogenic cells, becoming the mesoderm precursors. There, Sp-Delta activates *Sp-GataE* (Lee and Davidson 2004) and subsequently the transcription factors *Sp-Prox1, Sp-Ese,* and *Sp-GataC* (and others) in the oral and *Sp-Six1/2* (plus others) in the aboral mesoderm (Materna and Davidson 2012). After ingression of the primary mesenchyme cells (PMC), *Sp-Delta* ceases to be expressed there and turns on in the non-skeletogenic mesoderm (NSM). This second wave of Sp-Delta does not affect the surrounding presumptive endoderm cells, although they are now in direct contact with the Sp-Delta source. On the contrary, it serves to deactivate endodermal genes in the NSM

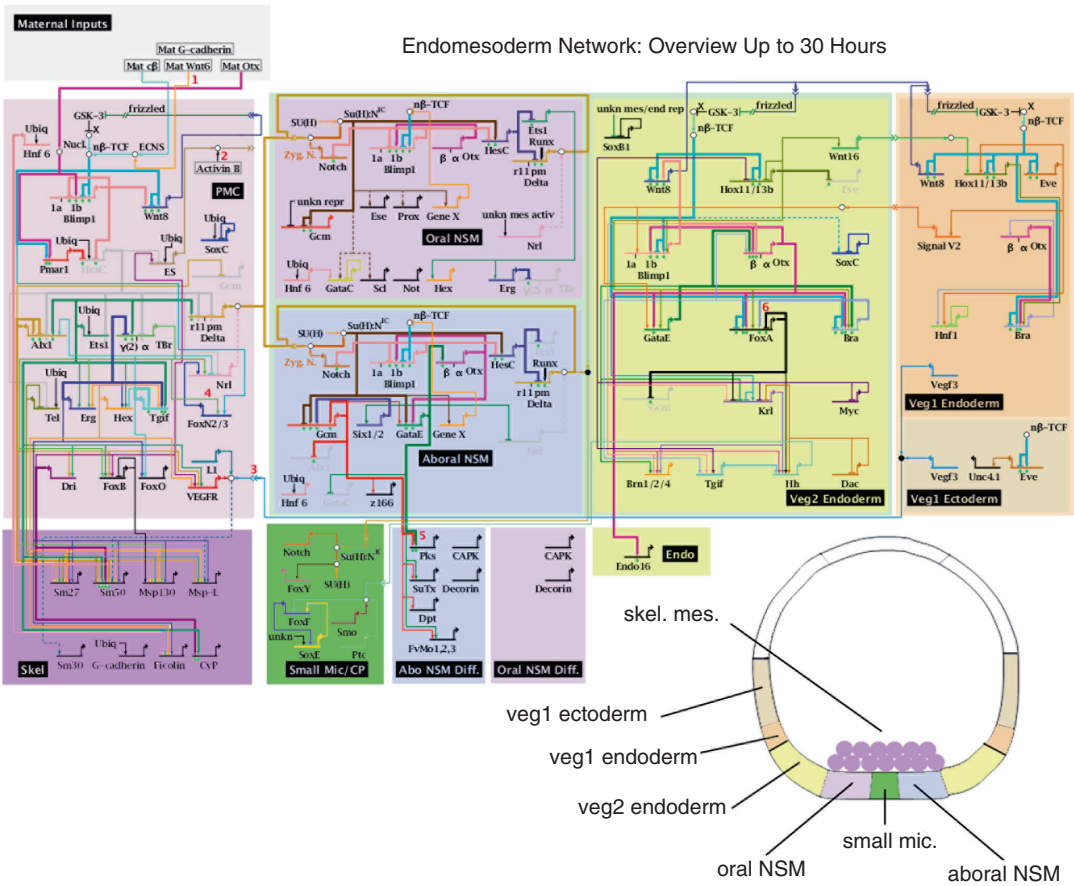


Fig. 1.13 Biotapestry diagram summarizing the gene regulatory interactions occurring during endomesoderm specification in *Strongylocentrotus purpuratus*. The last updated diagram is schematized (11/2011). The diagram is also available on the E. H. Davidson's laboratory webpage (http://sugp.caltech.edu/endomes). Colors label the different embryonic territories. Connecting lines indicate gene interactions

precursors (see below) (Croce and McClay 2010; Peter and Davidson 2011; Materna and Davidson 2012). It is known that mesodermal Sp-Delta provides a "switch" input to small micromeres and particularly to *Sp-FoxY* expression and that this later Sp-Delta signal is required for the specification of late mesoderm derivatives such as coelomic pouches and muscles (Sweet et al. 2002; Materna and Davidson 2012).

One of the first known direct outcomes of the first Delta/Notch pathway is the activation of the transcription factor *Sp-Gcm*. The *Sp-Gcm* promoter contains several Suppressor-of-Hairless (SuH) binding sites that mediate *Sp-Gcm* activation (Ransick and Davidson 2006), and the Notch pathway is known to directly activate *SuH* (Fortini and Artavanis-Tsakonas 1994). *Sp-Gcm* is later required for the development of the pigment cells by becoming involved in a positive intergenic feedback loop with *Sp-Six1/2* (Ransick and Davidson 2006). In the process of progressive segregation of fates within the endomesoderm, other transcription factors are relevant, for instance, *Sp-FoxA*. Reports on this gene indicate that *Sp-FoxA* is expressed in the definitive endoderm, where it promotes endoderm specification (Oliveri et al. 2006).

The endodermal regulatory state is dependent on a Wnt/β-catenin signaling under the spatial control of genes mediated by TCF regulatory sites. This Wnt/TCF system, together with a maternal/early zygotic form of *Sp-Otx*, activates

the endodermal regulatory genes *Sp-Blimp1b, Sp-Eve,* and *Sp-Hox11/13b* (Yuh et al. 2002; Arenas-Mena et al. 2006; Smith et al. 2008a), which will then activate *Sp-Brachyury, Sp-FoxA,* and *Sp-GataE. Sp-Gcm* at that time (12–16hpf) is coexpressed with *Sp-FoxA* in the veg2 tier and until a few hours later (18 h), when the expression domains of the two become exclusive, with *Sp-Gcm* being expressed only in the veg2L and *Sp-FoxA* in the veg2U cells. The repression of the endodermal genes in the mesodermal ring of cells (veg2L) occurs through an elegant regulatory-state exclusion mechanism: the same TCF sites that are used to initiate the endoderm GRN in the veg2 lineage are used again to extinguish it in the mesoderm precursors. The mechanism seems to depend on Delta/Notch signaling, via a MAP kinase pathway (Rottinger et al. 2006).

On the other hand, *Sp-FoxA* represses mesoderm development in the endoderm tier by preventing *Sp-Gcm* expression (Oliveri et al. 2006). All these molecular events driving the initial segregation of fates within the endomesoderm show the complexity of regulatory events needed to ensure the proper development of tissues and cell types within embryos.

The approach to study GRNs in development can obviously be applied to any developmental process in any embryo that allows for high-throughput gene perturbation analyses. Several studies are emerging which use this approach, for instance, and within echinoderms, the GRN which controls gut regionalization in the post-gastrular sea urchin embryo (Annunziata and Arnone 2014), the network responsible for oral and aboral ectoderm differentiation and ecto-endoderm boundary formation (Su et al. 2009; Li et al. 2014), or the network that defines the distribution of ciliary band-associated neurons in the bipinnaria larva of the sea star (Yankura et al. 2013). Other recent examples of the use of the same approaches outside echinoderms are the deciphering of the primary cardiac gene regulatory network in the invertebrate chordate *Ciona intestinalis* (Woznica et al. 2012) or the GRNs that underlie the compartmentalization of the *Ciona* central nervous system (Imai et al. 2009) (see Chapter 4).

