Development 138, 2639-2648 (2011) doi:10.1242/dev.048967
© 2011. Published by The Company of Biologists Ltd

Evolutionary crossroads in developmental biology: sea urchins

David R. McClay*

Summary

Embryos of the echinoderms, especially those of sea urchins and sea stars, have been studied as model organisms for over 100 years. The simplicity of their early development, and the ease of experimentally perturbing this development, provides an excellent platform for mechanistic studies of cell specification and morphogenesis. As a result, echinoderms have contributed significantly to our understanding of many developmental mechanisms, including those that govern the structure and design of gene regulatory networks, those that direct cell lineage specification, and those that regulate the dynamic morphogenetic events that shape the early embryo.

Key words: Sea urchin, Sea star, Echinoderm, Gastrula, Gene regulatory network

Introduction

Echinoderms and chordates are deuterostomes (see Glossary, Box 1), although echinoderms branched from the chordate lineage prior to the Cambrian period, more than 500 million years ago (Fig. 1A). Nevertheless, this shared relationship places echinoderms in an important position for developmental and evolutionary studies. The term echinoderm means 'spiny skin', and the Echinodermata phylum includes sea stars (Asteroidea), sea urchins and sand dollars (Echinoidea), brittle stars (Ophiuroidea), sea cucumbers (Holothuroidea), and sea lilies (Crinoidea) (Fig. 1B). Because their development is relatively simple and rapid, and because gene function in these organisms is easy to perturb and manipulate, echinoderms, especially sea urchins and sea stars, have long been used for testing hypotheses about how early development works.

Specification (see Glossary, Box 1) in echinoderms begins early in development. Zygotic gene transcription begins shortly after fertilization and zygotic mRNA quickly becomes responsible for the diverse specification activities. This is in contrast to the extended delay in the appearance of zygotic mRNA seen in *Xenopus* and *Drosophila*. In sea urchin embryos, by the time gastrulation (see Glossary, Box 1) is reached, each cell of the embryo is differentially specified to one of at least 14 early cell fates. Gastrulation then involves a series of complex and dynamic movements that ultimately lead to the formation of the free-floating larval form. In the final stage of development, metamorphosis (see Glossary, Box 1) occurs and the larva transforms into the juvenile sea urchin. Each of these stages in sea urchin development has been studied in great detail, and, most importantly, many of these details have provided us with insights into the conservation of these events across deuterostomes.

Department of Biology, Duke University, Durham, NC 27708, USA.

*Author for correspondence (dmcclay@duke.edu)

This primer provides an overview of the reproduction, biology and development of the echinoderms, focusing on the sea urchin, as used in studies of evolutionary developmental biology. Importantly, this article highlights how discoveries in the sea urchin have provided us with insights into some of the key mechanisms used to build a deuterostome organism.

Echinoderm evolution and model species

The echinoderm phylum dates at least to the Cambrian period, about 500-540 million years ago, when crinoids, animals similar to today's sea lilies, appear in the fossil record (Shu et al., 2001). From the Cambrian period onwards, fossil deposits of echinoderms track a good evolutionary history of this ancient and exclusively marine phylum of animals. Echinoderms were included with deuterostomes by early systematists, based on the shared embryonic character of the mouth as the second invagination during development. Genomic sequence data has since verified the deuterostome clade as the correct phylogenetic grouping of these animals (Swalla and Smith, 2008). This poses a basic question: to what extent is the genetic basis of development and morphogenesis conserved among deuterostomes? Research on echinoderms is of great value for addressing this question, as it offers an opportunity to discover remnants of the common template upon which the deuterostome style of development is built.

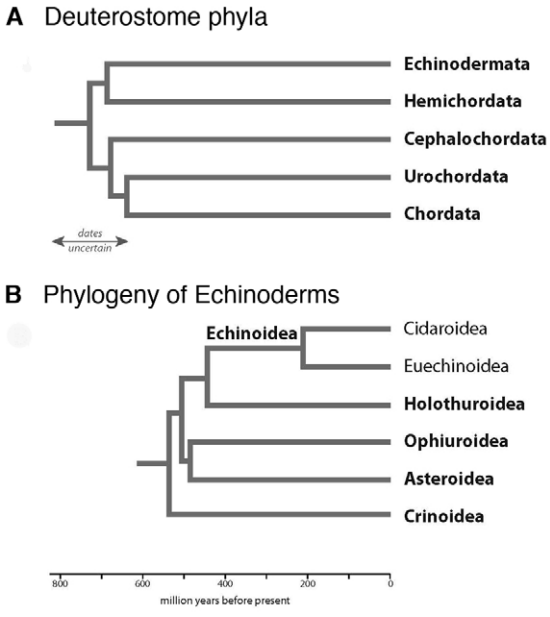


Fig. 1. Phylogeny of the deuterostomes and the echinoderms. (**A**) A cladogram of deuterostome phyla. The timing of phylum separation is uncertain. (**B**) Phylogeny of echinoderms. Images courtesy of Greg Wray.

Most developmental research on echinoderms is restricted to a small number of model sea urchin and sea star species (Fig. 2). On the west coast of the USA, *Strongylocentrotus purpuratus* is studied at the molecular, cellular and genomic levels, and its genome was the first of this phylum to be sequenced (Sodergren et al., 2006). On the eastern coast of the USA, *Lytechinus variagatus* is commonly studied and valued for the transparency of its eggs. In Japan, *Hemicentrotus pulcherrimus* is the most commonly studied species. Around the Mediterranean, *Paracentrotus lividus* is the sea urchin species of choice for most of the European research community, and was the echinoderm species used by late 19th and early 20th century embryologists for the discovery of many principles of early development (see Box 2). Several sea star species are also studied by developmental and evo-devo labs around the world, including *Asterias amurensis* and *Asterina pectinifera* from Japan, *Asterias forbesi* and *Asterina miniata* (now called *Patiria miniata*) in the USA, and *Astropecten aranciacus* in the Mediterranean.

Echinoderm morphology and development

Morphology

Adults appear five-sided or pentaradial, although this apparent symmetry is actually superimposed on a true bilaterian underlying body plan (Sprinkle, 1992). Adults have a thin epithelium covering an internal calcium carbonate skeleton. This skeleton consists of many plates that collectively form five ambulacra (see Glossary, Box 1), which extend from the mouth to the anus and are penetrated by many holes through which tube feet extend. The tube feet are part of a water-based vascular system that connects externally via a single opening next to the anus, called the madreporite. Side branches of this system connect to the tube feet, enabling them to extend/withdraw by hydraulic inflation/deflation. Muscles lining the tube feet provide the adult with a mode of movement, and objects are grasped via suckers at the end of the tube feet. The mouth of an adult usually faces downwards towards the substrate and is opened by a unique pentaradial arrangement of muscles and teeth used for biting and scraping food. This arrangement is known as 'Aristotle's lantern', after the great scientist and philosopher who described this structure and how it works. Food is digested in a five-part gut that branches from the mouth and then converges again at the anus on the top of the animal. The echinoderm nervous system consists of a diffuse network of neurons with ganglia but there is no central nervous system.

Lifecycle and reproduction

In most species the sexes are separate, and indirect development produces a free-swimming larva that then undergoes metamorphosis, at which point the juvenile emerges with the characteristic fivefold symmetry and grows into the adult. About one-fifth of all sea urchin species follow a direct development strategy. *Heliocidaris erythrogramma*, for example, gastrulates and reaches the juvenile stage 3 days after fertilization (Wray and Raff, 1989). Such direct developing species generally have large eggs (~300 μm in diameter), allowing them to bypass the larval feeding stage (Parks et al., 1988; Wray and Raff, 1989). Direct developing strategies arose relatively recently in several groups, and because of this it is likely that molecular remnants of the transition from indirect to direct development will be found in the not too distant future. The lifespan of species varies from a few years to more than 200 years (Ebert, 2008). Most echinoderms also have the capacity to develop asexually. Sea stars, for example, can regenerate a complete animal from an isolated arm or half. Furthermore, sea urchin larvae have recently been found to 'clone' themselves (Eaves and Palmer, 2003) as another form of asexual reproduction.

