Published in final edited form as:
Biol Bull. 2008 June ; 214(3): 266–273.

The Sea Urchin Genome as a Window on Function

STEFAN C. MATERNA and **R. ANDREW CAMERON***
Division of Biology 156-29, California Institute of Technology, 1200 E. California Blvd., Pasadena, California 91125

Abstract

The emphasis on the sequencing of genomes seems to make this task an end in itself. However, genome sequences and the genes that are predicted from them are really an opportunity to examine the biological function of the organism constructed by that genome. This point is illustrated here by examples in which the newly annotated gene complement reveals surprises about the way *Strongylocentrotus purpuratus*, the purple sea urchin, goes about its business. The three topics considered here are the nature of the innate immune system; the unexpected complexity of sensory function implied by genes encoding sensory proteins; and the remarkable intricacy of the regulatory gene complement in embryogenesis.

Introduction

Everywhere in the scientific and popular literature, on journal web sites and personal blogs, the advances in reading DNA sequences and the implications of genomic analysis are discussed. While the focus of this attention is easily placed on the genomic data bits that are inundating the public databases, the real value of genomic data lies in its capacity to explain biological mechanisms in a new way. This may be even more true for the sea urchin than it is for other organisms: while many years of continual effort have revealed in fine molecular detail the developmental mechanisms leading to the construction of a sea urchin embryo, the development and molecular function of other life stages is less well understood. Knowledge of how an urchin defends itself against pathogen intruders and how it senses its environment is especially lacking.

The first wave of analysis to extract functional information from genomic data is usually gene discovery. A list of all the genes within a genome is a first approximation of all the factors available to an organism for executing the biological functions required during its lifespan. Some conclusion may be drawn almost immediately from browsing and comparing such lists between organisms: that mice have three times as many olfactory receptors as humans is a fitting explanation for the much keener sense of smell possessed by rodents (Young *et al.*, 2002). A slightly less obvious example from the sea urchin genome is the expansion of Toll-like receptor (TLR) proteins (Hibino *et al.*, 2006). These proteins, which function in innate immunity, were found in only small numbers in bilaterian genome sequences until now. The sea urchin genome carries a greatly expanded number of these molecules. These data reveal a different solution for diversifying the animal's immune repertoire in the absence of an immunoglobulin-based adaptive immune system. Analogous to genes involved in immunity, genes that are indicative of neural functions can be sought by querying the genome. For example, sensory receptors constitute the biggest group of genes in the sea urchin genome (Materna *et al.*, 2006a). This indicates that the sea urchin,

*To whom correspondence should be addressed. acameron@caltech.edu.

despite the lack of obvious sensory organs, has an outstanding ability to sense its environment.

Of course, biological function is not explained by merely identifying parts; the answer to many biological questions lies in how the parts interact. Nowhere is this more obvious than in the regulatory processes that underlie establishment of the larval body plan of the sea urchin. How regulatory genes interact is not yet discernible from mere inspection of the genomic sequence (although it eventually may be—at least in the case of sequence-specific protein-DNA interactions), but it can be revealed through demanding experimental approaches. What the genomic sequence can deliver is a complete list of all the regulatory factors encoded in it. This list, as compiled for *Strongylocentrotus purpuratus*, the purple sea urchin, now serves as the basis for complementing and expanding our knowledge of the gene regulatory network of early development.

Direct evidence for function emerges only from experiments that establish the developmental or physiological consequences of perturbations—for example, the loss of a gene's activity. But as the following cases compellingly demonstrate, knowledge of the genome allows us to reformulate hypotheses and develop more targeted experiments to deepen our understanding of sea urchin biology.

The Sea Urchin Genome and Immunity

Although the sea urchin has been a favorite subject of embryologists and a good deal is known about the molecular processes of its early development, information about its immune system is scarce. Recent interest in invertebrate immunity has been fueled by the discovery of alternative ways to create immune diversity by means other than recombination of immunoglobulin genes (Cooper and Alder, 2006). Lampreys, for example, use antigen-binding receptors that establish immune diversity by homologous recombination of leucine-rich repeats (LRR) (Pancer *et al.*, 2004). Whereas vertebrates have few LRR genes, lampreys and hagfish possess an LRR locus that allows assembly of a hugely diverse set of these receptors. It is reminiscent of the vertebrate V(D)J system for generation of antibodies and T-cell receptors, but it is based on homologous recombination rather than being mediated by the Rag recombinase genes. Fruit flies achieve complexity in their immune receptor repertoire by alternative splicing of the Down syndrome cell adhesion factor (DSCAM), a molecule that is also involved in guiding neural growth (Watson *et al.*, 2005).

A first experimental insight into how the sea urchin deals with unwelcome intruders was the discovery that coelomocytes and pigment cells have a macrophage-like function. In response to an immune challenge such as an injection with bacteria, the coelomocytes up-regulate expression of several classes of molecules, most prominently scavenger receptors, and a newly identified family, called185/333 proteins (Pancer, 2000; Terwilliger *et al.*, 2007). Despite earlier indications pointing toward an expanded set of these gene families in the sea urchin, the magnitude of their expansion—as well as that of another family, the Toll-like receptors (TLRs)—became known only by inspecting the genomic sequence.