GRN studies not only provide explanation of how regulatory states are established in particular cells during development and how these states eventually determine the final morphology of the embryo but also provide a powerful tool, through comparisons of GRN architectures, to reveal the molecular evolution of developmental programs among different organisms (Hinman et al. 2003a; Hinman and Davidson 2007; McCauley et al. 2010).

As previously described, in both sea urchin and sea star embryos, the endomesodermal territories arise from the vegetal plate, where the invagination movements of gastrulation start. Mesoderm progenitors are located in the center of this plate and are the first to invaginate. The outer tiers of cells will progressively invaginate to form the fore-, mid- and hindgut. In this processes, the sea urchin and the starfish are very similar. However, sea urchins have a micromere set of cells that will give rise to the larval skeleton (this territory, missing in sea star, is represented in pink in Fig. 1.14A). When the sea urchin and sea star GRNs for endomesoderm specification are compared, an almost perfectly conserved five-gene network subcircuit, required for endoderm specification, becomes evident (highlighted in red in Fig. 1.14B). However, beyond this so-called "conserved regulatory kernel" (Davidson and Erwin 2006), the GRN structure, upstream and downstream of the kernel, has diverged extensively. These changes are translated into specific phenotypic effects. For example, mesoderm specification occurs quite differently: in sea urchins, mesoderm specification is induced by the Delta-Notch signal (originated from the micromere lineage at the center of the vegetal pole) which impinges on the cis-regulatory apparatus of the *gcm* gene, while in the sea star the Delta-Notch signal has the contrary effect of preventing mesoderm specification. A second type of change observed in GRN structure is mediated by regulatory gene co-option, the redeployment of network regulatory genes in new locations, and/or different times leading to new functions. For instance, instead of the skeletogenic functions executed by the *tbrain* regulator in the micromere lineages of the sea urchin (Oliveri et al. 2002), the *tbrain* gene is required in the sea

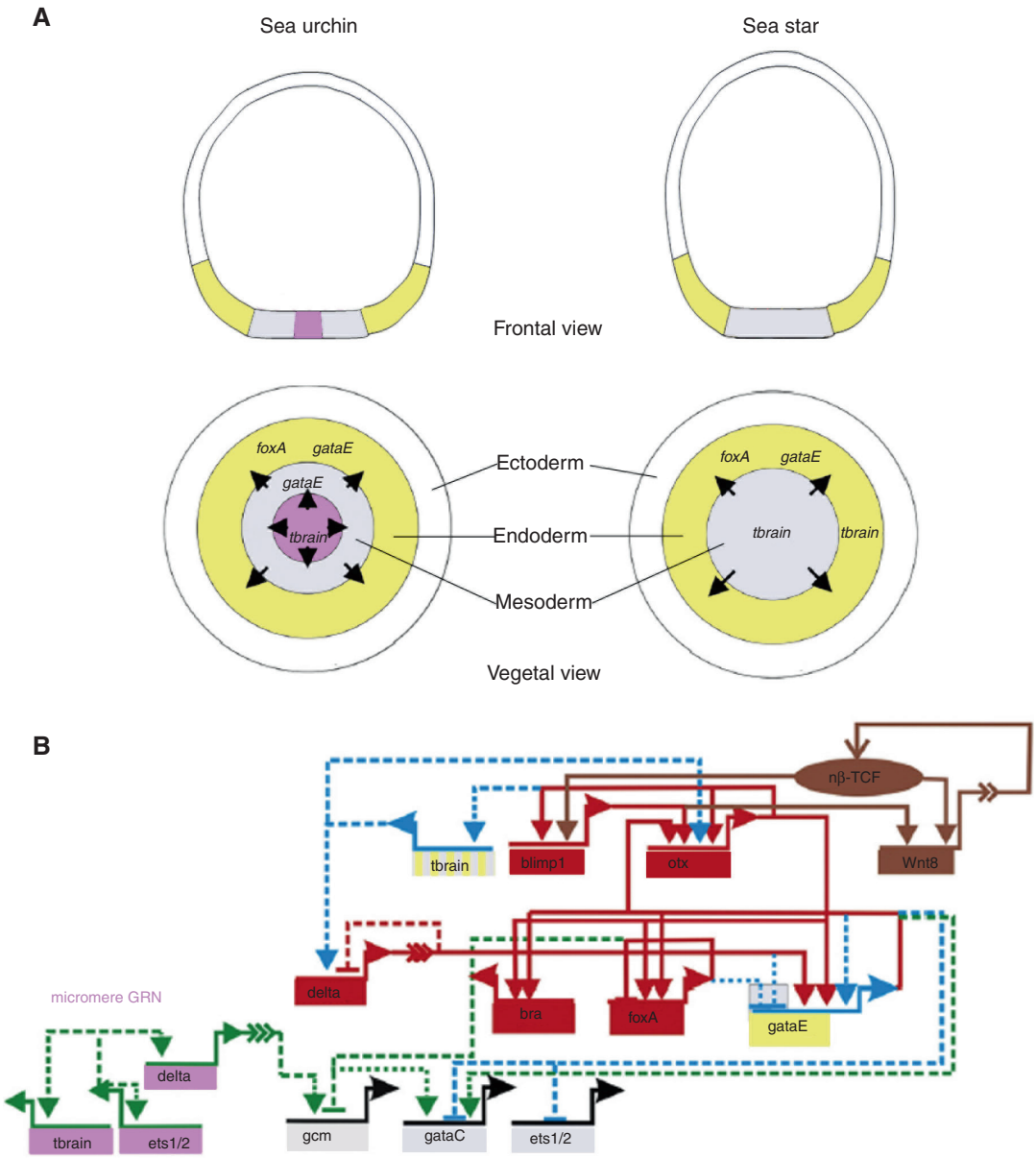


Fig. 1.14 Conservation and divergence in endomesoderm specification in sea urchins and sea stars. (**A**) Schematic representation of sea urchin and sea star blastulae. In the vegetal view of the embryos (*lower* part of the panel), some gene names are overlaid on their domains of expression, e.g., *tbrain* is expressed in the micromere cell lineage (*pink*) in the sea urchin and in the mesodermal and endodermal territories (*gray*) in the sea star and *gataE* is expressed within the endoderm and mesoderm in sea urchin but only in the endoderm in sea star. The *black arrows* represent Delta-Notch signaling from one cell territory to another. (**B**) The GRN depicting endomesoderm specification in sea urchins and sea stars at blastula stage. The regulatory interactions found in common in both taxa are shown in *red* (*solid lines*), while those occurring in the sea urchin only are shown in *dashed green lines*, and those only occurring in the sea star are shown in *dashed blue lines*. In sea urchins, the nuclearization of β-catenin is critical for the establishment of endomesoderm and forms a positive feedback loop with blimp1 (shown in *brown*). The role of nuclear β-catenin has not been examined in sea stars, but is likely to be conserved (Modified and adapted from Hinman et al. (2009))

star embryo for archenteron formation, a role performed under the control of endodermal regulators (*otx* and *gatae*), genes that do not affect the sea urchin *tbrain* gene expression at any time of development (see blue dashed arrows in Fig. 1.14B). A third difference between networks is the use of the *foxa* gene to repress mesoderm formation in sea urchin, a role taken by *gatae* in sea star embryos (compare blue and green dashed arrows in Fig. 1.14B); see Table 1.2 for comparison.

These observations demonstrate that GRNs are formed by discrete functional subcircuits which are affected by diverse selective pressures. Comparative GRN analyses provide us with key insights into the evolutionary processes that model body plans at the DNA regulatory level. As a general rule, it is assumed that the GRN subcircuits involving positive feedback tend to be conserved, generating constraints during development. This conservation may reflect a specific arrangement of transcription factor binding sites in cis-regulatory modules.