Sea star development is similar to sea urchin development, although the bipinnaria (Fig. 2H) and brachiolaria larvae are not the same shape as the pluteus larva of the sea urchin (Fig. 2E-G). In addition, there is no internal skeleton initially. Furthermore, some sea stars species brood their embryos until metamorphosis.

Box 1. Glossary

Ambulacra. Endoskeletal plates of the adult sea urchin.
Archenteron. Primitive gut formed by invagination.
Coelomic pouch. A mesodermal structure that begins as a bud off the anterior end of the gut and eventually lines the coelomic cavity. In sea urchins, the left coelomic pouch becomes the rudiment of the adult. At metamorphosis that rudiment transforms into the juvenile animal.
Deuterostome. A group of animals in which the first invagination at gastrulation becomes the anus and the second invagination becomes the mouth. Deuterostomes include chordates, hemichordates and echinoderms.
Ectoderm. The outer germ layer that gives rise to the skin and organs of the skin plus the nervous system.
Endoderm. The inner germ layer that gives rise to the gut and to the organs that bud from the gut.
Gastrulation. The early period of development during which cells rearrange to form the germ layers and the primitive body plan of the animal.
Gene regulatory network (GRN). The transcription factors and signal transduction devices that specify cells of the embryo towards their many fates. As cell types become more diverse, the GRNs that control this diversification become increasingly complex.
Macromere. One of four large vegetal blastomeres at the 16-cell stage produced as a consequence of an unequal vegetal cleavage.
Mesoderm. Literally, the middle layer. This germ layer gives rise to muscle, skeleton, pigment cells, coelomic pouches and blastocoelar cells in the embryo and in the adult.
Mesomere. One of eight middle-sized cells in the animal half of the 16 cell-stage embryo. These cells primarily are fated to become ectoderm and neural ectoderm.
Metamorphosis. The process of transformation from an immature to an adult form. In sea urchins, this corresponds to the termination of a larval stage by a hormonally induced major anatomical transformation.
Micromere. One of four small cells at the vegetal pole at the 16-cell stage, fated to become skeletal cells.
Morphogenesis. The term used to describe cell rearrangements that generate the structures of the larva.
Morpholino. A chemically modified oligonucleotide in which a morpholine group substitutes the ribose of each base. This oligonucleotide resists RNAase digestion and binds to the translational start site of mRNAs (by base pair complementarity). It is thus used experimentally to block translation.
Regulative development. The ability of an embryo or a tissue to adjust to changed conditions. Usually, this refers to embryonic replacement of missing cell types.
Specification. The process of programming a cell toward its normal fate. This process uses transcription factors and signals to build a specific gene regulatory network.
Stomodeum. The ectodermal invagination that forms the mouth and oral cavity.
Transfate. A molecular mechanism whereby a cell switches from one fate to a fate that it normally does not adapt.

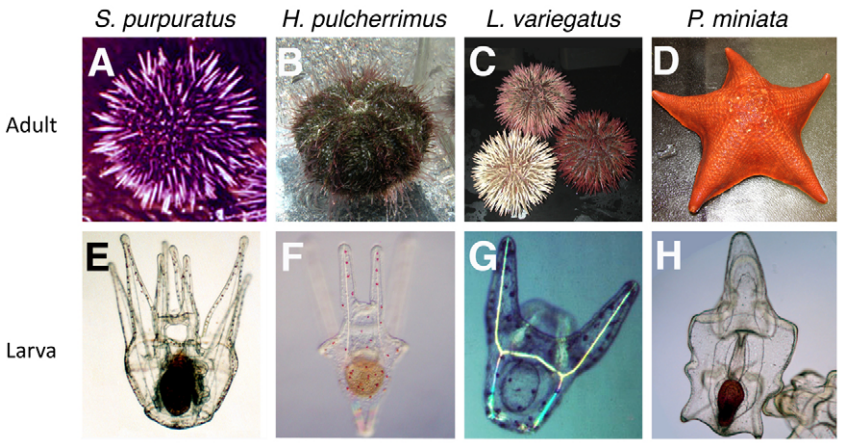


Fig. 2. Commonly used sea urchin and star fish species. (**A**) Adult *Strongylocentrotus purpuratus* (photo courtesy of Spbase). (**B**) Adult *Hemicentrotus pulcherrimus* (courtesy of Takuya Minokawa) (**C**) Adult *Lytechinus variegatus*, showing three color variants. (**D**) The adult star fish *Patiria miniata*. (**E**) Two-week-old larva of *S. purpuratus*. (**F**) Pluteus larva of *H. pulcherrimus*. (**G**) Pluteus larva of *L. variegatus* visualized by polarized light to show the larval skeleton (courtesy of Rachel Fink). (**H**) Bipinnaria larva of *P. miniata* (courtesy of Veronica Hinman).

Embryogenesis

The gravid season for echinoderms varies greatly between species: some species are gravid year round and other species are gravid for very short periods of time; others spawn with a lunar periodicity. Eggs and sperm of sea urchins are spawned into the seawater and fertilization occurs externally. Development to the larval stage is simple relative to other deuterostomes. Fertilization triggers activation of a high rate of metabolism (Epel, 1975), and the zygote begins a stereotypic radial pattern of cleavage (Fig. 3). The fourth and fifth cleavages are unequal in the vegetal half of the embryo, giving rise to the following groups of micromeres, macromeres and mesomeres (see Glossary, Box 1) arranged in tiers, beginning at the vegetal pole: four small micromeres (fated to the coelomic pouches, see Glossary, Box 1), four large micromeres (the future skeletogenic cells), four macromeres (future endoderm, see Glossary, Box 1, and nonskeletogenic mesoderm) and eight mesomeres (future ectoderm, see Glossary, Box 1, and neural ectoderm) (Fig. 3A-C). By the sixth cleavage, the embryo hollows, forming a blastocoel cavity in the center, and is now called a blastula. At mid-blastula stage, cilia appear and the embryo hatches from its fertilization envelope and enters the plankton community (Fig. 3D). Gastrulation then occurs in two temporally distinct phases. First, the vegetal half of the embryo flattens and the primary mesenchyme cells (the skeletogenic cells) ingress at the center of the vegetal plate (Fig. 3A). A short time after ingression, invagination of the archenteron (see Glossary, Box 1) begins. Non-skeletogenic mesoderm (NSM) cells in the center of the vegetal plate initiate invagination by changing shape to produce an inward bending sheet of cells. The archenteron then elongates to extend across the blastocoel by recruitment of endoderm cells into the archenteron, by elongation via convergent extension movements of cells and by extension of thin filopodia by the NSM cells to help pull the archenteron toward the animal pole (Fig. 3A,E). The archenteron reaches a target site near the animal pole and, shortly thereafter, the oral ectodermal epithelium invaginates to form the stomodeum (see Glossary, Box 1), which then fuses with the foregut epithelium.

To form the pluteus larva, the ectoderm changes shape, driven in part by growth of the underlying skeleton (Fig. 3E). The skeletal rods elongate in a species-specific pattern, whereas the gut subdivides into the foregut, midgut and hindgut, with muscular sphincters forming at the compartment junctions (Fig. 3F). Lateral to the foregut, coelomic pouches, progeny of the non-skeletogenic mesoderm and small micromeres, bulge to either side. The pluteus larva differentiates to produce a number of cells necessary for the skeleton, for neural transmission and for feeding (Fig. 4). The pluteus locomotes and feeds on plankton using coordinated beating of cilia growing in the ciliary band (Fig. 4C). As the larva grows, the rudiment of the adult grows from the left coelomic pouch (Fig. 4B). At metamorphosis, the larva settles and the mature rudiment transforms into the juvenile sea urchin.

Programming cells for diversification and morphogenetic movements

By the sixth cleavage, zygotic transcription is active in all cells and specification of the germ layers is well under way. To depict the sequence of specification, models of gene regulatory networks (GRNs, see Glossary, Box 1) in progeny of the macromeres, mesomeres and micromeres are used to show the relationship of

Box 2. Seminal discoveries in sea urchin development

For more than 150 years, the sea urchin embryo has been used for experimental purposes. During this time, a number of seminal discoveries have provided insights that dramatically influenced the field of biology. There are many discoveries of significance, but a few of the most prominent include the following.