TLRs are transmembrane proteins with a characteristic extracellular domain consisting of more than 20 LRRs that are arranged in a half-moon (Jin *et al.*, 2007). In vertebrates, TLRs bind antigens on both the concave and convex side, leading to conformational changes in the intracellular domain and in the recruitment of adapter proteins. In contrast to the genome of the chordates, that of the sea urchin contains a vastly expanded set of at least 214 TLR genes and is accompanied by a moderate expansion of downstream adaptors (Fig. 1; Hibino *et al.*, 2006). Signaling from some TLRs results in the activation of NF-κB, a transcription factor that has a locally restricted expression pattern in early sea urchin development (A. Ransick,

California Institute of Technology, Pasadena, CA, pers.comm.). Its restriction to pigment cells supports their role in immunity (Hibino *et al.*, 2006).

Scavenger receptors constitute a second expanded family of genes involved in immunity. They are membrane proteins characterized by several extracellular scavenger receptor cysteine-rich (SRCR) domains that are thought to provide specific binding capacity. There are more than a thousand SRCR domains in the sea urchin genome, distributed in about 218 different predicted gene models, thus outnumbering those found in vertebrate genomes by a factor of 10 (Fig. 1). In vertebrates, scavenger receptors are found in a number of immune cells and are involved in, among others, activation of T-cells, inhibition of B-lymphocyte proliferation, and regulation of the macrophage response (Mukhopadhyay and Gordon, 2004). Their specific role in sea urchin immunity is currently unknown and awaits experimental investigation.

No obvious orthologs of the 185/333 genes have been identified in organisms other than sea urchins, thus pointing to a novel acquisition in the sea urchin/echinoderm lineage (Fig. 1; Hibino *et al.*, 2006). Although not much is known about their biological function, 185/333 genes were shown to be highly expressed in coelomocytes. Only 10 gene models could be identified in the genome, which stands in sharp contrast to the high diversity that has been observed between their transcripts (Terwilliger *et al.*, 2007). This diversity might indicate that these genes are substrates that, by means of alternative splicing, or possibly a recombination-based process, contribute to immune diversity.

Although no direct evidence has been brought forward in support of a mechanism for rearranging genes in somatic cells, two orthologs of the above-mentioned mammalian *Rag* genes were found in the sea urchin genome (Fugmann *et al.*, 2006; reviewed in Rast *et al.*, 2006). In jawed vertebrates these recombinases mediate the rearrangement process. Although it is by no means certain that they are employed in a similar manner in sea urchins, their presence in the sea urchin genome at least indicates that the foundation on which the mammalian adaptive immune system evolved had already been laid in the last common ancestor of sea urchins and mammals.

In conclusion, it seems that the sea urchin is equipped with a diverse immune repertoire through a vastly expanded set of immune genes. The presence of a sea urchin immune system highly diverged from the basal bilaterian and based on different gene families than that of the vertebrates brings into better focus how sophisticated the biological functions of this apparently simple organism really are. It is also consistent with recent studies showing that sea urchins are long-lived animals that can survive for 30 years or more.

The Sea Urchin Genome and Sensory Reception

The sea urchin has no head. This lack of cephalization is a derived feature diagnostic for the pentamerally symmetrical echinoderms. When correlated with fossil evidence, glimpses of gene expression patterns during the formation of the adult body suggest that the original AP axis of these animals is the oral-aboral axis of the adult echinoderm body plan (Mooi and David, 1997; Peterson *et al.*, 2000). Since a head serves as a site for the concentration of sensory structures at the anterior end of a bilaterally symmetrical animal, it might be expected that an animal without a head or any notable structures for the support of sensory apparatus like antennae or eyestalks would lack complex sensory functions. But the gene complement of the sea urchin includes representatives of many of the sensory proteins found in the chordate deuterostomes (Burke *et al.*, 2006).

Nerve cells first appear in the sea urchin embryo at about 60 h postfertilization as single neurons that stain with an antibody for serotonin (Bisgrove and Burke, 1986). The cell

lineage giving rise to these cells is not invariant and, therefore, must be specified through intercellular communication (Fig. 2; Cameron *et al.*, 1993; reviewed in Burke *et al.*, 2006). A variety of studies using morphological observations and immunocytochemical staining with anti-synaptotagmin and anti-serotonin antibodies indicate that the nerve cells elaborate as the embryo feeds and grows (reviewed in Burke *et al.*, 2006). Evidence from the gene catalog confirms that sea urchins have, among others, serotonergic, GABAnergic, and dopaminergic neurons. However, no evidence for adrenalin- or melatonin-mediated neurotransmission has emerged (Burke *et al.*, 2006). The cell bodies of the diffuse nervous system of the larva lie in the apical plate and around the ciliated band that borders the oral ectoderm. Axons extend beneath the ciliated bands and to the muscles derived from the coeloms (Lacalli and West, 1993). A TGFβ ligand, Nodal, is necessary for oral ectoderm specification in embryos (Duboc *et al.*, 2004) and probably patterns the neural plate region of the embryo containing the earliest neurons. Smad transcription factors are known downstream effectors of the nodal signal, and sea urchin Smad2/3 has been shown to mediate patterning of the neurogenic ectoderm (Yaguchi *et al.*, 2007). The identification of a Smad2/3 homolog in the genome annotation formed the basis for the Sp-Smad2/3 in this study (Howard-Ashby *et al.*, 2006a). Given the presence of neural specification genes and genes of the neuron differentiation gene battery but the absence of elaborate sensory support structures, it will be interesting to see how sensory anatomy is specified in sea urchins.