For quite a long time echinoderm biology has been greatly contributing to shed light on fundamental questions in developmental biology. The experimental availability of embryos belonging to different species, all separated by various evolutionary distances and accessible to the tools of modern regulatory biology, has proven invaluable. In the last two decades, this group of animals has been instrumental in addressing key biological questions such as how gene regulatory networks control development and how they evolved. In other words, echinoderm models have the potential to greatly contribute to solve central questions in the evolution of development, particularly from a gene regulation point of view. The larvae of echinoderms provide the rich source of morphological variation necessary to address relevant questions such as the evolution of novelties. There are many differences among echinoderm larval forms, but perhaps the most dramatic and obvious is the larval skeleton, which provides the structural material that gives the larva its typical morphology. Larval skeletons are found in the sea urchin echinoplutei and in the brittle star ophioplutei, but not in sea star larvae (see previous sections). Small larval spicules and ossicles are also found in the auricularia larvae of holothurians (see above). All the echinoderm embryos that produce larval skeletal elements share an early ingression of the mesenchyme cells, prior to gastrulation, although it appears that only sea urchins establish their skeletogenic cell lineage via an asymmetric blastomere cleavage that leads to micromere formation. The micromere skeletogenic lineage can therefore be considered a novelty in echinoids. However, it is important to point out that due to some unresolved uncertainties in echinoderm evolution, it is not clear when a larval skeleton was first invented (see Fig. 1.15 for alternative scenarios). It has been proposed, for instance, that the gene regulatory network that controls larval skeleton formation in sea urchins was co-opted from its adult skeletogenic program (Gao and Davidson 2008; Koga et al. 2014). However, it is not clear when this happened. One way to address this question would be to analyze the molecular mechanisms which control specification of larval skeletogenic lineages in other echinoderm taxa, particularly in brittle stars. This approach would shed light on the question of whether the echinoplutei and the ophioplutei are homologs or not. Additionally, and perhaps more importantly, approaching this or other developmental questions, at a deep gene regulatory network level, will provide us with new insights into the understanding of GRN evolution. The example given here is, perhaps, one of the most obvious, but questions from polarity to the specification of different cell lineages or the morphological arrangement of tissues are putative targets for undertaking similar approaches.

LATE DEVELOPMENT

Echinoderms are unique among bilaterians in that the adults have a pentameral radial body plan, the phylotypic character of the Echinodermata. The larvae, however, are bilateral with some asymmetry conveyed by the expansion of the coeloms on the left side (Hyman 1955; David and Mooi 1998). This “asymmetrical

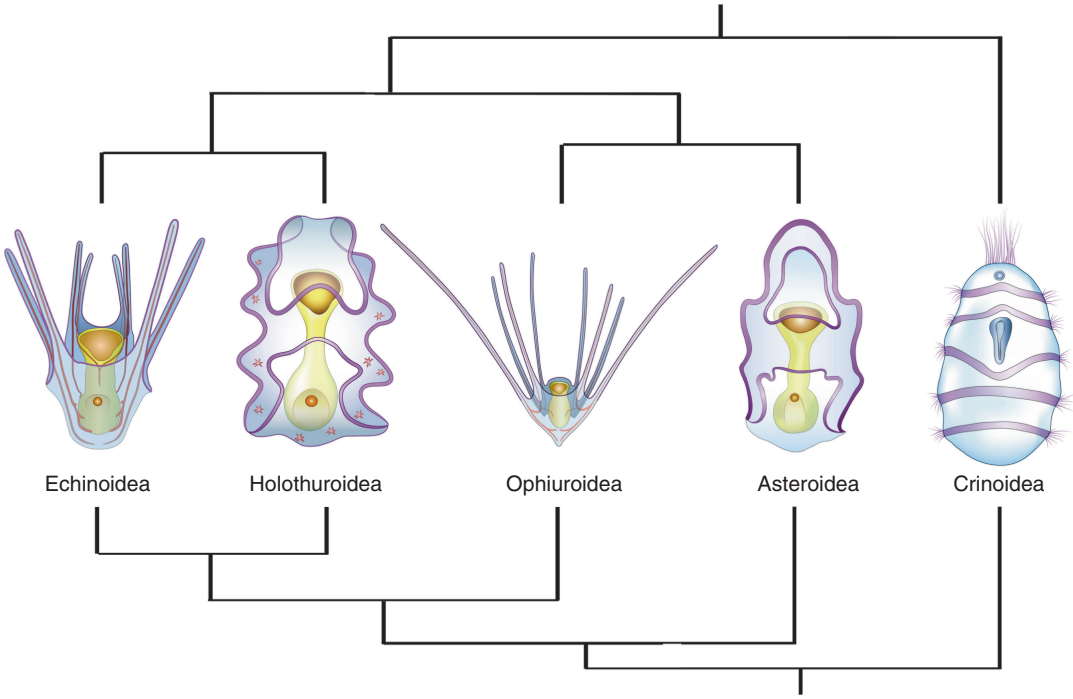


Fig. 1.15 Distribution of larval types in echinoderm phylogeny. Representative echinoderm larvae are displayed according to the two alternative phylogenetic scenarios illustrated in Fig. 1.3

bilaterality" is particularly prominent in species with nonfeeding larvae (Smith et al. 2009; Morris 2011, 2012; Morris et al. 2011). At metamorphosis, the bilateral larva transforms into the radial juvenile with a new main body axis, the oral-aboral axis. This change from a bilateral larva to a radial adult is of great interest and there are many reviews on echinoderm metamorphosis and the morphological changes that occur (Bury 1895; Hyman 1955; Chia and Burke 1978; Burke 1989).

From Bilateral to Radial Symmetry: Larval and Adult Polarities

Transformation from the larval to the adult echinoderm involves two major phases: (i) coelomogenesis, including formation of the hydrocoel and the origin of the pentameral plan, and (ii) metamorphosis. Coelomogenesis starts early, shortly after gastrulation. Although details of coelom development differ among groups, in most echinoderms the left coelom gives rise to the adult hydrocoel and somatocoel. The hydrocoel and its five lobes are the core of the body plan. These lobes form the primary podia. In juvenile development these podia give rise to the radial canals of the adult water vascular system. Each radial canal extends from a growth zone at the base of the primary podium (Morris 2012). In all echinoderms coelomic development on the larval left side is the basis for construction of the adult. The left somatocoel becomes the body coelom of the adult echinoderm (Burke 1989), while the right coelom of the sea star larva also contributes to the adult body coelom (Morris et al. 2011).

The interaction between the hydrocoel and overlying ectoderm is important during development of the juvenile body – called the juvenile rudiment. In euechinoid sea urchins, crinoids, and holothuroids, an ectodermal invagination, the vestibule, forms adjacent to the hydrocoel and the juvenile develops within the vestibule-like invagination (Smiley 1986; Burke 1989; Holland 1991; Smiley et al. 1991; Ramafofia et al. 2003). In other groups, such as the cidaroid sea urchins,

asteroids, and ophiuroids, the juvenile develops on the external surface of the larva (Emlet 1988; Byrne and Barker 1991; Selvakumaraswamy and Byrne 2006).