- In 1892, Hans Driesch isolated blastomeres from sea urchin embryos at the two- and four-cell stages, and demonstrated that the isolated blastomeres were capable of producing complete embryos, thereby demonstrating regulative development (Driesch, 1892).
- In 1902, Theodore Boveri, using sea urchin eggs fertilized with two sperm, showed that only when nuclei inherited a complete set of chromosomes would development occur correctly. This was early evidence that established the chromosomal mechanism of inheritance (Boveri, 1902).
- In 1924, Sven Horstadius, using microsurgical techniques to recombine cells, demonstrated the inductive capacity of sea urchin embryo blastomeres (Horstadius, 1924).
- In 1982, Tim Hunt discovered cyclin proteins in sea urchin eggs (see Evans et al., 1983); this opened up research into the mechanism of cell cycle control. For this discovery, he was co-recipient of the Nobel Prize in 2001.
- In 1996, Eric Davidson and Cathy Yuh demonstrated the complexity of gene enhancers by showing the modular arrangement of the cis-regulatory apparatus of the *endo-16* gene in sea urchin development (Yuh and Davidson, 1996).
- In 2002, Eric Davidson and many other researchers in the sea urchin community produced a gene regulatory network model of sea urchin endomesoderm development, showing the basic mechanisms of cell specification at a systems level (Davidson et al., 2002).

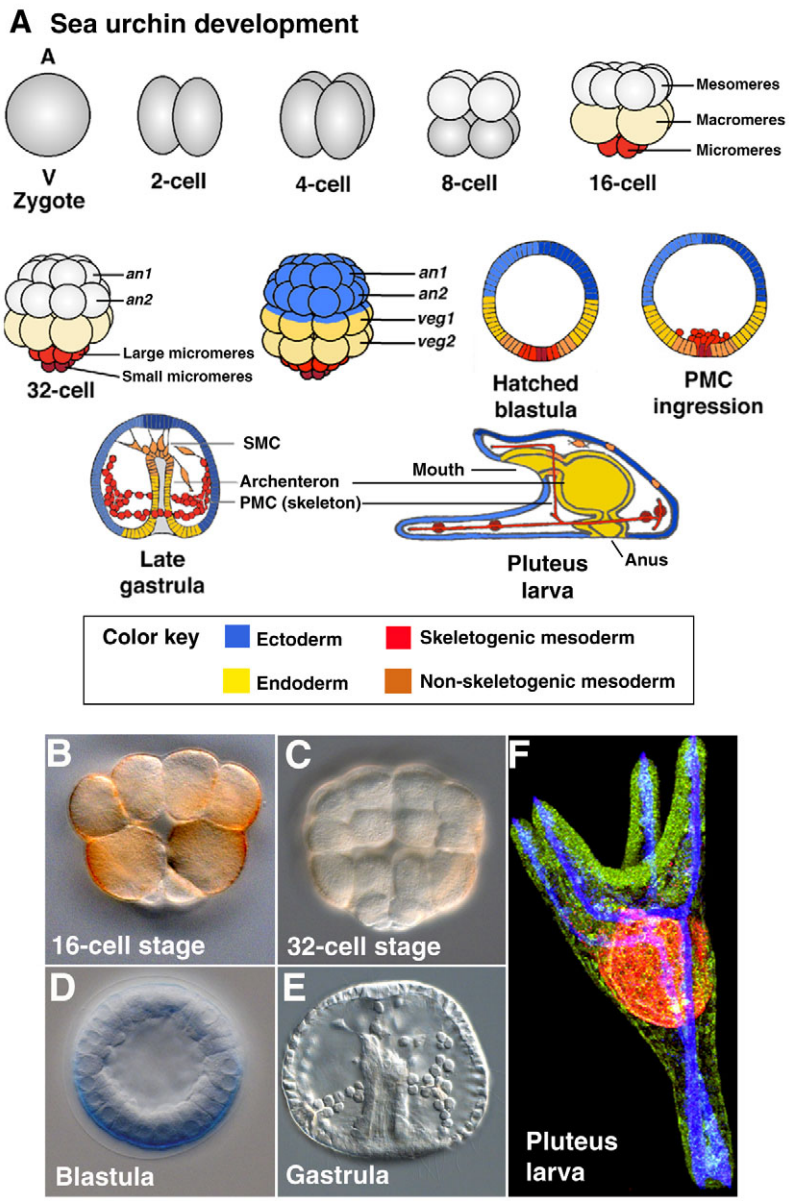


Fig. 3. Early sea urchin development. (**A**) Sequence of sea urchin development from the zygote to the pluteus larva stage. At the 16-cell stage there are four micromeres (red) at the vegetal (V) pole, four central macromeres (light yellow) and eight mesomeres (grey) at the animal (A) pole. From the hatched blastula stage onwards, the embryo is shown as a mid-sagittal section. The colors indicate when the cells begin to be specified toward ectoderm (blue), mesoderm (red) and endomesoderm (yellow). Later, the ectoderm becomes subdivided (as indicated by different shades of blue), and the mesoderm (orange) separates from endoderm (dark yellow). (**B-E**) Selected stages of *Paracentrotus lividus* development: (B) 16-cell stage; (C) 32-cell stage; (D) blastula stage; and (E) mid-gastrula stage, showing the gut invaginating and the skeletogenic cells forming a ring of cells around the gut and beginning to synthesize the skeleton. (**F**) Pluteus larva stained to show the gut (red), the skeleton (blue) and the ectoderm (green). an, animal; veg, vegetal; PMC, primary mesenchyme cells (skeletogenic cells); SMC, secondary mesenchyme cells (non-skeletogenic mesoderm).

transcription factors and signals over time (Fig. 5 and Box 3). This specification continues through the mesenchyme blastula stage, so that, as the embryo nears gastrulation (which starts at around tenth cleavage), the GRN activates cell biological mechanisms that drive morphogenesis. Thus, by gastrulation, each cell type reaches a distinct GRN state, and that specification state provides the unique instructions required by each cell for successful morphogenesis and for cell type-specific differentiation.

Experimental techniques in Echinoderm research

Many approaches enable mechanistic studies of the development of echinoderms, in particular that of sea urchins [discussed in detail by Ettensohn et al. (Ettensohn et al., 2004)]. Adult sea urchins are collected or obtained from commercial shippers when the animals are gravid. Eggs and sperm are obtained from different species either by shaking adults vigorously, by injection of 0.5 M KCl, by isolating gonads from the adults, by electrical stimulation or by neurotransmitter stimulation to cause gamete release. To fertilize eggs, concentrated sperm is diluted in seawater and a few drops of this suspension are added to the eggs. The embryos grow well in artificial seawater, making them appropriate for both laboratory and classroom use, and amenable to many experimental techniques.

Biochemical and cell biological analysis

Millions of sea urchin eggs can be fertilized at the same time and will develop synchronously, providing ample material for biochemical studies. There are many ways to isolate and purify individual cell types of the embryo for biochemical purification and/or molecular and cell biological analysis. Embryos can be dissociated by removal of calcium (McClay, 2004), and specific cells can be isolated using gradient centrifugation (Hynes and Gross, 1970), lectin adhesive affinity, which selectively isolates skeletogenic cells (Ettensohn and McClay, 1987), or by fluorescent cell sorting following expression of fluorescent reporters that are expressed in specific cell types (Arnone et al., 2004). Large quantities of RNA, DNA, proteins or other cellular components for study can be isolated from such cultures at any stage (Foltz et al., 2004).