A new portion of the nervous system appears in the adult sea urchin and reflects the pentameral symmetry of the adult body. The circumoral nerve ring lies near the peristomium on the oral surface and extends nerve fibers along the gut and into the Aristotle's Lantern, the jaw apparatus. Body wall structures are enervated by five radial nerves that extend toward the anal region along the inside of the calcareous test. The radial nerves send branches into each of the tube feet, the locomotory appendages that extend through the test. This part of the nervous system arises in the rudiment, a primordium for the adult oral surface that develops in the larval stage. The earliest structure that will give rise to the adult nerve cords is a thickening in the ectoderm-derived epithelium in the floor of the rudiment (Hyman, 1955). Synaptotagmin immunofluorescence shows that the adult nerve ring forms in the area where these thickenings first form (Burke *et al.*, 2006). mRNA transcribed from the Hox3 gene appears in these structures at about 4 weeks of development, but its role is not known (Arenas-Mena *et al.*, 2000). Taken together, these expression patterns suggest candidate genes for more targeted functional studies of the development of the sea urchin nervous system, both the diffuse and probably ancestral nerve net found in the larva and adult as well as the adult nerve tracts.

The behavioral response to a variety of environmental stimuli is another window into the enigmatic nervous system of sea urchins. In this respect their discriminatory powers are quite sophisticated. For example, they can distinguish between a nearby active or inactive predator (Phillips, 1978) or between food resources in a Y maze (Pisut, 2004; reviewed in Raible *et al.*, 2006). These studies of sensory capabilities imply the presence of a suite of receptor molecules. By homology among the bilaterians, the sensory molecules involved in photoreception and chemoreception come from subdivisions of the rhodopsin-type G-protein-coupled receptors (GPCR), more than 900 of which are found in the sequence of the first assembly of the sea urchin genome (Materna *et al.*, 2006a; Raible *et al.*, 2006). GPCRs transduce signals across cell membranes. While many function internally—as receptors for neurotransmitters, for example—these 7-transmembrane receptors are also involved in sensing environmental signals. A detailed analysis of the phylogenetic relationships of the sea urchin GPCRs revealed four independent groups of rather divergent proteins, many of which are clustered in the genome (Raible *et al.*, 2006). A suite of five representative members of these protein families is almost exclusively expressed in the early larva and in the tube foot and pedicellariae of the adult animal. In addition to the locomotory tube foot,

the pedicellariae are appendages that extend from the body wall. Both of these structures have been implicated in sensory activity. Typically, the jawed pedicellariae comprise three or more types that respond to different chemical substances.

GPCRs respond to light as well as to ions and organic odorants. Sea urchins exhibit a wide range of behaviors with respect to light, including predator avoidance, covering reactions, and shade seeking (Millott, 1975). A rhabdomeric GPCR has been putatively identified among the divergent classes of GPCRs. It is one of the four that are expressed in the structures that probably bear sensory cells (Raible *et al.*, 2006). The expression of this protein in the tips of the arms in early larvae is puzzling since no distinct sensory structures have been identified there.

The induction of metamorphosis, the transition from larval to adult habit, is initiated by a chemoreceptive event that in many species is a response to a bacterial biofilm (reviewed in Unabia and Hadfield, 1999). It occurs very rapidly through a massive reorganization of the larval tissues around the rudiment of the adult oral surface that has developed as the larva grows (reviewed in Pearse and Cameron, 1991). The mature larvae become competent to undergo this metamorphosis but remain in the water column until a suitable cue is encountered. Furthermore, many studies have shown that various neurotransmitter agonists and antagonists can influence the onset of metamorphosis (reviewed in Bishop and Brandhorst, 2003). Combined behavioral and histochemical studies have implicated cells that express nitrous oxide synthase and cyclic guanidine monophosphate in the inhibition of metamorphosis in the sea urchin *Lytechinus pictus* (Bishop and Brandhorst, 2001, 2003; Bishop *et al.*, 2001). The sea urchin homolog of a neuronal nitric oxide synthase gene has now been identified in the genome (Goel and Mushegian, 2006).

The Sea Urchin Genome and the Embryonic Regulome

While the inspection of the whole gene catalog directly yields insights into the immunobiology and sensory system of the sea urchin, such a parts lists can also be an invaluable resource for addressing more intricate problems. The repertoire of regulatory genes is a good example. Transcription factors are highly conserved genes and, despite minor expansions and reductions, the sets of transcription factors found in animal genomes are quite similar; more similar than predicted from diverse animal body plans (Davidson, 2006). Thus, how body plans are established cannot be deduced from gene lists alone but requires a rich knowledge of the regulatory processes in which these genes are used. This information is summarized in gene regulatory networks, as demonstrated for different developmental phenomena, *e.g.*, the regulatory processes underlying specification of endomesodermal cells in the sea urchin embryo (Ben-Tabou de Leon and Davidson, 2006, 2007). Identification and transcriptional profiling of all transcription factors in the sea urchin genome afforded a global view of the regulatory landscape of the embryo and now provides the information on which to expand and complete our understanding of the regulatory networks covering sea urchin development.

