CHAPTER 14

ECHINODERM IMMUNITY

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Abstract: A survey for immune genes in the genome for the purple sea urchin has shown that the immune system is complex and sophisticated. By inference, immune responses of all echinoderms may be similar. The immune system is mediated by several types of coelomocytes that are also useful as sensors of environmental stresses. There are a number of large gene families in the purple sea urchin genome that function in immunity and of which at least one appears to employ novel approaches for sequence diversification. Echinoderms have a simpler complement system, a large set of lectin genes and a number of antimicrobial peptides. Profiling the immune genes expressed by coelomocytes and the proteins in the coelomic fluid provide detailed information about immune functions in the sea urchin. The importance of echinoderms in maintaining marine ecosystem stability and the disastrous effects of their removal due to disease will require future collaborations between ecologists and immunologists working towards understanding and preserving marine habitats.

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INTRODUCTION

Echinoderms are a phylum of ubiquitous benthic marine invertebrates, found in a continuous distribution from the intertidal zone to the deepest depths of the ocean explored to date. They are the sister group to the chordates and are the basal deuterostomes.[1] Approximately 7000 extant echinoderm species have been described, falling into five classes—echinoids (sea urchins and sand dollars), holothurians (sea cucumbers), asteroids (sea stars), crinoids (sea lilies and feather stars) and ophiuroids (brittle stars), as well as a number of extinct classes known from the fossil record. Many members of the phylum are large and long lived.[2]

Echinoderms are central players in many benthic ecosystems, variously as herbivores, carnivores and detritivores. They possess a simple gut which opens into an anus on the aboral surface, or, for crinoids, on the oral surface of the animal.[1] All echinoderms have basic pentameric radial symmetry in their adult forms and most metamorphose from bilateral larvae found in the plankton that function as the dispersal stage, feed and produce the adult rudiment. Adults lack obvious sensory organs or any kind of head and locomote largely by means of tube feet, which function by means of a water vascular system unique to the phylum. Activities are coordinated by a simple neural ring that communicates to five nerve bundles into the five ambulacral grooves that run longitudinally along each radial segment of the body. All echinoderms have some form of calcified skeleton composed of aragonite, which is more or less extensive in the various classes. At one extreme, echinoids are completely enclosed in a calcified test, with only a thin layer of muscular tissue lining the interior of the body cavity (or coelom) while holothurians possess a highly muscularized body wall in which the exoskeleton is reduced to microscopic ossicles dispersed throughout the dermis. The coelomic cavity contains the gonads and gut of the animal, bathed in coelomic fluid. This coelomic fluid is key to the immunological capabilities of echinoderms, being the medium in which the immunocytes or coelomocytes reside, and which also contains antimicrobial molecules.

A larval sea star was first used by Metchnikoff[3] to demonstrate that invertebrate immune cells could recognize the presence of and respond to (encapsulate) a rose prickle. Subsequent characterization of the echinoderm immune system in adults relied on allograft rejection assays in sea cucumbers, sea stars,[4,5] and sea urchins,[6-8] and demonstrated the ability of echinoderms to recognize self from nonself. Later evaluation of the sea urchin graft rejection kinetics illustrated the innate characteristics of echinoderm immunity.[9] Although the immune system is entirely innate, it has recently been found to be highly complex and sophisticated[10,11] and is clearly effective given that echinoderms have survived as a phylum for 450-500 million years and that individual echinoderms can survive the constant assault by pathogens in the marine environment (reviewed by ref. 12) for upwards of 100 years.[2]

COELOMOCYTES, THE IMMUNE MEDIATORS IN ECHINODERMS

The body cavity of echinoderms is filled with coelomic fluid,[13] which bathes the internal organs and forms the fluid medium in which the coelomocytes are suspended. The composition of coelomic fluid (CF) is similar to sea water in terms of minor dissolved salts and other minerals and contains proteins, the best-characterized of which are those involved in antipathogen responses. Indeed, whole CF (wCF) is a complex tissue that