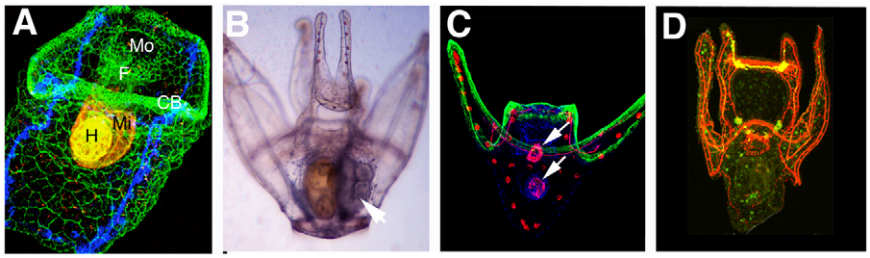


Fig. 4. Sea urchin larval structures. (**A**) Larva of *S. pururatus* at 72 hours stained to show the hindgut (H) and midgut (Mi, red), skeleton (blue) and ectoderm (green). The mouth (Mo), foregut (F) and ciliary band (CB) are also shown. (**B**) Larva of *H. pulcherrimus* at 7 days showing the rudiment to the side of the gut (arrow). Image courtesy of Takuya Minokawa. (**C**) Larva of *L. variegatus* at 48 hours showing the sphincter muscles between the foregut and midgut (upper arrow), and between the midgut and hindgut (lower arrow). The ciliary band (green), the foregut (blue) and skeletogenic cells (red) are also shown. (**D**) Larva of *L. variegatus* at 72 hours showing the neural cells that include serotonergic neurons (yellow) and non-serotonergic neurons (red). The cell bodies and nerves of this system run within the ciliary band, with an additional circle of neurons surrounding the sphincter muscle between the foregut and midgut (compare C with D). Cell nuclei are shown in green.

Genetic manipulations, perturbations and lineage tracing

Injections can be used to perturb embryos at the molecular level, or to introduce various molecular reagents into eggs or individual cells (Cheers and Ettensohn, 2004). Thousands of eggs, if necessary, can be injected in one sitting for a wide range of molecular studies. For example, the injection of morpholinos (see Glossary, Box 1) can be used to block translation of specific mRNAs (or of splice junctions in RNA processing) and, thus, to assess protein function. The expression of dominant-negative molecules, the ectopic expression of molecules, the overexpression of a molecule, or the inclusion of inhibitors that block specific signals or pathways can similarly be used to assess gene function. The advantage to the investigator is that endpoint assays involving in situ hybridization, antibody staining or PCR allow one to gain an impression of gene function during development. These approaches allow the investigator to assess whether a gene function is necessary and whether it is sufficient. This, in turn, progresses to epistatic studies that aim to determine which protein depends on which and to establish the regulatory relationships between genes. For lineage-tracing studies, individual cells can be injected up to the 16-cell stage with constructs or fluorescent markers. Mosaic approaches allow the investigator to study how the progeny of a single inserted founder cell develop relative to a control founder cell, starting at that same position in the embryo. Immunostaining or in situ analyses at the end of the study then provide spatial information about the expression of a specific protein or mRNA relative to controls. Time-lapse microscopy enables one to follow the trajectory and behavior of fluorescently labeled cells in whole mounts, with the small size and transparency of the embryo allowing it to be visualized in its entirety.

Temporary transgenesis

The ability to inject DNA into eggs also provides an easy method for the production of transgenic embryos. If the construct contains an appropriate enhancer and promoter, it will be activated and transcribed in the correct cells and at the correct time in development. Such constructs usually are introduced along with a reporter (Arnone et al., 2004) that allows the visualization of expression of that molecule. This approach has many uses, one of the most common being cis-regulatory analysis (see below). BACs (bacterial artificial chromosomes) that are modified by insertion of a reporter to replace the coding sequence of the gene offer another transgenic approach.

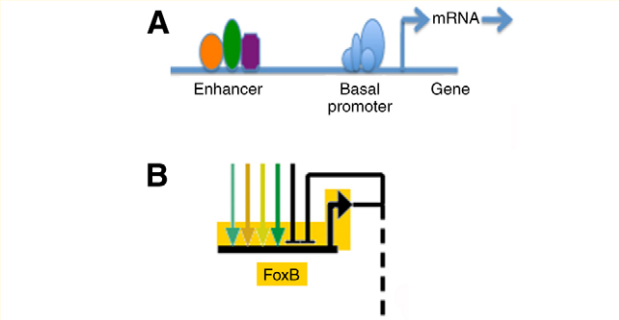
Cis-regulatory analysis reveals how the expression of a gene is controlled

In cis-regulatory analyses, a reporter gene that expresses a fluorescent or colorimetric marker and replaces the protein-coding sequence of the gene is constructed. This gene construct is then injected into eggs and the spatial and temporal expression of the reporter is followed (Arnone et al., 2004). If the reporter

Box 3. Gene regulatory networks

A gene regulatory network (GRN) (e.g. that shown in Fig. 5) is a graphic model of the transcription factors and signals necessary to specify a particular cell type. Each gene in a developmental GRN is depicted to show the transcription factors binding to its enhancer and the initiation factors binding to its basal promoter. If the enhancer information is sufficient to activate the gene (as shown below in A), an mRNA is produced (arrow) and presumably will be translated into a functional protein. For each gene within the network, gene activation is represented by arrows and gene inactivation is represented by repressive bars. For example, in the skeletogenic GRN, FoxB (forkhead B transcription factor; shown below in B) is activated by four inputs and repressed by two inputs, one of which corresponds to its own feedback repression.

GRNs are assembled experimentally by sequentially perturbing each gene and noting the effect on the other genes in the network compared with controls. GRNs are useful because they allow investigators to model complex information and gain insight into how cells work. For example, they provide insight into developmental phenomena such as competence, induction, commitment, reversible/irreversible differentiation and other properties displayed by cells as they contribute to the development of an embryo.