Transcription factors and signaling molecules are the elements that convey the information necessary to produce differential expression of genes. Transcription factors bind to the regulatory regions and activate target genes in a cell-specific manner, while signaling pathways connect gene activity to intercellular communication. These pathways ultimately alter expression levels of, most prominently, transcription factors (Davidson, 2006). Transcription factors have a canonical structure featuring a highly conserved DNA-binding domain and a variety of functional regions that modulate the activity of the transcriptional complex. Compared to the identification of genes coding for structurally more variable proteins, identification of full sets of transcription factors is rather easy. Searching the sea

urchin genome with a reference database consisting of mouse, human, and fly transcription factors revealed a minimal set of 283 transcription factors (Howard-Ashby *et al.*, 2006a, b; Tu *et al.*, 2006; Rizzo *et al.*, 2006). Among others, this includes homeobox genes, bHLH factors, nuclear receptors, basic zippers, and forkhead genes, which in this order represent the largest classes in the sea urchin genome (Table 1). Another large group of genes that may also include transcription factors are zinc finger genes. However, the zinc finger domain is also known to bind to RNA or other proteins. Overall there are about 380 zinc finger genes in the sea urchin genome, of which about 30 have been shown to be orthologs to vertebrate transcription factors (Materna *et al.*, 2006b). Although some transcription factors may have been missed due to assembly errors and sequencing problems posed by repetitive sequence, several lines of evidence indicate that more than 90% of all sea urchin transcription factors have now been identified. The overall number of transcription factors—excluding zinc fingers—is similar to that of *Drosophila*, and about half the number found in humans. A sea urchin member can be found for virtually all known classes of vertebrate transcription factors and, in fact, even for most subclasses (Howard-Ashby *et al.*, 2006a, b). Thus the diversification of transcription factor families must predate the echinoderm-chordate split with lineage-specific expansions, a theme that is emerging for most gene families in the different deuterostome lineages (Materna *et al.*, 2006a).

Transcriptional profiling revealed that transcription factor genes become activated at a more or less constant rate during the first 48 hours postfertilization—that is, up to late gastrulation. At this point about 80% of all transcription factors are, or have been, transcribed zygotically (Fig. 3A, B; Howard-Ashby *et al.*, 2006c). This finding speaks volumes about the complexity of the molecular processes that partition the embryo into distinct territories: before the ingression of the skeletogenic cells, a suite of embryonic territories have been established, and each now expresses a set of genes characteristic of its regulatory state (Davidson, 2006). At this point the embryo is not much more than a ball of cells with few defining morphological features, but already it has made use of about 50% of its transcription factors. Of course, in preparation for gastrulation more genes are recruited to prepare the embryo for the morphological changes that are about to ensue. The extensive use of regulatory genes reflects the tasks that need to be completed, as territories are defined, boundaries established, and alternative cell fates repressed. Many, but not all, of the newly identified regulatory genes are expressed in a localized manner in one or another region of the embryo (Howard-Ashby *et al.*, 2006a, b; Materna *et al.*, 2006b). Although ubiquitous factors cannot be directly responsible for localized expression of downstream genes, they still fill an important role in the mechanics of transcription, ensuring the stability and dynamics of the transcription program (Yuh *et al.*, 2001).

Many regulatory genes need to act in concert in order to drive development forward. In experiments that perturb the expression of individual genes, the links between regulatory genes can be queried through a precise quantitative monitoring of the effects. From systematic observations on gene expression under perturbed conditions, maps of gene regulatory networks are assembled. These maps reveal the complex topology of the network and expose feedback loops and other motifs responsible for the fail-safe execution of the developmental program (Alon, 2007; Materna and Davidson, 2007). A glimpse of this complexity is offered by the endomesoderm GRN (a small part of which is shown in Fig. 3C, D). Although this GRN covers only half of the embryo for a limited period of development, it contains more than 40 localized regulatory genes (Ben-Tabou de Leon and Davidson, 2007).

The set of newly identified regulatory genes from the genome annotation supports the effort to complete the GRN and expand it to other territories of the sea urchin, such as the endoderm. This is a big advance over previous methods of gene discovery such as

subtractive RNA screens (Rast *et al.,* 2002). Although they do successfully identify regulatory molecules, subtractive RNA screens are very labor intensive and may produce many false positives. Furthermore, there is no guarantee that all the important players have been identified. How the genome changed the identification of network genes is well illustrated by the recent identification of HesC as the Repressor of Micromeres (Revilla-i-Domingo *et al.,* 2007). This factor had been predicted on the basis of the results of perturbation experiments involving the *Pmar1* gene, but its identity had remained elusive. *Pmar1* drives specification of skeletogenic cells—the micromere descendants (Oliveri *et al.,* 2002). Because Pmar1 is a micromere-specific repressor, it must repress a second repressor —the Repressor of Micromeres—that prevents expression of skeletogenic genes in ectopic locations (Fig. 3C, D). When *Pmar1* is expressed ubiquitously, as can be achieved by mRNA injection, the Repressor of Micromeres is repressed and many cells in ectopic locations adopt a skeletogenic cell fate (Oliveri *et al.,* 2002). This double repression system assures that only micromeres express skeletogenic genes. Among the newly identified transcription factors were about 20 whose expression profile matched the expectations for the postulated repressor. Monitoring expression levels of these candidates following *Pmar1* overexpression, which causes an ectopic loss of the Repressor of Micromeres, shortened this list. Then, knockout of HesC proved that this gene is the searched-for repressor: It causes the same changes as can be observed in *Pmar1* overexpression, that is, ectopic specification of skeletogenic cells. Thus, knowledge of the transcription factor set afforded the possibility to both validate the GRN architecture and quickly identify the missing factor.