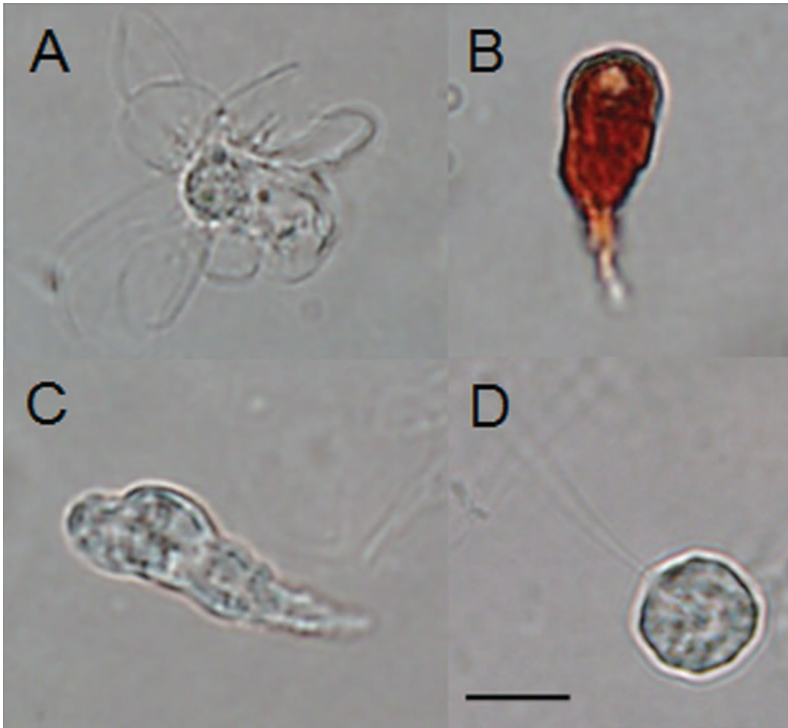


Figure 1. Live coelomocytes from the sea urchin, *Paracentrotus lividus*. A) petaloid phagocyte. B) red spherule cell. C) colorless spherule cell. D) vibratile cell. Scale bar = 5 microns. Images taken by R. Bonaventura.

mediates responses to wounding and microbial infections by undergoing reactions such as opsonization, coagulation, encapsulation and phagocytosis. Initial publications on echinoderm coelomocytes appeared in the late 19th century (e.g., ref. 14) and since then many reports have appeared describing the morphologies and functions of the various coelomocytes[9,15-17] (reviewed by refs. 18,19). Observations of live cells in CF from sea urchins suggest three basic categories of coelomocytes; phagocytes, spherule cells (also called amoebocytes,[20,21] or morula cells) and vibratile cells (Fig. 1); however further analyses suggest additional categories (Table 1) (reviewed in ref. 22). Within the spherule cell category, there are red and colorless spherules within the cells (Fig. 1B,C) and there are three different categories of phagocytes depending on cytoskeletal morphology and size (Fig. 2). Additional cell types have been noted in nonechinoid species including crystal cells, fusiform cells and progenitor cells, about which very little is known. The descriptions below refer to sea urchin coelomocytes, which are the best studied.

Phagocytes

Phagocytes have been variously referred to as leukocytes, or as bladder, petaloid or filoform phagocytes in the literature and constitute the most abundant type of coelomocyte (Table 1) in the coelomic fluid. In suspension the cells appear in a petaloid form (Fig. 1A;

Table 1. Coelomocytes in sea urchins

Cell Type	% in Coelomic Fluid	Function
Phagocyte Types Type 1—Discoidal cells Type 2—Polygonal cells Type 3—Small phagocytes	Total phagocytes *Sp** 40-80% *Sd* 67% *Pl* 80%	Encapsulation, Opsonization, Graft rejection, Chemotaxis, Phagocytosis, Antibacterial activity, Cellular clotting
Red spherule cells	*Sp* 7-40% *Sd* 8% *Pl* 4.7%	Oxygen transport. Antibacterial activity from echinochrome A.
Colorless spherule cells	*Sp* 3.7-25% *Sd* 6.5% *Pl* 7.8%	Cytotoxicity, Clotting?
Vibratile cells	*Sp* 11.9-20% *Sd* 18.5% *Pl* 7.5%	Movement or agitation of coelomic fluid? Associated with clotting.

**Sp*, *Strongylocentrotus purpuratus*. *Sd*, *Strongylocentrotus droebachiensis*. *Pl*, *Paracentrotus lividus*
Information collected from references 7, 16, 17, 19, 22, 24, 28, 33, 34, 38, 39, 50, 95, 222 and 223.