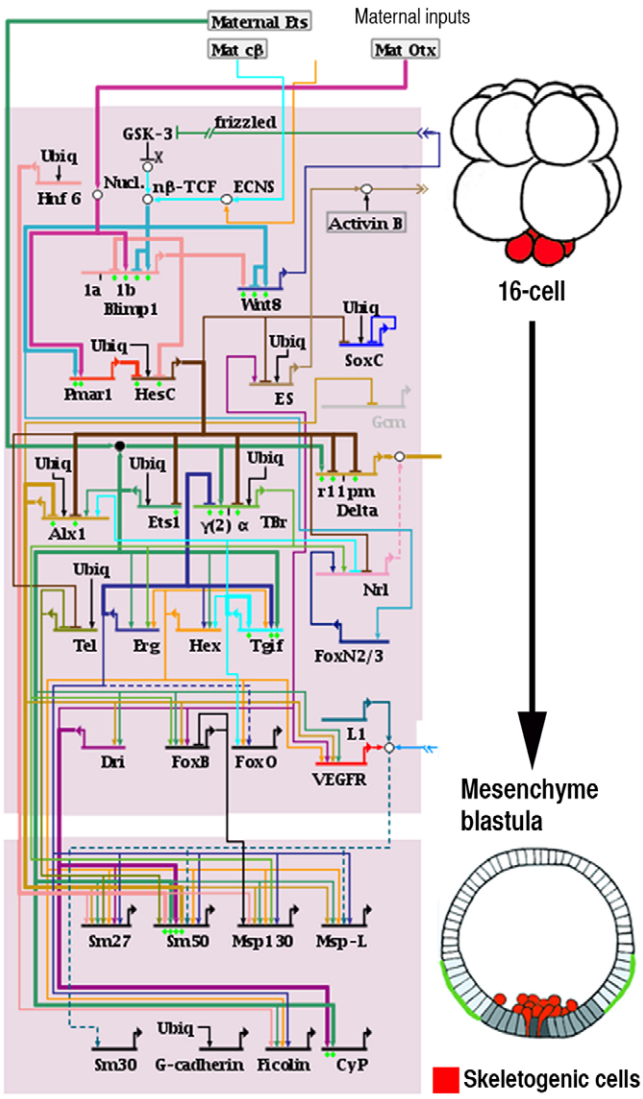


Fig. 5. Gene regulatory network model for specification of the skeletogenic mesoderm lineage. A graphic model representing the GRN involved in skeletogenic micromere specification (see Box 3 for further details). At the top of the model, known maternal transcription factor inputs initiate specification in the micromeres at the 16-cell stage (shown as red cells in the embryo on the right). These cells activate Pmar1 [a transcription factor, (TF) and an obligate repressor], which then represses another repressor (HesC, TF), thereby activating Alx1 (TF), Ets1 (TF), Tbr (TF) and Delta [a ligand (L) for the Notch signal transduction pathway]. Over time, additional genes are activated as the embryo forms the mesenchyme blastula stage (depicted by the lower embryo on the right), at which point the skeletogenic cells (red) are fully specified and ingress into the blastocoel. Light gray indicates endoderm; dark gray indicates nonskeletogenic mesoderm. Modified, with permission, from E. Davidson (http://sugp.caltech.edu/endomes/). γ(2)α, cis regulatory sites of the Tbr transcription factor (TF); Blimp, TF; cβ, cytoplasmic β-catenin; CyP, a differentiation gene (DG); Dri, TF; ECNS, early cytoplasmic nuclearization system; Erg, TF; ES, early signal; Ets, TF; Fox, TF; GSK-3, enzyme in canonical Wnt signal transduction pathway; Hex, TF; Hnf6, TF; L1, transmembrane co-receptor of VEGFR; Msp, DG; nβ, TF; Nr1, neuralized, a ubiquitin ligase; Nucl., nucleus; Otx, TF; r11pm, cis-regulatory module of Delta signal; Sm, skeletal matrix; SoxC, TF; TCF, TF; Tel, TF; Tgif, TF; Ubiq, ubiquitous TF; VEGFR, VEGF signal transduction receptor; Wnt8, a signal in the Wnt signal transduction pathway.

gene construct contains the entire enhancer region of the gene, the expression pattern of the reporter should match that of the endogenous gene. If that is the case, the investigator then fragments the non-coding sequence of the construct until the enhancer region(s) is/are narrowed to one or more modules (Yuh and Davidson, 1996). Recent advances in cis-regulatory analysis have shortened what traditionally is a long and labor-intensive process. First, computational approaches that compare non-coding sequences for a given gene between two species provide information on putative cis-regulatory modules for this analysis. Then, in order to accelerate the analysis, 'bar coded' putative cis-regulatory modules allow simultaneous analysis of up to one hundred or more reporters simultaneously (Nam et al., 2010). This greatly reduces the time needed for an analysis. Another recent advance for cis-regulatory analysis uses BAC expression reporters to provide constructs containing 100 kb or more of non-coding DNA, in order to ensure that the entire enhancer region is present (Smith et al., 2007; Ben-Tabou de-Leon and Davidson, 2010).

Genomic information enabling cis-regulatory analysis for several species can be found on a frequently updated website (see SpBase at http://www.spbase.org/SpBase/). These databases provide increasingly valuable resource information for cis-regulatory analysis, and for evo-devo studies that examine how genomes compare and how they evolved.

Key recent discoveries and impact on the field

Because of its accessibility, its rapid synchronous development, its amenability to experimental manipulation and its optical transparency, the sea urchin embryo continues to be a valuable model organism for studying the mechanisms that pattern embryonic tissues and for constructing the GRNs that control this early development. With the addition of molecular and genomic information, the sea urchin is a leading model for determining specification mechanisms that establish the major tissue territories in the embryo. Furthermore, the recent advances in GRN construction have allowed us both to understand how these specification events occur and to track how these complex networks have evolved. Some of the key findings enabled by research with this model are discussed below.

Elucidation of the mechanisms that pattern the embryo

The Wnt pathway controls endomesoderm specification

Three laboratories independently discovered that the Wnt pathway is crucial for the activation of endomesoderm development in sea urchins (Emily-Fenouil et al., 1998; Wikramanayake et al., 1998; Logan et al., 1999). β-Catenin, a key component of the Wnt signaling pathway, was observed to enter the nucleus of micromeres at fourth cleavage and later entered the nucleus of macromeres and their progeny (Logan et al., 1999). As a transcriptional co-activator, β-catenin was found to be essential for activation of the endomesoderm GRN and further analyses have established that β-catenin activates *pmar1* (paired-class micromere anti-repressor, a transcription factor, see Fig. 5) to launch the micromere GRN (Oliveri et al., 2002; Oliveri et al., 2003) and that it activates Wnt8 and homeobox (Hox) 11/13b to initiate the endoderm GRN (Peter and Davidson, 2010).

Delta-Notch signaling controls mesoderm formation

Elimination of Notch signaling was observed to prevent specification of mesoderm (Sherwood and McClay, 1997; Sherwood and McClay, 1999). It was soon discovered that Delta,

a Notch ligand that is produced first by micromeres and later by the non-skeletogenic mesoderm, activates mesoderm specification in two intervals of signaling (Sweet et al., 2002; Croce and McClay, 2010). The pattern of Delta expression is controlled dynamically, allowing progressive expression of Delta in cells more distant from the vegetal pole (Smith and Davidson, 2008).

Nodal and BMP govern formation of new embryonic axes

The Nodal pathway, along with BMP (bone morphogenetic protein) signaling, Lefty and Chordin, was discovered to organize oral-aboral (also called ventral-dorsal) territories of ectoderm (Duboc et al., 2004; Duboc et al., 2005; Duboc et al., 2008; Bradham et al., 2009). An apparent initial asymmetry involving subtle redox differences (Coffman and Denegre, 2007), plus a possibly related asymmetric inactivation of p38 mitogen-activated protein kinase (Bradham and McClay, 2006), are somehow necessary for the asymmetric expression of Nodal on the oral ectoderm. Nodal expression then controls activation of both the oral GRN state and activation of BMP2/4, which is important for aboral ectoderm specification (Angerer et al., 2000; Duboc et al., 2004). Nodal later provides a signaling input that is important for specifying left-right asymmetries (Duboc et al., 2005), although, surprisingly, Nodal operates on the right side (compared with the situation in vertebrates where it operates on the left). In addition, Hedgehog signaling contributes to left-right patterning in the sea urchin (Walton et al., 2009), a property that is also shared with left-right specification in vertebrates (Levin et al., 1995).

A small territory at the animal pole controls neural specification

A small group of cells at the animal pole organizes into a unique territory of the embryo called the apical plate. This territory is defined during specification of the ectoderm by progressive restriction of FoxQ2 (forkhead Q2) and Six3 (sine oculis-related homeobox 3 homolog) expression (Yaguchi et al., 2008; Wei et al., 2009). Some of the cells within this territory initiate specification of the first neural cells of the embryo (Burke et al., 2006a; Burke et al., 2006b; Yaguchi et al., 2006; Yaguchi et al., 2007; Yaguchi et al., 2008; Wei et al., 2009). The area of neural cell specification is restricted initially to a small region of the animal pole domain by Nodal signaling from the oral side and by BMP signaling from the aboral side (Nakajima et al., 2004; Yaguchi et al., 2007; Bradham et al., 2009), although the details of these regulatory mechanisms are unclear. Neurogenesis also occurs later in a subset of cells in the ciliary band (Saudemont et al., 2010), establishing an elaborate neural network in the pluteus larva (Fig. 4D).

Small micromeres contribute to germ cell precursors

Small micromeres appear at the vegetal pole as products of the unequal fourth and fifth cleavages. These cells divide only once more and then the majority of the eight small micromeres are incorporated into the left coelomic pouch, which becomes the rudiment of the adult. When small micromeres were removed and embryos grown to adulthood, few, if any, gametes were present in the gonad, suggesting that small micromeres contribute to formation of the germ cell precursors (Yajima and Wessel, 2011). Small micromeres also express many of the markers that characterize germ cell precursors in other animals (Juliano et al., 2010; Gustafson et al., 2011), further supporting their role as germline precursors.

Early development is regulative

Cells of the sea urchin embryo have the capacity to transfate (see Glossary, Box 1) or to switch from one cell type to another (Ettensohn and McClay, 1988; Ettensohn et al., 2007). Interestingly, transfating was discovered to take shortcuts: rather than reverting back to the top of a GRN and repeating the specification sequence, the switch is somehow accomplished by moving to the middle of the network of the alternate fate; the cells then switch their GRN state to the newly adopted cell type (Ettensohn et al., 2007). Transfating is a regulative capacity of many sea urchin embryonic cells, originally discovered more than a century ago (Driesch, 1892). This capacity has been described as a ‘failsafe’ mechanism, at least in micromeres, in which one set of experiments shows reactivation of the micromere network after blocking upstream initiation of specification (Smith and Davidson, 2009). The full range of this regulative development (see Glossary, Box 1) is not understood but must use both signaling and altered expression of transcription factors to detect and then replace missing or incorrectly specified cells.