Conclusions

Knowledge of the genomic sequence and the annotated genes derived from it can sidestep experimental difficulties by providing a nearly complete list of the genes whose function may provide direct insights into how the sea urchin lives. As the above examples illustrate, this list has revealed some dramatic changes that distinguish the sea urchin from its deuterostome relatives. Foremost, in light of the recombination-based mechanisms that other deuterostomes use to create immune diversity, was the way the sea urchin achieves this goal, which was unexpected and surprising. Some insights from the genome underline that the sea urchin found similar, yet independent, solutions to other problems, as the expanded repertoire of sensory receptors shows. Although initial information about the behavior of this creature has been available for decades, the developmental and anatomical underpinnings of these processes still await thorough examination. Despite these extraordinary findings, the sea urchin genome information also enables many smaller, but nonetheless important, discoveries. The recent identification of HesC as the Repressor of Micromeres may be typical of the impact the sea urchin genome has for studies that are not directly concerned with the genome. In whatever way it will be used, the sea urchin genome is a tremendous resource that has made, and will continue to make, a difference.

Acknowledgments

We thank Dr. Eric H. Davidson and two anonymous reviewers whose comments improved the manuscript. The original work from the authors that was reviewed here received support from the National Science Foundation and the National Institutes of Health (NCRR and NICHD).

Literature Cited

Alon U. Network motifs: theory and experimental approaches. Nat Rev Genet. 2007; 8:450–461. [PubMed: 17510665]

Arenas-Mena C, Cameron RA, Davidson EH. Spatial expression of Hox cluster genes in the ontogeny of a sea urchin. Development. 2000; 127:4631–4643. [PubMed: 11023866]

Ben-Tabou de-Leon S, Davidson EH. Deciphering the underlying mechanism of specification and differentiation: the sea urchin gene regulatory network. Science STKE. 2006; 361:pe47.

Ben-Tabou de-Leon S, Davidson EH. Gene regulation: gene control network in development. Annu Rev Biophys Biomol Struct. 2007; 36:191–212. [PubMed: 17291181]

Bisgrove BW, Burke RD. Development of the nervous system of the pluteus larva of *Strongylocentrotus droebachiensis*. Cell Tissue Res. 1987; 248:335–343.

Bishop CD, Brandhorst BP. NO/cGMP signaling and HSP90 activity represses metamorphosis in the sea urchin *Lytechinus pictus*. Biol Bull. 2001; 201:394–404. [PubMed: 11751251]

Bishop CD, Brandhorst BP. On nitric oxide signaling, metamorphosis and the evolution of biphasic life cycles. Evol Dev. 2003; 5:542–550. [PubMed: 12950632]

Bishop CD, Bates WR, Brandhorst BP. Regulation of metamorphosis in ascidians involves NO/cGMP signaling and HSP90. J Exp Zool. 2001; 289:374–384. [PubMed: 11351325]

Burke RD, Angerer LM, Elphick MR, Humphrey GW, Yaguchi S, Kiyama T, Liang S, Mu X, Agca C, Klein WH, et al. A genomic view of the sea urchin nervous system. Dev Biol. 2006; 300:434–460. [PubMed: 16965768]

Cameron RA, Britten RJ, Davidson EH. The ciliated band of the sea urchin derives from both aboral and oral ectoderm territories. Dev Biol. 1993; 160:369–376. [PubMed: 8253270]

Cooper MD, Alder N. The evolution of adaptive immune systems. Cell. 2006; 124:815–822. [PubMed: 16497590]

Davidson, EH. The Regulatory Genome: Gene Regulatory Networks in Development and Evolution. Academic Press; San Diego: 2006.

Davidson EH, Rast JP, Oliveri P, Ransick A, Calestani C, Yuh CH, Minokawa T, Amore G, Hinman V, Arenas-Mena C, et al. A provisional regulatory gene network for specification of endomesoderm in the sea urchin embryo. Dev Biol. 2002; 246:162–190. [PubMed: 12027441]

Duboc V, Rottinger E, Besnardeau L, Lepage T. Nodal and BMP2/4 signaling organizes the oral–aboral axis of the sea urchin embryo. Dev Cell. 2004; 6:397–410. [PubMed: 15030762]

Fugmann SD, Messier C, Novack LA, Cameron RA, Rast JP. An ancient evolutionary origin of the Rag1/2 gene locus. Proc Natl Acad Sci USA. 2006; 103:3728–3733. [PubMed: 16505374]

Goel M, Mushegian A. Intermediary metabolism in sea urchin: the first inferences from the genome sequence. Dev Biol. 2006; 300:282–292. [PubMed: 16979151]

Hibino T, Loza Coll M, Messier C, Majeske A, Cohen A, Terwilliger D, Buckley K, Brockton V, Nair S, Berney K, et al. The immune gene repertoire encoded in the purple sea urchin genome. Dev Biol. 2006; 300:349–365. [PubMed: 17027739]