The timing of development of the coeloms and the rudiment differs in species with development through a feeding larva and those that develop through a nonfeeding larva. In species with feeding larvae, the time between the initiation of coelomogenesis and rudiment development can be days to months, as the larva accrues sufficient nutrients to support metamorphosis (Byrne and Barker 1991; Smith et al. 2008b). Species with nonfeeding larvae, in contrast, have considerable maternal nutrients and start building the rudiment shortly after gastrulation (Minsuk and Raff 2002; Raff and Byrne 2006; Smith et al. 2009; Morris 2012). The rapid formation of the juvenile in species with nonfeeding larvae is facilitated by a heterochronic shift in the early development of the left coelom (Raff and Smith 2009; Smith et al. 2009).

Morphogenesis of the developing juvenile is complex. This is best documented for sea urchins, in an species with planktotrophic larvae, *Strongylocentrotus purpuratus* and *Paracentrotus lividus* (Gosselin and Jangoux 1998; Smith et al. 2008b), and in species with lecithotrophic larvae, *Heliocidaris erythrogramma* (Minsuk and Raff 2002; Morris 2011) and *Holopneustes purpurascens* (Morris 2012). There are also good descriptions of metamorphosis in the other echinoid groups with feeding larvae (Emlet 1988; Vellutini and Migotto 2010). Rapid development (3–5 days) of a comparatively large rudiment in echinoids with lecithotrophic larvae has been particularly important in generating insights into coelomogenesis and metamorphosis (Minsuk and Raff 2002; Minsuk et al. 2009; Smith et al. 2009; Morris 2011, 2012).

In euechinoids, the vestibule and invagination of the ectoderm forms on the left side of the larvae. This structure forms from ectoderm overlying the region where the hydrocoel forms. The ectoderm in this region thickens and invaginates to form the vestibule. The vestibule floor develops an intimate contact with the primary podia. This mesoderm-ectoderm communication is important in development of the adult rudiment centered on the oral pole of the future oral-aboral axis (Burke 1989; Minsuk and Raff 2002; Smith et al. 2008b; Minsuk et al. 2009). The center of the vestibule becomes the adult mouth. Inductive signals from the left coelom are important for development of the rudiment (Minsuk et al. 2009). The five primary podia and the developing spines that develop between the podia project into the vestibule so that the thickened epithelium of the vestibule floor forms the external outer cover of these structures. The vestibule ectoderm also forms the nervous system, as indicated by the expression of neural genes such as *otx* in this region (Morris et al. 2004). Between the podia a thickening of tissue forms, the epineural folds. These rise up and fuse to close over the developing neural tissue (von Ubisch 1913). The skeleton is formed by associated mesoderm. Prior to metamorphosis, the vestibule and the developing rudiment dominate the left side of the euechinoid larva. In contrast, cidaroid larvae do not form a vestibule. In these echinoids the rudiment is exposed on the left side of the larva (Emlet 1988). In echinoids, the oral-aboral axis of the future adult is positioned on the respective left-right axis of the larva.

Morphogenesis of the developing juvenile asteroid is described for species with planktotrophic larvae, particularly *Asterias rubens* and *Patiriella regularis* (Gemmill 1914; Byrne and Barker 1991; Gondolf 2000) and with lecithotrophic larvae, for instance, *Asterina gibbosa*, *Leptasterias hexactis*, and *Parvulastra exigua* (Chia 1968; MacBride 1896; Morris et al. 2009). The hydrocoel and rudiment develops on the left side of the larva, and as in echinoids, the oral-aboral axis of the juvenile is positioned on the respective left-right axis of the larva. The juvenile asteroid develops as the larval body is absorbed into the future oral region of the sea star.

In holothuroids and ophiuroids, the hydrocoel originates on the left side but shifts in position during rudiment development. In holothuroids, a vestibule-like structure forms at the anterior end of the larva in the oral region and the oral-aboral axis of the future adult is positioned on the anterior-posterior axis of the larva (Hyman 1955; Smiley 1986; Smiley et al. 1991). In ophiuroids, the juvenile oral-aboral axis develops along the dorsoventral axis of the larvae (Hyman 1955; Hendler 1991). The juvenile ophiuroid develops externally. Crinoids differ from the other groups

in that the hydrocoel and vestibule originate ventrally and then become positioned at the anterior end of the larvae as the rudiment develops (Holland 1991). Thus, the juvenile crinoid oral-aboral axis is positioned along the anterior-posterior axis of the larvae.

The patterning mechanisms underlying development of the pentameral body plan are poorly understood. Several studies document expression of signaling and homeobox genes in the coeloms (e.g., *eng*, *wnt*, *hox4*), indicating a role for these genes in early development of the juvenile (Peterson et al. 2000a; Ferkowicz and Raff 2001; Byrne et al. 2005; Cisternas and Byrne 2009). Hox genes are expressed in a spatial and collinear sequence in the coeloms of sea urchin and crinoid larvae (Table 1.2; see Peterson et al. 2000a; Hara et al. 2006). The initial specification of the left coelomic pouch seems to depend on the activation of the BMP signaling pathway (Luo and Su 2012; Warner et al. 2012).

In the developing juvenile of the echinoid *Holopneustes purpurescens*, oral-aboral identity appears to be specified by Hox genes as indicated by the oral expression of *hox3* and aboral expression of *hox11/13* (Morris and Byrne 2014).

Once the rudiment has formed, expression patterns of several genes reflect different aspects of the typical echinoderm body plan (Arenas-Mena et al. 1998; Ferkowicz and Raff 2001; Lowe et al. 2002; Sly et al. 2002; Morris and Byrne 2005; Wilson et al. 2005; Morris and Byrne 2014). The developing five-rayed central nervous system has a distinct pentameral expression of many neural genes (Sly et al. 2002; Morris et al. 2004; Byrne et al. 2005; Morris and Byrne 2005). Some of these genes (e.g., *otx*) are also expressed in development of the peripheral nervous system of the tube feet, indicating a potential role in patterning a so-called "metameric-type" series of outgrowths from the radial canals (Table 1.2; see Byrne et al. 2005; Morris and Byrne 2005).

Metamorphosis

Metamorphosis can occur in the water column (e.g., in ophiuroids) or following settlement of competent larvae (e.g., in echinoids). In echinoids, ophiuroids, and holothuroids, the primary podia are used to select settlement sites and attach to the substrate. In many asteroid and crinoid species, the larvae have specialized attachment structures that they use for settlement. Metamorphosis involves degeneration of the larval body and can take minutes to hours (Chia and Burke 1978). The larval tissue of most echinoderms is discarded or resorbed. In holothuroids, however, the larval body is retained as the ectoderm of the juvenile (Smiley et al. 1991).

In euechinoids, the primary podia extend through the vestibule opening to attach to the substrate, and metamorphosis ensues with eversion of the vestibule. The vestibular ectoderm thus becomes the juvenile epidermis. What remains of the larval tissue becomes positioned as a clump of tissue on the aboral surface of the juvenile and is eventually resorbed. In asteroids, as the juvenile develops in the attached larva, the larval body bends so that the left side of the larva – the oral side of the juvenile – is directed towards the substrate and the right side becomes the upper one. The larval body degenerates into a stalk and is resorbed into the oral region of the young sea star and then the tube feet take over the role of attachment and benthic locomotion.

The bilateral larval axis of holothuroid larvae is congruent with the bilateral axis of the juvenile and adult (Smiley 1986). These echinoderms have a bilateral symmetry as adults superimposed on pentamery (Hyman 1955). The feeding larva transforms into a bilateral juvenile with the primary podia at the anterior end giving rise to the buccal tentacles that are later used for feeding (Smiley et al. 1991; Ramofafia et al. 2001). Pentamery is evident in the five buccal tentacles, which are in a radial position. In holothuroids the canals of the water vascular system form directly from the ring canal in an interradial position and thus are not homologous to the ambulacral canals of other echinoderms.