Analysis of sea urchin GRNs reveals complex specification sequences

After a decade of transcription factor and signal discovery, a landmark GRN graphic model was produced based on perturbation analyses of transcription factors and signals used in the early sea urchin embryo (Davidson et al., 2002). Many laboratories added additional nodes and refined existing connections (Angerer et al., 2000; Angerer et al., 2001; Ettensohn et al., 2003; Wikramanayake et al., 2004; Smith et al., 2007; Oliveri et al., 2008; Smith and Davidson, 2008). As the GRN models advanced, cis-regulatory analyses of transcription factors in the network were added to authenticate, correct and expand the relationship between signals and transcription factors that control endomesoderm specification (Yuh et al., 2001; Minokawa et al., 2005; Ransick and Davidson, 2006; Nam et al., 2007; Range et al., 2007; Wahl et al., 2009; Ben-Tabou de-Leon and Davidson, 2010). The skeletogenic cell GRN is shown in Fig. 5, and a website for the current gene regulatory network model and the data that underpin it can be found at http://sugp.caltech.edu/endomes/.

Knowledge of the gene regulatory network that operates in early sea urchin embryo development also has provided a systems-level glimpse of the mechanisms that are used to control subcircuits within the network. Many regulatory mechanisms reported in other systems are included as control devices in the sea urchin GRN circuits. These include the ‘double-negative gate’ (Revilla-i-Domingo et al., 2007), a mechanism by which a repressor represses an early repressor, thereby allowing activation of early genes in the network. Other devices include ‘lockdown’ or ‘feed-forward’ loops, which are mechanisms in which one or more transcription factors in a series feed back to activate another in that series, creating a positive feed-forward regulatory mechanism and often simultaneously providing a lockdown mechanism that tends to prevent network reversal (Davidson and Levine, 2008; Oliveri et al., 2008). A number of self-repression regulatory elements have been discovered whereby a gene first activates a pathway and then, as its expression increases, begins to repress itself (Davidson, 2009). Additional mechanisms have been found to control more complex dynamics (Smith and Davidson, 2008).

The evolution of GRNs

A comparative analysis of the endomesoderm GRN between sea urchins and sea stars showed that much of the early circuitry responsible for activation of endoderm is conserved, despite an

evolutionary separation from a common ancestor more than 500 million years ago (Hinman et al., 2003; Hinman et al., 2007). More distant comparisons show that many of the genes that operate early in the sea urchin GRN are also likely to govern the activation of GRNs in hemichordates (Lowe et al., 2006; Lemons et al., 2010), and components of the early network may also be conserved in other deuterostomes. The Wnt pathway, as an example, has a role in the vegetal activation of eggs in all deuterostomes studied (Moon et al., 1997; Croce and McClay, 2006). Many other 'developmental genes' work together in developmental processes throughout the deuterostomes. Goosecoid, for example, works with Nodal in many organisms, including the sea urchin (Duboc et al., 2004). The processes these genes control may differ, but cassettes of genes seem to be conserved by working together. With comparative knowledge of GRNs, it becomes possible to infer mechanisms of evolutionary change (Davidson and Erwin, 2009), with the sea urchin representing a basal deuterostome. A recent analysis shows that the sea urchin skeletogenic GRN is similar between species as distant as sea urchins and sea stars (Gao and Davidson, 2008). In modern sea urchins, the progeny of the micromeres produces the larval skeleton. However, a recent study shows that non-micromere mesoderm produces juvenile and adult stage skeleton (Yajima, 2007). Those data support the notion that skeletogenesis is an ancient function of mesoderm and, in euechinoids, the skeletogenic cells co-opted the skeletogenic GRN and precociously produced the larval skeleton.

Limitations and future directions

The biggest limitation in echinoderm research is the relative inability to use genetics as a research tool. The generation time (egg to egg) is too long for mutagenesis to be used practically as a tool for genetic discovery of mechanism, and handicaps the establishment of permanent transgenic lines. This limitation is of much less significance than a decade ago when the current array of molecular tools was unavailable. Another limitation is that there are no echinoderm cell lines. Therefore, processes can be studied effectively only in vivo. Fortunately, the simplicity of the embryo provides an excellent opportunity for the discovery of the mechanisms that govern many developmental processes, such as fertilization, cleavage asymmetry, epithelial-mesenchyme transitions and morphogenetic movements, all processes that cannot be imitated in cell culture.

Future research in echinoderm biology, as in every other system, will benefit from advances in technology. Additional sequenced genomes will inform cis-regulatory analyses and will improve our understanding of the function of non-coding sequences. Furthermore, comparative genomic approaches will expand our evolutionary insights. High-throughput sequencing and increased speed and sophistication of software will accelerate methods that currently are slow. The recently developed ability to perform cis-regulatory analyses on many genes at once, rather than laboriously dissecting one gene at a time (Nam et al., 2010), is an example of that rapidity, and will enable cis-regulatory analysis to be a central component of systems biology and of genomic analysis. Expanded databases will also enable comparisons between control and experimentally perturbed cells. Hopefully, tools will become available to introduce specific perturbations at any time and into at any location in the embryo. This will allow embryos, with the exception of the perturbed cell/tissue, to develop normally except for the time and place of the perturbation, thereby enhancing our ability to understand the mechanisms behind specific developmental events or the production of distinct structures during development. Furthermore, improved imaging technologies will expand our capacity to examine cellular function in this transparent embryo; with new fluorescent probes, an ability to uncage probes and novel fluorescent reporters available, we will be better able to address molecular function in the regulation of both gene expression and signaling. Such experimental advances should also provide the tools with which to dissect more thoroughly morphogenetic processes and evolutionary mechanisms.

Conclusions

Early specification events and the control of morphogenesis are rapidly being understood at a molecular and systems level in the sea urchin. The penetration of the complexity of early development in this relatively simple system provides important information about sea urchin development and, importantly, enables comparative studies with other deuterostomes. Many recent discoveries provide seminal insights into conserved developmental mechanisms, as well as providing examples in which processes appear to be co-opted for novel function. Much remains to be learned, however, about how specification works and how it controls morphogenesis, how morphogenetic movements are conducted at a cell biological level, how evolution acts on embryos, and, finally, how variation in embryonic development acts on evolution.

Acknowledgements

The author appreciates input from lab members and from anonymous reviewers, and images provided by a number of colleagues. The author's research is support by the NIH. Deposited in PMC for release after 12 months.

Competing interests statement

The author declares no competing financial interests.

References

Angerer, L. M., Oleksyn, D. W., Logan, C. Y., McClay, D. R., Dale, L. and Angerer, R. C. (2000). A BMP pathway regulates cell fate allocation along the sea urchin animal-vegetal embryonic axis. *Development* **127**, 1105-1114.

Angerer, L. M., Oleksyn, D. W., Levine, A. M., Li, X., Klein, W. H. and Angerer, R. C. (2001). Sea urchin goosecoid function links fate specification along the animal-vegetal and oral-aboral embryonic axes. *Development* **128**, 4393-4404.

Arnone, M. I., Dmochowski, I. J. and Gache, C. (2004). Using reporter genes to study cis-regulatory elements. *Methods Cell Biol.* **74**, 621-652.

Ben-Tabou de-Leon, S. and Davidson, E. H. (2010). Information processing at the foxa node of the sea urchin endomesoderm specfication network. *Proc. Natl. Acad. Sci. USA* **107**, 10103-10108.

Boveri, T. (1902). Uber mehrpolige mitosen als mittel zur analyse des zellkerns. *Verh. d. phys.-med. Ges. Wursburg N. F.* **35**, 67-90.

Bradham, C. A. and McClay, D. R. (2006). p38 MAPK is essential for secondary axis specification and patterning in sea urchin embryos. *Development* **133**, 21-32.

Bradham, C. A., Oikonomou, C., Kuhn, A., Core, A. B., Modell, J. W., McClay, D. R. and Poustka, A. J. (2009). Chordin is required for neural but not axial development in sea urchin embryos. *Dev. Biol.* **328**, 221-233.