Howard-Ashby M, Materna SC, Brown CT, Chen L, Cameron RA, Davidson EH. The identification and characterization of transcription factor families in early *Strongylocentrotus* development. Dev Biol. 2006a; 300:90–107. [PubMed: 17054934]

Howard-Ashby M, Materna SC, Brown CT, Chen L, Cameron RA, Davidson EH. Identification and characterization of homeobox transcription factor genes in *Strongylocentrotus purpuratus*, and their expression in embryonic development. Dev Biol. 2006b; 300:74–89. [PubMed: 17055477]

Howard-Ashby M, Materna SC, Brown CT, Tu Q, Oliveri P, Cameron RA, Davidson EH. High regulatory gene use in sea urchin embryogenesis: implications for bilaterian development and evolution. Dev Biol. 2006c; 300:27–34. [PubMed: 17101125]

Hyman, LH. The Invertebrates: Echinodermata. McGraw-Hill; New York: 1955.

Jin MS, Kim SE, Heo JY, Lee ME, Kim HM, Paik SG, Lee H, Lee JO. Crystal structure of the TLR1-TLR2 heterodimer induced by binding of a triacylated lipopeptide. Cell. 2007; 130:1071–1082. [PubMed: 17889651]

Lacalli TC, West JE. A distinctive nerve cell type common to diverse deuterostome larvae: comparative data from echinoderms, hemichordates and amphioxus. Acta Zool. 1993; 74:1–8.

Materna SC, Davidson EH. Logic of gene regulatory networks. Curr Opin Biotechnol. 2007; 18:351–354. [PubMed: 17689240]

Materna SC, Berney K, Cameron RA. The *S. purpuratus* genome: a comparative perspective. Dev Biol. 2006a; 300:485–495. [PubMed: 17056028]

Materna SC, Howard-Ashby M, Gray RF, Davidson EH. The C_2H_2 zinc finger genes of *Strongylocentrotus purpuratus* and their expression in embryonic development. Dev Biol. 2006b; 300:108–120. [PubMed: 16997293]

Millott N. The photosensitivity of echinoderms. Adv Mar Biol. 1975; 13:1–52.

Mooi R, David B. Skeletal homologies of echinoderms. Paleontol Soc Papers. 1997; 3:305–335.

Mukhopadhyay S, Gordon S. The role of scavenger receptors in pathogen recognition and innate immunity. Immunobiology. 2004; 209:39–49. [PubMed: 15481139]

Oliveri P, Carrick DM, Davidson EH. A regulatory gene network that directs micromere specification in the sea urchin embryo. Dev Biol. 2002; 246:209–228. [PubMed: 12027443]

Pancer Z. Dynamic expression of multiple scavenger receptor cysteine-rich genes in coelomocytes of the purple sea urchin. Proc Natl Acad Sci USA. 2000; 97:13156–13161. [PubMed: 11069281]

Pancer Z, Amemiya CT, Ehrhardt GR, Ceitlin J, Gartland GL, Cooper MD. Somatic diversification of variable lymphocyte receptors in the agnathan sea lamprey. Nature. 2004; 430:174–180. [PubMed: 15241406]

Pearse, JS.; Cameron, RA. Echinodermata: Echinoidea. In: Giese, AC.; Pearse, JS.; Pearse, VB., editors. Reproduction of Marine Invertebrates. Vol. 6. Boxwood Press; Pacific Grove, CA: 1991. p. 514-662.

Peterson KJ, Arenas-Mena C, Davidson EH. The A/P axis in echinoderm ontogeny and evolution: evidence from fossils and molecules. Evol Dev. 2000; 2:93–101. [PubMed: 11258395]

Phillips DW. Chemical mediation of invertebrate defensive behaviors and the ability to distinguish between foraging and inactive predators. Mar Biol. 1978; 49:237–243.

Pisut, DP. Master's thesis. Georgia Institute of Technology; Atlanta, GA: 2004. The distance chemosensory behavior of the sea urchin *Lytechinus variegatus*.

Raible F, Tessmar-Raible K, Arboleda E, Kaller T, Bork P, Arendt D, Arnone MI. Opsins and clusters of sensory G-protein-coupled receptors in the sea urchin genome. Dev Biol. 2006; 300:461–475. [PubMed: 17067569]

Rast JP, Cameron RA, Poustka AJ, Davidson EH. *brachyury* target genes in the early sea urchin embryo isolated by differential macroarray screening. Dev Biol. 2002; 246:191–208. [PubMed: 12027442]

Rast JP, Smith LC, Litman G, Coll ML. Genomic insights into the immune system of the sea urchin. Science. 2006; 314:952–956. [PubMed: 17095692]

Revilla-i-Domingo R, Oliveri P, Davidson EH. A missing link in the sea urchin embryo gene regulatory network: *hesC* and the double-negative specification of micromeres. Proc Natl Acad Sci USA. 2007; 104:12383–12388. [PubMed: 17636127]

Rizzo F, Fernandez-Serra M, Squarzoni P, Archimandritis A, Arnone MI. Identification and developmental expression of the *ets* gene family in the sea urchin *(Strongylocentrotus purpuratus)*. Dev Biol. 2006; 300:35–48. [PubMed: 16997294]