The larval gut serves as a primordium of parts of the adult echinoderm gut. During metamorphosis there is considerable degeneration of digestive tract cells and reorganization of other digestive tract cells (Chia and Burke 1978). The larval stomach forms the adult stomach. The

mouth appears to form through perforation of the hydrocoel (Gemmill 1914; Bury 1989; Minsuk et al. 2009; Morris et al. 2011). Later, growth of the digestive tract in the perimetamorphic period (sensu Gosselin and Jangoux 1998) is required to complete its morphogenesis. Formation of a functional gut to development of the anus can take days or weeks, depending on the species, and has been described in detail for *Paracentrotus lividus* (Gosselin and Jangoux 1998). The final development of the gut marks the end of metamorphosis.

EVOLUTION OF RADIAL (PENTAMERAL) SYMMETRY: POTENTIAL AXIAL HOMOLOGIES WITH OTHER DEUTEROSTOMES

The most conspicuous characteristic of extant Echinodermata is their adult pentameral (five-fold) symmetry. This symmetry evolved secondarily, as revealed by the presence of bilateral fossils (Smith 2005; Zamora et al. 2012) and the last common ancestor of Bilateria which predates the origin of Echinodermata by many millions of years. The adult echinoderm body is organized along the major body axis, the oral-aboral axis.

It is not clear how this echinoderm body plan relates to the bilaterian anterior-posterior (AP) axis. There are two main hypotheses on echinoderm body plan evolution: (1) the bilateral AP axis in echinoderms is derived from the stacking of the coeloms in development (Mooi and David 2008; Peterson et al. 2000a) and (2) the rays are in line with the chordate AP axis – the rays as the chordate body axis (Raff and Popodi 1996; Heinzeller and Welsch 1999; Morris 2011, 2012).

Coelomic Stacking Hypothesis

Several lines of evidence suggest that the bilateral AP axis in adult echinoderms is derived from the stacking of coelomic compartments that occurs during development (Peterson et al. 2000a; Mooi and David 2008; Smith et al. 2008b). These arguments are based on the expression of regulatory genes (e.g., Hox genes) during postembryonic development, comparative analysis of coelom development in echinoderms, and the analysis of skeletal plate morphology in both extant and fossil echinoderms. This hypothesis uses mesoderm derivatives as the key structures for understanding axial homologies. It is expressed in three steps, along the following lines:

(i) The coelomic stacking theory suggests that the coeloms in sea urchin larvae stack in the order: left hydrocoel-left somatocoel-right somatocoel. This arrangement is seen in development of echinoids with a feeding larva. These coeloms in an oral-aboral direction are hydrocoel, somatocoel, and right coelom. Morris (2012) also derives the AP axis from the oral-aboral arrangement of the coeloms in echinoids with a nonfeeding larva. In this case, the arrangement is derived by bending the chordate AP axis at the junction between the head of the archenteron and the forming coeloms. Thus, both Peterson et al. (2000a) and Morris (2012) get a similar sequence of coeloms from oral to aboral and both homologize this echinoderm adult axis with the AP axis of the deuterostome ancestor.

(ii) The Hox genes seem to work as a vectorial system in all bilaterian animals, providing cells along the major (AP) body axis with positional information. Their regulatory activities extend to all germ layers, although preferentially to the ectoderm and mesoderm. The main feature that characterizes this group of genes is that they are expressed in nested domains along the AP axis, with gene expression domains following the order of the genes on the respective chromosome. It is particularly relevant that some Hox genes are expressed only in the larval somatocoels, again with nested domains of expression, where the most "anterior" Hox genes are expressed in more apical/anterior domains and the "posterior" Hox genes in more blastoporal/posterior domains (Table 1.2; see Arenas-Mena et al. 2000; Hara et al. 2006).

These expression domains indicate the organization of axial domains within the somatocoels and hence in their derivatives. The use of Hox genes in both the specification of the bilaterian AP axis and in the coeloms suggests that the stacking of coeloms might be the best evidence we have for the orientation of the major echinoderm body axis (although co-option cannot be ruled out). During this part of development, there is no expression of Hox genes in the gut or nervous system.

(iii) It has been recognized that all echinoderms, extant and fossil, have body walls with two areas of skeletons, the so-called axial and extraxial skeletons (Mooi and David 1997, 2008). Although both types are composed of the same biomineral matrix, it is suggested that they may be patterned by different sets of regulatory genes (Mooi et al. 2005; Mooi and David 2008). While the axial skeleton is associated with the water vascular system, the extraxial is formed outside the axial system and comprises two subregions: the perforate extraxial (including, for instance, the anus and gonopores in sea urchins) and the imperforate extraxial, covering the coeloms in the most aboral parts (see Fig. 1.16). While the perforate axial skeleton may be associated with the left somatocoel, the imperforate one is associated with the right somatocoel. Strikingly,

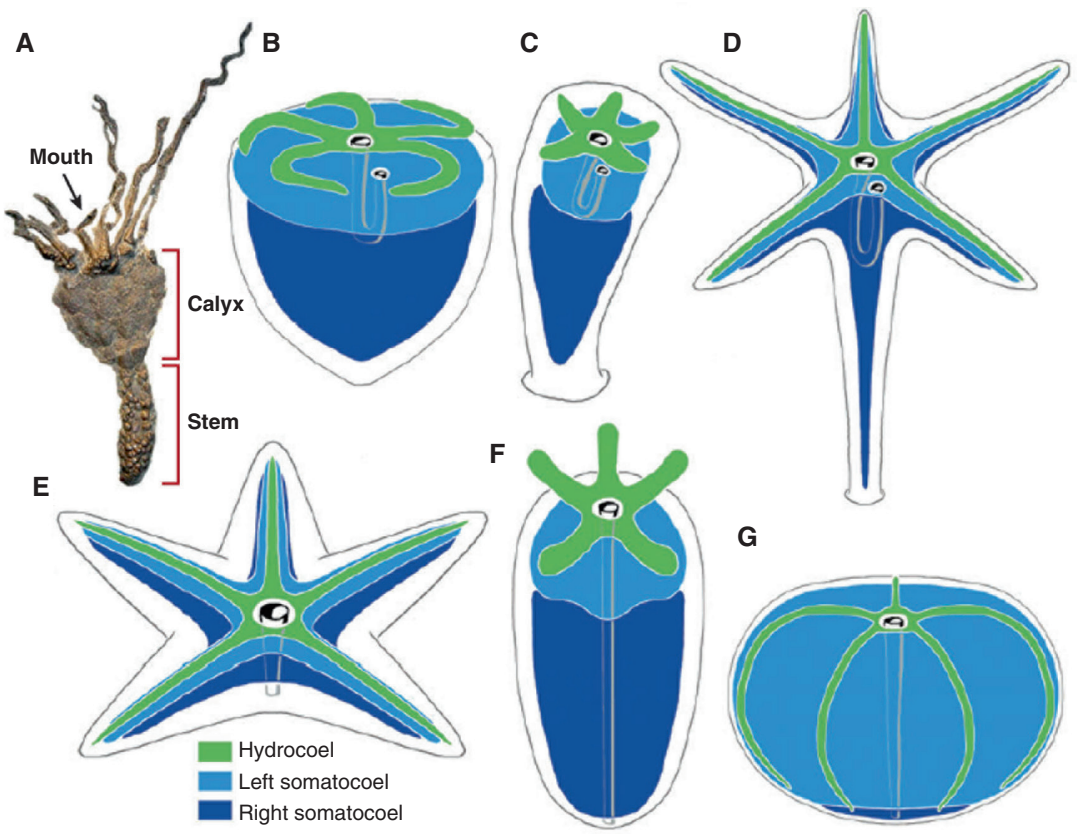


Fig. 1.16 Stacking of coelomic derivatives in all extant (but also in fossil) echinoderm classes. Different colors mark derivatives of the three coelom compartments. The arrangement of derivatives is a manifestation of the AP axis of animals, following the extraxial-axial theory (*EAT*). (**A**) Cambrian fossil *Gogia spiralis*, showing the sequential arrangement of mouth, calyx, and stem. (**B**) Cambrian fossil *Camptostroma*. (**C**) Crinoid larva. (**D**) Extant adult crinoid. (**E**) Extant asteroid. (**F**) Extant holothuroid. (**G**) Extant echinoid (The diagram is taken from Mooi and David (2008)©)