Burke, R. D., Angerer, L. M., Elphick, M. R., Humphrey, G. W., Yaguchi, S., Kiyama, T., Liang, S., Mu, X., Agca, C., Klein, W. H. et al. (2006a). A genomic view of the sea urchin nervous system. *Dev. Biol.* **300**, 434-460.

Burke, R. D., Osborne, L., Wang, D., Murabe, N., Yaguchi, S. and Nakajima, Y. (2006b). Neuron-specific expression of a synaptotagmin gene in the sea urchin Strongylocentrotus purpuratus. *J. Comp. Neurol.* **496**, 244-251.

Cheers, M. S. and Ettensohn, C. A. (2004). Rapid microinjection of fertilized eggs. *Methods Cell Biol.* **74**, 287-310.

Coffman, J. A. and Denegre, J. M. (2007). Mitochondria, redox signaling and axis specification in metazoan embryos. *Dev. Biol.* **308**, 266-280.

Croce, J. C. and McClay, D. R. (2006). The canonical Wnt pathway in embryonic axis polarity. *Semin. Cell Dev. Biol.* **17**, 168-174.

Croce, J. C. and McClay, D. R. (2010). Dynamics of Delta/Notch signaling on endomesoderm segregation in the sea urchin embryo. *Development* **137**, 83-91.

Davidson, E. H. (2009). Network design principles from the sea urchin embryo. *Curr. Opin. Genet. Dev.* **19**, 535-540.

Davidson, E. H. and Levine, M. S. (2008). Properties of developmental gene regulatory networks. *Proc. Natl. Acad. Sci. USA* **105**, 20063-20066.

Davidson, E. H. and Erwin, D. H. (2009). Evolutionary innovation and stability in animal gene networks. *J. Exp. Zool. B Mol. Dev. Evol.* **314**, 182-186.

Davidson, E. H., Rast, J. P., Oliveri, P., Ransick, A., Calestani, C., Yuh, C. H., Minokawa, T., Amore, G., Hinman, V., Arenas-Mena, C. et al. (2002). A genomic regulatory network for development. *Science* **295**, 1669-1678.
Driesch, H. (1892). The potency of the first two cleavage cells in echinoderm development. Experimental production of partial and double formations. *In Foundations of Experimental Embryology* (ed. B. H. Willier and J. M. Oppenheimer), pp. 3-50. New York: Hafner.
Duboc, V., Rottinger, E., Besnardeau, L. and Lepage, T. (2004). Nodal and BMP2/4 signaling organizes the oral-aboral axis of the sea urchin embryo. *Dev. Cell* **6**, 397-410.
Duboc, V., Rottinger, E., Lapraz, F., Besnardeau, L. and Lepage, T. (2005). Left-right asymmetry in the sea urchin embryo is regulated by nodal signaling on the right side. *Dev. Cell* **9**, 147-158.
Duboc, V., Lapraz, F., Besnardeau, L. and Lepage, T. (2008). Lefty acts as an essential modulator of Nodal activity during sea urchin oral-aboral axis formation. *Dev. Biol.* **320**, 49-59.
Eaves, A. A. and Palmer, A. R. (2003). Reproduction: widespread cloning in echinoderm larvae. *Nature* **425**, 146.
Ebert, T. A. (2008). Longevity and lack of senescence in the red sea urchin Strongylocentrotus franciscanus. *Exp. Gerontol.* **43**, 734-738.
Emily-Fenouil, F., Ghiglione, C., Lhomond, G., Lepage, T. and Gache, C. (1998). GSK3beta/shaggy mediates patterning along the animal-vegetal axis of the sea urchin embryo. *Development* **125**, 2489-2498.
Epel, D. (1975). The program and mechanisms of fertilization in the echinoderm egg. *Am. Zool.* **15**, 507-522.
Ettensohn, C., Wessel, G. and Wray, G. A. (2004). *Development of Sea Urchins, Ascidians, and other Invertebrate Deuterostomes: Experimental Approaches*. San Diego: Elsevier, Academic Press.
Ettensohn, C. A. and McClay, D. R. (1987). A new method for isolating primary mesenchyme cells of the sea urchin embryo. Panning on wheat germ agglutinin-coated dishes. *Exp. Cell Res.* **168**, 431-438.
Ettensohn, C. A. and McClay, D. R. (1988). Cell lineage conversion in the sea urchin embryo. *Dev. Biol.* **125**, 396-409.
Ettensohn, C. A., Illies, M. R., Oliveri, P. and De Jong, D. L. (2003). Alx1, a member of the Cart1/Alx3/Alx4 subfamily of Paired-class homeodomain proteins, is an essential component of the gene network controlling skeletogenic fate specification in the sea urchin embryo. *Development* **130**, 2917-2928.
Ettensohn, C. A., Kitazawa, C., Cheers, M. S., Leonard, J. D. and Sharma, T. (2007). Gene regulatory networks and developmental plasticity in the early sea urchin embryo: alternative deployment of the skeletogenic gene regulatory network. *Development* **134**, 3077-3087.
Evans, T., Rosenthal, E. T., Youngblom, J., Distel, D. and Hunt, T. (1983). Cyclin: a protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division. *Cell* **33**, 389-396.
Foltz, K. R., Adams, N. L. and Runft, L. L. (2004). Echinoderm eggs and embryos: procurement and culture. *Methods Cell Biol.* **74**, 39-74.
Gao, F. and Davidson, E. H. (2008). Transfer of a large gene regulatory apparatus to a new developmental address in echinoid evolution. *Proc. Natl. Acad. Sci. USA* **105**, 6091-6096.
Gustafson, E. A., Yajima, M., Juliano, C. E. and Wessel, G. M. (2011). Post-translational regulation by gustavus contributes to selective Vasa protein accumulation in multipotent cells during embryogenesis. *Dev. Biol.* **349**, 440-450.
Hinman, V. F., Nguyen, A. T., Cameron, R. A. and Davidson, E. H. (2003). Developmental gene regulatory network architecture across 500 million years of echinoderm evolution. *Proc. Natl. Acad. Sci. USA* **100**, 13356-13361.
Hinman, V. F., Nguyen, A. and Davidson, E. H. (2007). Caught in the evolutionary act: precise cis-regulatory basis of difference in the organization of gene networks of sea stars and sea urchins. *Dev. Biol.* **312**, 584-595.
Hynes, R. O. and Gross, P. R. (1970). A method for separating cells from early sea urchin embryos. *Dev. Biol.* **21**, 383-402.
Juliano, C. E., Swartz, S. Z. and Wessel, G. M. (2010). A conserved germline multipotency program. *Development* **137**, 4113-4126.
Lemons, D., Fritzenwanker, J. H., Gerhart, J., Lowe, C. J. and McGinnis, W. (2010). Co-option of an anteroposterior head axis patterning system for proximodistal patterning of appendages in early bilaterian evolution. *Dev. Biol.* **344**, 358-362.
Levin, M., Johnson, R. L., Stern, C. D., Kuehn, M. and Tabin, C. (1995). A molecular pathway determining left-right asymmetry in chick embryogenesis. *Cell* **82**, 803-814.
Logan, C. Y., Miller, J. R., Ferkowicz, M. J. and McClay, D. R. (1999). Nuclear β-catenin is required to specify vegetal cell fates in the sea urchin embryo. *Development* **126**, 345-357.
Lowe, C. J., Terasaki, M., Wu, M., Freeman, R. M., Jr, Runft, L., Kwan, K., Haigo, S., Aronowicz, J., Lander, E., Gruber, C. et al. (2006). Dorsoventral patterning in hemichordates: insights into early chordate evolution. *PLoS Biol.* **4**, e291.
McClay, D. R. (2004). Methods for embryo dissociation and analysis of cell adhesion. *Methods Cell Biol.* **74**, 311-329.
Minokawa, T., Wikramanayake, A. H. and Davidson, E. H. (2005). cis-Regulatory inputs of the wnt8 gene in the sea urchin endomesoderm network. *Dev. Biol.* **288**, 545-558.
Moon, R. T., Brown, J. D. and Torres, M. (1997). WNTs modulate cell fate and behavior during vertebrate development. *Trends Genet.* **13**, 157-162.
Nakajima, Y., Kaneko, H., Murray, G. and Burke, R. D. (2004). Divergent patterns of neural development in larval echinoids and asteroids. *Evol. Dev.* **6**, 95-104.
Nam, J., Su, Y. H., Lee, P. Y., Robertson, A. J., Coffman, J. A. and Davidson, E. H. (2007). Cis-regulatory control of the nodal gene, initiator of the sea urchin oral ectoderm gene network. *Dev. Biol.* **306**, 860-869.
Nam, J., Dong, P., Tarpine, R., Istrail, S. and Davidson, E. H. (2010). Functional cis-regulatory genomics for systems biology. *Proc. Natl. Acad. Sci. USA* **107**, 3930-3935.
Oliveri, P., Carrick, D. M. and Davidson, E. H. (2002). A regulatory gene network that directs micromere specification in the sea urchin embryo. *Dev. Biol.* **246**, 209-228.
Oliveri, P., Davidson, E. H. and McClay, D. R. (2003). Activation of pmar1 controls specification of micromeres in the sea urchin embryo. *Dev. Biol.* **258**, 32-43.
Oliveri, P., Tu, Q. and Davidson, E. H. (2008). Global regulatory logic for specification of an embryonic cell lineage. *Proc. Natl. Acad. Sci. USA* **105**, 5955-5962.
Parks, A. L., Parr, B. A., Chin, J.-E., Leaf, D. S. and Raff, R. A. (1988). Molecular analysis of heterochronic changes in the evolution of direct developing sea urchins. *J. Evol. Biol.* **1**, 27-44.
Peter, I. S. and Davidson, E. H. (2010). The endoderm gene regulatory network in sea urchin embryos up to mid-blastula stage. *Dev. Biol.* **340**, 188-199.
Range, R., Lapraz, F., Quirin, M., Marro, S., Besnardeau, L. and Lepage, T. (2007). Cis-regulatory analysis of nodal and maternal control of dorsal-ventral axis formation by Univin, a TGF-beta related to Vg1. *Development* **134**, 3649-3664.
Ransick, A. and Davidson, E. H. (2006). cis-regulatory processing of Notch signaling input to the sea urchin glial cells missing gene during mesoderm specification. *Dev. Biol.* **297**, 587-602.
Revilla-i-Domingo, R., Oliveri, P. and Davidson, E. H. (2007). A missing link in the sea urchin embryo gene regulatory network: hesC and the double-negative specification of micromeres. *Proc. Natl. Acad. Sci. USA* **104**, 12383-12388.
Saudemont, A., Haillot, E., Mekpoh, F., Bessodes, N., Quirin, M., Lapraz, F., Duboc, V., Rottinger, E., Range, R., Oisel, A. et al. (2010). Ancestral regulatory circuits governing ectoderm patterning downstream of Nodal and BMP2/4 revealed by gene regulatory network analysis in an echinoderm. *PLoS Genet.* **6**, e1001259.
Sherwood, D. R. and McClay, D. R. (1997). Identification and localization of a sea urchin Notch homologue: insights into vegetal plate regionalization and Notch receptor regulation. *Development* **124**, 3363-3374.
Sherwood, D. R. and McClay, D. R. (1999). LvNotch signaling mediates secondary mesenchyme specification in the sea urchin embryo. *Development* **126**, 1703-1713.
Shu, D. G., Morris, S. C., Han, J., Chen, L., Zhang, X. L., Zhang, Z. F., Liu, H. Q., Li, Y. and Liu, J. N. (2001). Primitive deuterostomes from the Chengjiang Lagerstatte (Lower Cambrian, China). *Nature* **414**, 419-424.
Smith, J. and Davidson, E. H. (2008). Gene regulatory network subcircuit controlling a dynamic spatial pattern of signaling in the sea urchin embryo. *Proc. Natl. Acad. Sci. USA* **105**, 20089-20094.
Smith, J. and Davidson, E. H. (2009). Regulative recovery in the sea urchin embryo and the stabilizing role of fail-safe gene network wiring. *Proc. Natl. Acad. Sci. USA* **106**, 18291-18296.
Smith, J., Theodoris, C. and Davidson, E. H. (2007). A gene regulatory network subcircuit drives a dynamic pattern of gene expression. *Science* **318**, 794-797.
Sodergren, E., Weinstock, G. M., Davidson, E. H., Cameron, R. A., Gibbs, R. A., Angerer, R. C., Angerer, L. M., Arnone, M. I., Burgess, D. R., Burke, R. D. et al. (2006). The genome of the sea urchin Strongylocentrotus purpuratus. *Science* **314**, 941-952.
Sprinkle, J. (1992). Radiation of the echinodermata. In *Origin and Early Evolution of the Metazoa* (ed. J. H. Lipps and P. W. Signor), pp. 375-398. New York: Plenum Press.
Swalla, B. J. and Smith, A. B. (2008). Deciphering deuterostome phylogeny: molecular, morphological and palaeontological perspectives. *Philos. Trans. R. Soc. Lond. B* **363**, 1557-1568.
Sweet, H. C., Gehring, M. and Ettensohn, C. A. (2002). LvDelta is a mesoderm-inducing signal in the sea urchin embryo and can endow blastomeres with organizer-like properties. *Development* **129**, 1945-1955.
Wahl, M. E., Hahn, J., Gora, K., Davidson, E. H. and Oliveri, P. (2009). The cis-regulatory system of the tbrain gene: alternative use of multiple modules to promote skeletogenic expression in the sea urchin embryo. *Dev. Biol.* **335**, 428-441.
Walton, K. D., Warner, J., Hertzler, P. H. and McClay, D. R. (2009). Hedgehog signaling patterns mesoderm in the sea urchin. *Dev. Biol.* **331**, 26-37.