Terwilliger DP, Buckley KM, Brockton V, Ritter NJ, Smith LC. Distinctive expression patterns of *185/333* genes in the purple sea urchin, *Strongylocentrotus purpuratus:* an unexpectedly diverse family of transcripts in response to LPS, β-1,3-glucan, and dsRNA. BMC Mol Biol. 2007; 8:16. [PubMed: 17331248]

Tu Q, Brown CT, Davidson EH, Oliveri P. Sea urchin forkhead gene family: phylogeny and embryonic expression. Dev Biol. 2006; 300:49–62. [PubMed: 17081512]

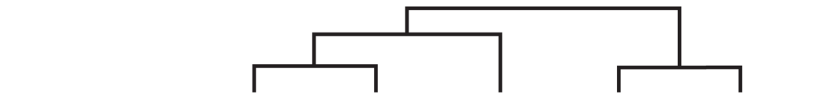
Unabia CRC, Hadfield MG. Role of bacteria in larval settlement and metamorphosis of the polychaete *Hydroides elegans*. Mar Biol. 1999; 133:55–64.

Watson FL, Püttmann-Holgado R, Thomas F, Lamar DL, Hughes M, Kondo M, Rebel VI, Schmucker D. Extensive diversity of Ig-superfamily proteins in the immune system of insects. Science. 2005; 309:1874–1878. [PubMed: 16109846]

Yaguchi S, Yaguchi J, Burke RD. Sp-Smad2/3 mediates patterning of neurogenic ectoderm by nodal in the sea urchin embryo. Dev Biol. 2007; 302:494–503. [PubMed: 17101124]

Young JM, Friedman C, Williams EM, Ross JA, Tonnes-Priddy L, Trask BJ. Different evolutionary processes shaped the mouse and human olfactory receptor gene families. Hum Mol Genet. 2002; 11:535–546. [PubMed: 11875048]

Yuh CH, Bolouri H, Davidson EH. *Cis*-regulatory logic in the *endo16* gene: switching from a specification to a differentiation mode of control. Development. 2001; 128:617–629. [PubMed: 11171388]



	H.s.	C.i.	S.p.	C.e.	D.m.
TIR	10	3	222	1	9
SRCR	16	8	218	3	14
185/333	0	0	10*	0	0

Figure 1.
Comparison of numbers of genes with immune function across the bilateria. Phylogenetic relationship is given by the tree above (H.s.—*Homo sapiens*, C.i.—*Ciona intestinalis*, S.p.—*Strongylocentrotus purpuratus*, C.e.—*Caenorhabditis elegans*, D.m.—*Drosophila melanogaster*; SRCR—scavenger receptors, TLR—Toll-like receptors, vertebrate and invertebrate types; *the number of sea urchin 185/333 genes is an approximation).