when these different skeletons are mapped onto the adult morphology of all echinoderms, we see that their relative disposition in the animal follows the stacking of the coelomic compartments, such that the hydrocoel derivatives are oral with respect to left somatocoel derivatives, which at the same time occupy oral positions with respect to the derivatives of the right somatocoel (see Fig. 1.4). The commonalities in the organization (and the ontogenies) of the different parts of the adult echinoderm body have allowed the elucidation of body wall homologies across different extant and also fossil groups (see below). These architectural and ontogenetic principles were termed extraxial-axial theory (EAT) (Mooi et al. 1994).

The EAT explains very well the anatomy of adult echinoderms with respect to the ambulacral and interambulacral regions and homologies between these body regions in the different classes. This hypothesis unites the disparate forms of the five extant echinoderm classes and some echinoderm fossils. A recent study (Hotchkiss 2012), however, reinterprets the designation of axial and extraxial skeletons in the asteroid arm by Mooi and David (2000), and this has implications for the rays as axis hypothesis (see below).

The coelomic stacking and the EAT hypotheses have been taken to suggest that the ambulacra are outgrowths, perpendicular to the major AP axis, and thus appendages. Two lines of evidence support this scenario. The first is derived from the theoretical models of Hotchkiss (Hotchkiss 1998), in which he suggests that the consideration of "rays as appendages" best explains the origin of the pentameral symmetry. Accordingly, a suggested characteristic of all echinoderms is the clear organization of structural elements along a major body axis, running from the anterior mouth (oral side = anterior) to the derivatives of the right somatocoel (aboral side = posterior). The adult echinoderm mouth thus corresponds to the anterior pole of other bilaterians. The relationship between the sequences of coelom development along the oral-aboral axis appears to be a basic feature of echinoderm anatomy (Peterson et al. 2000a; Mooi and David 2008; Morris 2012). However, the question concerning the evolution of a pentamerous arrangement of the arms remains unanswered in this scenario.

Insights into the affinity of the echinoderm ambulacrum are provided by data on expression of some regulatory genes during development, in particular the homologs of *distal-less*, which is normally expressed in the growing tips of several bilaterian appendages (e.g., annelid parapodia, tunicate ampullae, vertebrate limb buds) and in the podia of larval and juvenile echinoderms (Lowe and Wray 1997; Panganiban et al. 1997), although these expression data alone do not sufficiently argue for homology of echinoderm podia to other bilaterian appendages (e.g., (Winchell et al. 2010).

The "Rays as the Chordate Body Axis" Hypothesis

In this hypothesis the rays are axial in line with the chordate AP axis with one ambulacrum being the homolog of the chordate body axis (Fig. 1.17; see Raff and Popodi 1996; Heinzeller and Welsch 1999; Morris 2012). The echinoderm ambulacra are also interpreted as a metameric series (Turner 1998; Morris 2011, 2012). The other four ambulacra are thus hypothesized to be an evolutionary duplication from an ancestor with a single ambulacrum (Raff and Popodi 1996; Hotchkiss 1998; Heinzeller and Welsch 1999; Minsuk et al. 2009).

The "rays as the chordate body axis" hypothesis stems from development of coelom derivatives (Morris 2012; Morris and Byrne 2014) and the morphology of the adult nervous system (Heinzeller and Welsch 2001). Using the relative positions of mesodermal derivatives in both groups of animals, specific homologies between the hydrocoel and the notochord on the one hand and the secondary podia and somites on the other were suggested. The expression of some regulatory genes in coeloms would be compatible with this set of proposed homologies (however limited the number of genes is). Morris (2012) suggested that the five ambulacra arose as duplications of a

posterior growth zone – a series of duplications from an ancestor with a single ambulacrum. Thereby, the presence of repeated blocks of muscles and ossicles along the ambulacra is indicative of "segmentation," which also occurs along the major body axis (AP) of chordates. Accordingly, the posterior growth zones seen in the growth of the juvenile and adult echinoderm are the regions behind the primary podia, following the "ocular plate rule" of Mooi et al. (1994) with the oldest ossicles next to the mouth and the youngest at the end of the ambulacra. In echinoids the ocular plate is at the aboral pole and thus AP is readily seen to be parallel to oral-aboral. In asteroids, the equivalent growth zone at the terminal plate is at the end of the arms (see Hotchkiss 2012) and accordingly the AP axis would best be termed proximal-distal with regard to mouth and arm tip. Thus, the ray or ambulacrum in both echinoids and asteroids is interpreted as the chordate anterior-posterior axis (see Fig. 1.17).

Other arguments are based on the functional analogies between the chordate spinal and the echinoderm ectoneural chords, to the extent that a nervous system is required to control the movement of serial muscles and podia and its formation from ectodermal domains overlying these mesodermal structures. In fact, some authors have suggested that the radial nerves and the circumoral ring of the adult are "strong candidates" for a homolog of the chordate CNS (Haag 2005), a position that is also opposed by some (Nielsen 2006). Analysis of Hox gene expression in the adult rudiment of the direct-developing sea urchin *Holopneustes purpurascens* seems to lend support to this assumption, stressing the concept that echinoderms and chordates share structural homologies and that an echinoderm arm is organized metamerically (Morris and Byrne 2014), as is the main vertebrate axis. The reiterated expression of other genes involved in segmentation (e.g., *engrailed*) in some echinoderm arms may be interpreted in the same context. However, as for the first hypothesis, one has to be cautious about using patterns of gene expression as signs of homology due to the potential of basically all known developmental genes for having been co-opted into novel functions (Nielsen and Martinez 2003).

The homology of the ambulacrum of echinoderms to the AP axis could be interpreted as being supported by fossil data, which indicates that the earliest echinoderms have one ambulacrum and were bilaterally symmetric (Smith 2005; Zamora et al. 2012).

All in all, the axial homologies of echinoderms with other deuterostomes and the origins of the radial symmetry have generated much discussion

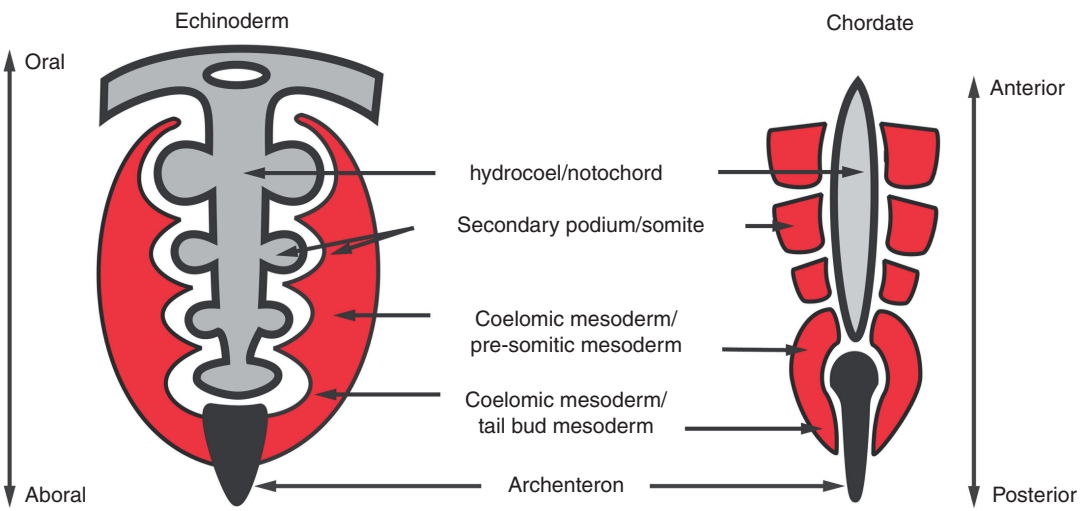


Fig. 1.17 Comparison of coelomic compartments of an echinoderm arm and the metameric anterior-posterior axis of a chordate (Courtesy of Valerie Morris)

and controversies with varying hypotheses proposed. The expected increase in comparative data on the ontogeny of adult structures and how gene regulatory networks specify them will undoubtedly continue to provide us with important insights in the future.