Wei, Z., Yaguchi, J., Yaguchi, S., Angerer, R. C. and Angerer, L. M. (2009). The sea urchin animal pole domain is a Six3-dependent neurogenic patterning center. *Development* **136**, 1179-1189.
Wikramanayake, A. H., Huang, L. and Klein, W. H. (1998). β-Catenin is essential for patterning the maternally specified animal-vegetal axis in the sea urchin embryo. *Proc. Natl. Acad. Sci. USA* **95**, 9343-9348.
Wikramanayake, A. H., Peterson, R., Chen, J., Huang, L., Bince, J. M., McClay, D. R. and Klein, W. H. (2004). Nuclear β-catenin-dependent Wnt8 signaling in vegetal cells of the early sea urchin embryo regulates gastrulation and differentiation of endoderm and mesodermal cell lineages. *Genesis* **39**, 194-205.
Wray, G. A. and Raff, R. A. (1989). Evolutionary modification of cell lineage in the direct-developing sea urchin Heliocidaris erythrogramma. *Dev. Biol.* **132**, 458-470.
Yaguchi, S., Yaguchi, J. and Burke, R. D. (2006). Specification of ectoderm restricts the size of the animal plate and patterns neurogenesis in sea urchin embryos. *Development* **133**, 2337-2346.
Yaguchi, S., Yaguchi, J. and Burke, R. D. (2007). Sp-Smad2/3 mediates patterning of neurogenic ectoderm by nodal in the sea urchin embryo. *Dev. Biol.* **302**, 494-503.
Yaguchi, S., Yaguchi, J., Angerer, R. C. and Angerer, L. M. (2008). A Wnt-FoxQ2-nodal pathway links primary and secondary axis specification in sea urchin embryos. *Dev. Cell* **14**, 97-107.
Yajima, M. (2007). A switch in the cellular basis of skeletogenesis in late-stage sea urchin larvae. *Dev. Biol.* **307**, 272-281.
Yajima, M. and Wessel, G. M. (2011). Small micromeres contribute to the germline in the sea urchin. *Development* **138**, 237-243.
Yuh, C. H. and Davidson, E. H. (1996). Modular cis-regulatory organization of Endo16, a gut-specific gene of the sea urchin embryo. *Development* **122**, 1069-1082.
Yuh, C. H., Bolouri, H. and Davidson, E. H. (2001). Cis-regulatory logic in the endo16 gene: switching from a specification to a differentiation mode of control. *Development* **128**, 617-629.