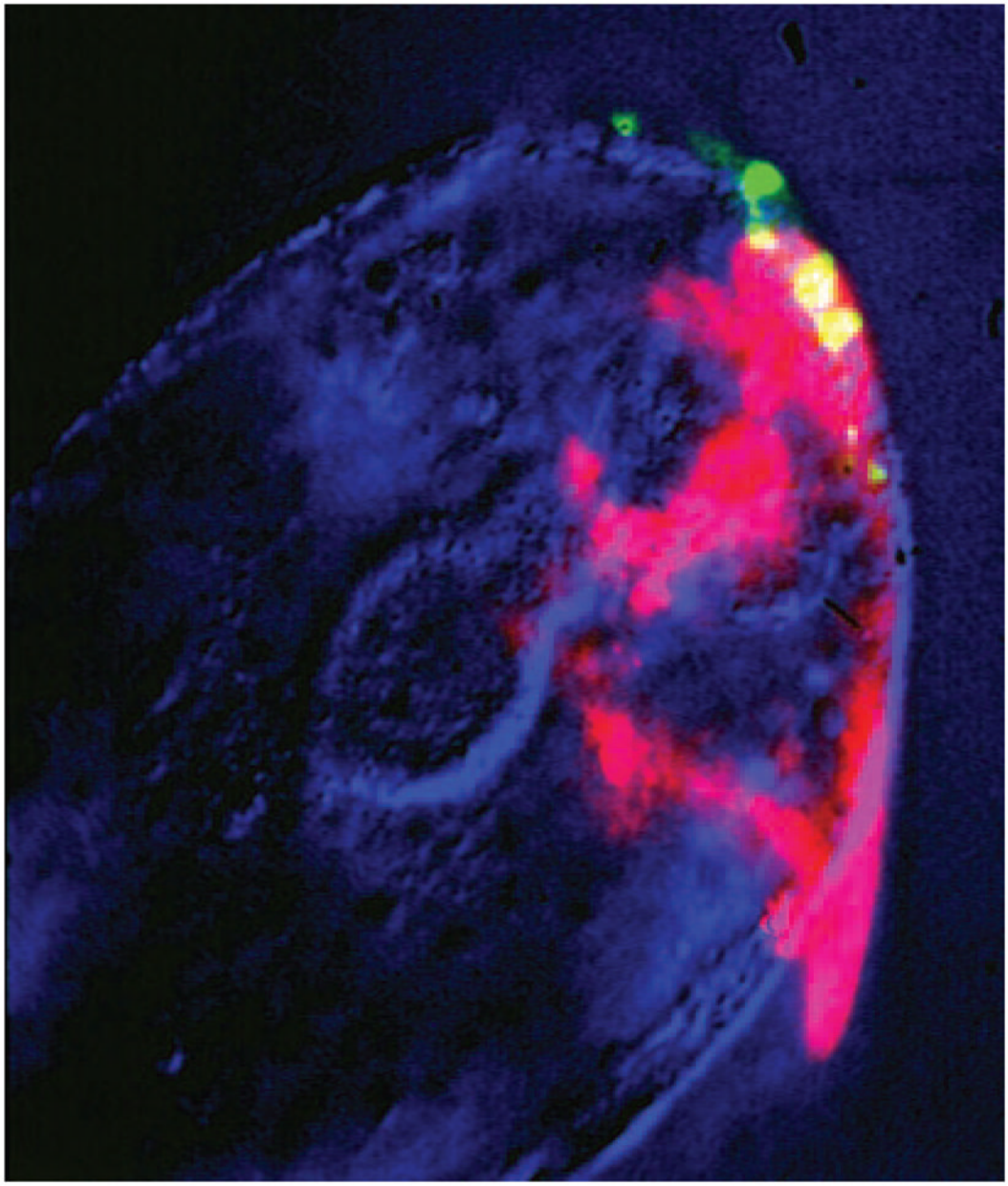


Figure 2.
A view of a sea urchin embryo that combines lineage tracing and serotonin immunocytochemistry (green). An oral ectoderm cell destined to contribute to the oral hood was injected with rhodamine dextran (red), and the neurons were subsequently stained with an anti-serotonin antibody (green). It is apparent that the neurons are descendants of both the ectoderm precursor and its aboral neighbor (single green cell). This shows that neurons arise from both oral and aboral ectoderm precursors.

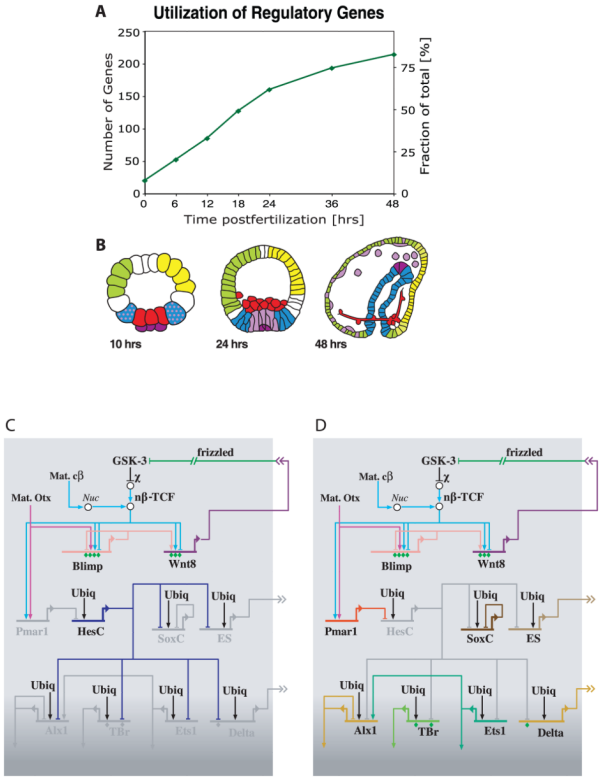


Figure 3.

Gene activity in early development. A & B: the regulome. (A) By late gastrulation (48 h postfertilization) almost 80% of the regulatory genes are or have been activated at least once. (B) Schematic representation of sea urchin development. Prior to the ingression of skeletogenic cells (red), cells in all regions have assumed distinct regulatory states; the regulatory complexity is not reflected in the simple morphology. (red—skeletogenic lineage, blue w/purple dots—endomesoderm, purple—mesoderm proper, blue—endoderm proper, yellow—oral ectoderm, green—aboral ectoderm, violet—small micromeres). C & D: Simplified gene regulatory network covering the skeletogenic lineage. (C) Initially, HesC represses the skeletogenic specification genes (Alx1, Tbr, Ets1) that control expression of the differentiation gene battery (not shown). Nuclearized *β*-catenin forms a complex with TCF in the nucleus and leads to activation of Blimp and Wnt8. Activation of these genes in turn leads to inhibition of TCF degradation and reenforces their own activation. (D) The *β*-catenin/TCF complex activates the *Pmar1* gene. Pmar1 represses *HesC* and releases the repression of skeletogenic specification genes. It also allows activation of signaling genes that induce specification of the neighboring mesoderm. (c*β*—cytoplasmic *β*-catenin, n*β*—nuclear *β*-catening, ES—early signal, Mat.—maternal, Nuc.—Nuclearization, Ubiq.—ubiquitous activator; see fig. 2 in Davidson *et al.*, 2002, for a full version of the endomesoderm GRN).

Table 1

Transcription factor (TF) utilization in early development, by family

TF family	Members	% Expressed[1]
Homeobox	85	71.8
bHLH	48	59.5
Nuclear receptors	33	69.7
Forkhead	22	95.5
bZip	14	84.6
ets	11	90.9
Sox/HMG	10	80
T-box	6	83.3
Smad	4	100
Other types	45	93.3
All transcription factors	283	77.6

[1] Genes that are or have been expressed by late gastrulation (48 h postfertilization).