ADULT DEVELOPMENTAL PROCESSES: REGENERATION IN ECHINODERMS

Regeneration may be defined in general as the formation of new tissues or organs to replace those lost or damaged due to injury (see Vol. 2, Chapter 4) for a detailed treatment of the subject). Although a response to injury is evoked in most animals, there is a remarkable variety in the degree of morphological and functional recovery, not only between individuals from unrelated groups but also between closely related species and even between organs and parts of the same individual. The regenerative capacity is generally rather limited in vertebrates compared to that of many invertebrates (Goss 1969; Mattson 1976; Tanaka and Reddien 2011). Some vertebrates, including some amphibians and reptiles, are capable of tail, limb, and/or digit replacement, but these properties pale in comparison to the amazing capacity of invertebrates to repair most organs, including the CNS (Goss 1969; Mattson 1976; Tanaka and Reddien 2011)

Among the invertebrates, the Echinodermata, together with the Platyhelminthes (see Vol. 2, Chapters 3 and 4), have a remarkable capacity to regenerate lost or amputated organs (Candia Carnevali and Bonasoro 2001a, b; Candia Carnevali 2006; Candia Carnavali and Burighel 2010). Larval and adult echinoderms from each of the extant classes exhibit natural, rapid regeneration of entire lost parts (Eaves and Palmer 2003; Candia Carnevali 2006). This striking regenerative capacity serves a range of biological purposes (Sköld et al. 1994). Of primary importance is the replacement of tissues following predation and, secondarily, regeneration has developed as part of a process of asexual reproduction where fission results in two (or occasionally more) individuals (Candia Carnevali 2006; Sanchez Alvarado and Tsonis 2006). Many echinoderms regenerate in a seasonal pattern following, for instance, fragmentation of the body for asexual reproduction (Lee et al. 2008). Clearly, these developments have been of substantial adaptive value and are responsible for the ecological success of echinoderms.

Approximately 70 % of the genes known from echinoderms have obvious human homologs (Sodergren et al. 2006). Therefore, the molecular processes involved in echinoderm regeneration are more likely to be shared with mammals than those observed in other classic models, such as cnidarians (e.g., *Hydra*) or planarian flatworms, which are more distantly related to chordates. Moreover, all the regenerative strategies that are currently described in animals are represented in echinoderms; arm regeneration in ophiuroids and crinoids is an epimorphic blastemal process, and in asteroids and echinoids, morphallaxis is the main process involved (Suarez-Castillo et al. 2004; Candia Carnevali 2006). We understand here morphallactic regeneration as that relying on cellular reorganization with only limited production of new cells, while we define epimorphic regeneration as that involving dedifferentiation of adult structures in order to form an undifferentiated mass of cells from which the new structures eventually develop. However, there is clear evidence that regeneration in echinoderms involves contributions from both processes. In fact, some studies have shown that under different experimental conditions, the same individuals employ both epimorphic and morphallactic mechanisms, the use of which depending on the specific needs of the moment (Candia Carnevali and Bonasoro 2001a).

Currently, the best understood processes in echinoderm regeneration are arm regeneration in crinoids, asteroids, and ophiuroids and visceral regeneration in holothurians (and, to a lesser extent, in crinoids). Regeneration of other structures, such the holothurian nervous system, has also attracted much interest over the last few years (Mashanov et al. 2008, 2013). Here, the current knowledge of echinoderm regenerative processes is summarized.

Arm Regeneration

Three classes of echinoderms, namely, crinoids, asteroids, and ophiuroids, are well known for their extraordinary potential to regenerate amputated limbs. This property and the ease with which many species can be handled in the laboratory have been instrumental in the selection of echinoderm species as models for regeneration studies. Many species of asteroids, ophiuroids and holothuroids reproduce asexually by splitting the body into pieces that undergo subsequent regeneration. Moreover, in a few asteroids, a whole animal can be regenerated from just a fragment of the limb, e.g., *Linckia* (Edmondson 1935). The process of arm regeneration has been studied in detail in the crinoid *Antedon mediterranea* (Candia Carnevali and Bonasoro 2001b), the asteroid *Asterias rubens* (Moss et al. 1998; Hernroth et al. 2010; Ben Khadra et al. 2014), and the ophiuroid *Amphiura filiformis* (Bannister et al. 2005; Dupont and Thorndyke 2006; Czarkwiani et al. 2013). While the overall morphological changes have been well documented, the cellular processes involved are still a matter of some debate. However, what is mostly lacking is a good understanding of the molecular processes involved.

In *Antedon mediterranea*, the regeneration of amputated arms has been described as a typical blastemal regeneration in which migratory cells derived from the brachial nerve (amoebocytes) and coelomic epithelium (coelomocytes) are the major contributors to the process. The extensive studies by Candia Carnevali and collaborators have shown that the mitotic activities are located in the blastema and in the coelomic epithelia (reviewed in Candia Carnevali 2006). Moreover, regeneration is under neural control, probably through the modulatory activities of neurotransmitters and growth factors (Thorndyke and Candia Carnevali 2001; Patruno et al. 2003). Interestingly, crinoid arm explants are able to survive and engage in regeneration for several weeks in culture, providing another interesting context for regeneration (Bonasoro et al. 1999).

Asteroid arm regeneration differs from that in crinoids and ophiuroids in that a blastema is not formed. In asteroids the cells contributing to the regrowth of the amputated limb are derived from coelomic epithelium and the pyloric cecum (Holm et al. 2008; Hernroth et al. 2010), most of them originating in locations far from the wound. In asteroids, such as *Antedon*, the regeneration process is dependent on the presence of the nervous system as it has been shown for *Asterina gibbosa* (Huet 1975). Very little is known about the molecular control of asteroid regeneration (Thorndyke and Candia Carnevali 2001). Up to date only a few homeobox genes and a BMP homolog have been identified in regenerating sea star arms (Thorndyke and Candia Carnevali 2001; Ben Khadra et al. 2014). A preliminary report also identified a few enzyme-encoding cDNAs in regenerating larvae (Vickery et al. 2001), but this study was not followed by a more exhaustive characterization of the genes.

Amphiura filiformis is the best-known regenerating model species for the Ophiuroidea (Dupont and Thorndyke 2006; Czarkwiani et al. 2013). However, what is known about the process is still very limited. Few studies have been carried out into the nature of the cells contributing to the growth of new structures, although coelomocytes are thought to be involved (Thorndyke et al. 2001). A few morphological studies have been performed on ophiuroid regeneration (Thorndyke et al. 2003), but these are focused on the ecological adaptive value of regeneration (Sköld and Rosenberg 1996). The only molecular study performed on *A. filiformis* suggests the participation of diverse transcription factors, for instance, several linked to the formation of mesoderm, including *foxb*, *gata, ets, alx*, and also homeobox family members (Czarkwiani et al. 2013; Ben Khadra et al. 2014). Moreover, a TGF growth factor has been identified in the regenerative process (Bannister et al. 2005). However, recent transcriptomic analysis (microarrays) of regenerating *Amphiura* tissues has the potential to open up new fruitful avenues in the study of ophiuroid regeneration (Burns et al. 2012).

Visceral Regeneration

Holothuroids and crinoids are able to regenerate their digestive system after evisceration (autotomy). The best-studied models are the holothuroids

(Mashanov and Garcia-Arraras 2011). Evisceration (discarding of the digestive tract) occurs in response to certain stimuli (e.g., predation) with the rupture of specific breakage planes and detachment from the anchoring mesentery, this porcess being under neural control (Emson and Wilkie 1980; Byrne 2001). Two types of evisceration occur: anterior and posterior. Anterior evisceration occurs in dendrochirotids and results in loss of the gut and anterior associated organs: the tentacles and the pharyngeal bulb. Posterior evisceration occurs in aspidochirotids and results in loss of the gut, from the esophagus to the cloaca, and associated structures such as the respiratory trees.

As in other regenerative processes in echinoderms, regrowth involves an initial phase of wound healing followed by tissue remodeling and growth. The wound is closed during the first few days and involves contraction of body wall muscles. The remaining stump of the digestive tube starts a process of outgrowth and the mesentery also regenerates to provide a path for extension of the new gut. This process involves the mobilization of multiple cells, including the dedifferentiation and transdifferentiation of different cell types. Evisceration necessarily involves a large wave of cell proliferation to replenish missing structures. The tubular rudiments grow along the free edges of the mesentery and eventually fuse to form a whole, continuous, gut. The morphogenetic process, and formation of the final structure, is accompanied by the destruction of some cells via apoptosis (Mashanov et al. 2010).

Visceral regeneration in holothurians is one of the few regenerative processes in echinoderms for which an increasing source of molecular data are available. Conventional cloning (gene candidate approaches) have been used to identify genes involved in the regenerative process, e.g., the homologs of *ependymin*, *wnt6*, and *hox6* (Suarez-Castillo et al. 2004; Sun et al. 2013b), but more recently, transcriptomic tools have also been incorporated to gain an understanding of the changes in global patterns of gene activity (Rojas-Cartagena et al. 2007; Ortiz-Pineda et al. 2009; Sun et al. 2013a). These technologies, and the eventual sequencing of genomes, will prove extremely useful in modeling the molecular events controlling visceral regeneration. However, detailed methods for in situ hybridization and gene knockdown are still lacking.

As mentioned above, crinoids are also able to regenerate the gut after evisceration. The process has been studied in the feather star *A. mediterranea* (Dolmatov et al. 2001; Mozzi et al. 2006). In this case, the wound is sealed through a clotting process, which recruits coelomic and hemal fluids. A process of cell proliferation follows, mostly in the coelomic epithelium. As described for holothurians, the mesenterial tissue is also involved. Moreover, dedifferentiation and transdifferentiation also occur in crinoid regeneration, with the coelomic epithelium being an important source of new cells.

Nervous System Regeneration

Regeneration of the nervous system is an integral part of regeneration of amputated limbs in crinoids, asteroids, and ophiuroids. However, it is from the recent study of holothuroids that new insights have been gained (see Mashanov et al. 2008, 2013). After transection of the radial nerve cord (RNC) in *Eupentacta fraudatrix*, the RNC regenerates and reconnects in about 20 days. This process involves the two components of the nerve cord, the so-called ectoneural and hyponeural cords. Cell proliferation and death (apoptosis) are involved, and radial glial cells are the major source of new cells (neurons and glia). Through a process of dedifferentiation, the radial glia enter into the mitotic cycle and produce the new cells (though some neurons are also seen entering mitosis). While initially dedifferentiation is located at the stump, later on it spreads to other regions of the RNC. Mitotic activity in both halves of the transected nerve cord leads to the growth of the stumps towards each other. During this period, mitotic cells in the areas behind the stump enter into differentiation and restore the normal cytoarchitecture of the nerve cord. The final process is the fusing of the growing tips, which gives rise to a fully functional cord.

Interestingly, it has been shown that in mammals, glial cells are also involved in the regenerative process, as in holothurians (and probably in

all echinoderms), but, while in echinoderms the radial glia are active in the regeneration process (Mashanov et al. 2013), in mammals glial cells become a factor that block the process (Shearer and Fawcett 2001). In early-branching vertebrates, the glial reaction is, instead, permissive of regeneration (Zukor et al. 2011). This reaction may be linked to the fact that early-branching vertebrates also keep, during adulthood, a population of competent radial glial cells. This is not the case with mammals. Overall, it appears that the glial reaction modulates the (limited) regenerative capacity of the nervous system across the vertebrates (Horner and Gage 2000).

Although our knowledge on regenerative processes in different echinoderms has recently improved, we are still missing key information regarding cellular and molecular aspects that control echinoderm regeneration. Gaining knowledge is mostly hampered by the lack of suitable techniques, particularly in the realms of gene knockdown and transgenesis. However, this situation may change over the next few years, given the speed with which new molecular technologies tend to move from the traditional model systems to others.

OUTLOOK

Echinoderms have been used as models in developmental biology for more than a century. Areas ranging from the analysis of early embryogenesis to the study of regeneration mechanisms have been illuminated by the use of echinoderm model systems. Moreover, the well-preserved fossil record of the group provides an excellent reference framework to analyze evolutionary innovations. The recent increase in papers describing genomic and transcriptomic analysis in several species of the phylum and the astounding success of incorporating high-throughput methods to analyze gene regulatory networks suggest that we are entering an era where many fundamental problems in EvoDevo will be tractable in the laboratory, also using echinoderms as model organisms. Challenges in understanding the changes, ranging from cell lineage specification to the evolution of larval forms, or the genesis of adult structures through metamorphosis, will be more amenable to address using experimental approaches.

However, there are still some research areas that will need particular attention. The development of non-echinoid echinoderms has to be further explored, including their molecular control. Our current knowledge of postembryonic development is limited, especially the development of adult structures, which is particularly relevant for modeling the origin of pentameral symmetry. The need of experimental techniques to analyze postembryonic development is urgent. These techniques should prove especially useful in the analysis of adult processes such as regeneration.

The future looks bright for the use of echinoderm models in EvoDevo, although this should not deter us from improving our knowledge on the last-mentioned (and mostly neglected) areas of research.

OPEN QUESTIONS

- The molecular control of echinoderm embryology (other than echinoids)
- The evolution of echinoderm embryogenesis
- The evolution of echinoderm genomes and morphologies (from populations to species and higher taxa)
- The evolution of gene regulatory networks (the mechanistic basis)
- The developmental and genetic basis of echinoderm life history evolution
- Larval morphogenesis and the development of adult echinoderm structures, from molecules to morphologies
- The axial affinities of the adult echinoderm body with the AP axis of other Bilateria
- The molecular control of regeneration

NOTE ADDED IN PROOFS

In the recent paper by Baughman et al. 2014 the authors show that the sea star *Acanthaster planci* has an almost complete HOX cluster, without any major reorganization as it is seen in the genome of the echinoid *Strongylocentrotus purpuratus*.

This would suggest that the HOX cluster was reorganized specifically in the echinoid lineage and that the other echinoderm classes do not share the structure described for *S. purpuratus.* Moreover, this indicates that the ancestral state for the Echinodermata is having an unmodified HOX cluster.

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