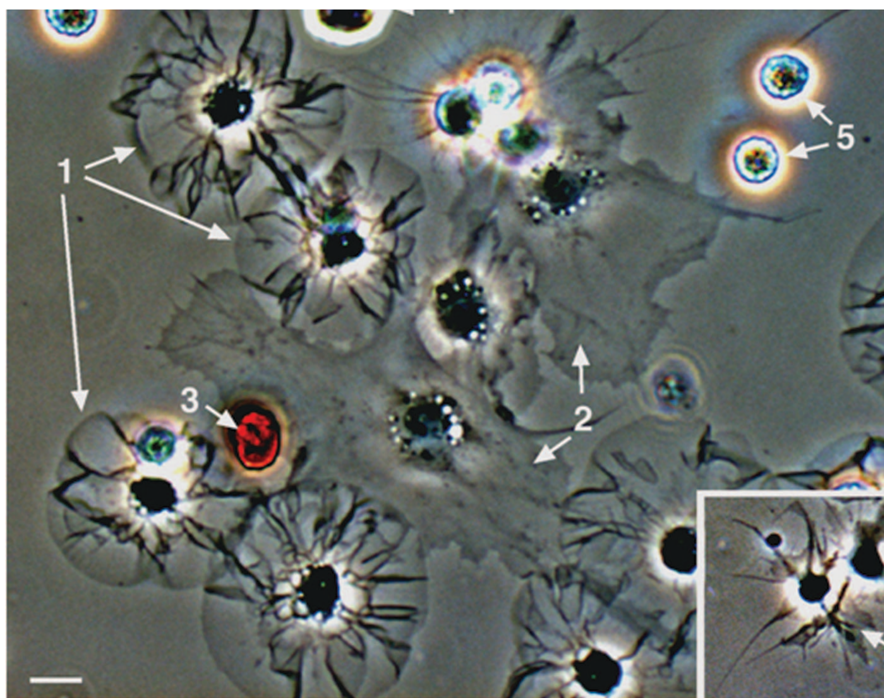


Figure 2. Coelomocytes from the sea urchin *S. droebachiensis*. Cells were withdrawn in anticoagulant and settled onto a glass coverslip. All cell types are shown and labeled with numbers. Large phagocytes; 1 = discoidal phagocyte; 2 = polygonal phagocyte; 3 = red spherule cell; 4 = colorless spherule cell; 5 = vibratile cell (the lower cell has lost the prominent flagellum seen in the upper cell). Inset; 6 = small phagocyte. Bar = 10 microns.

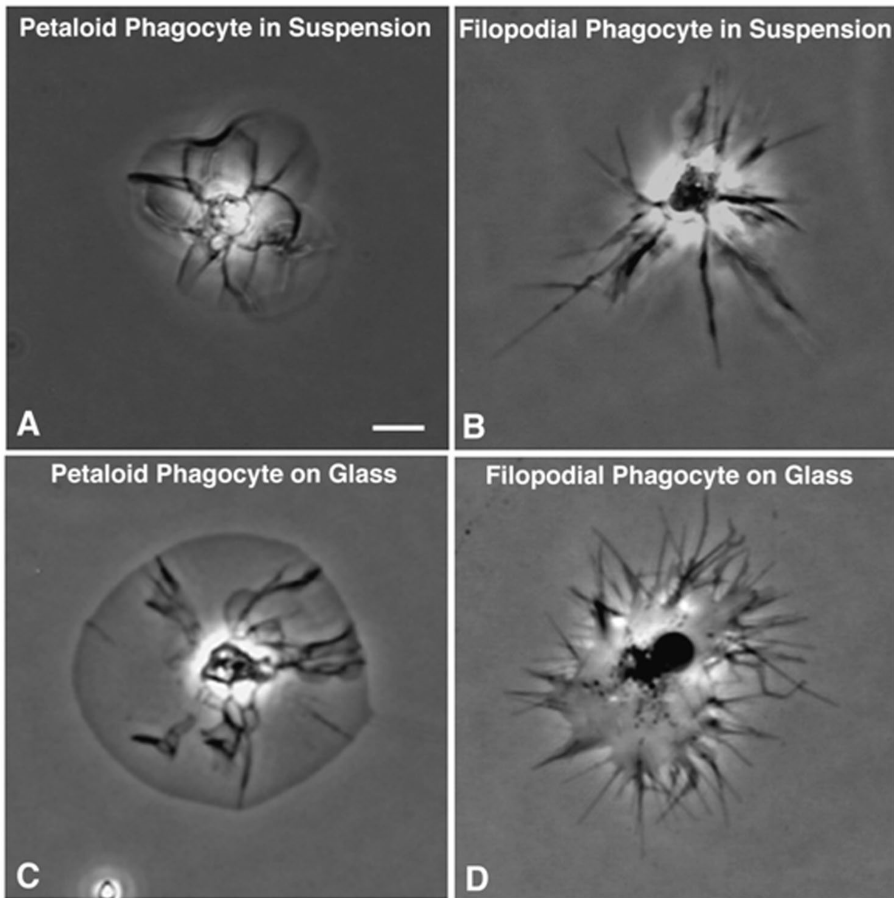


Figure 3. Phagocytes undergo an inducible petaloid/lamellipodial to filopodial/filiform shape change that is important in the coelomic fluid clotting process. This shape change occurs in cells in suspension; panel (A), petaloid cell; panel (B), filopodial cell. Shape change also occurs when cells are attached to glass substrate; panel (C), petaloid (lamellipodial) cell; panel (D), filopodial cell. Shape change was induced by hypotonic shock. Bar = 10 microns; magnifications of panels (A-D) are equivalent.

Fig. 3A) in which numerous cytoplasmic lamellipodia give the impression of the petals of a flower.[23] More recent work indicates that the phagocyte population actually consists of several different cell types that can be distinguished based on cytoskeletal morphology and organization, actin-based motility patterns,[24-27] and differential gene and protein expression.[19,28,29] Phagocytes are most easily distinguished as three morphologies in spread cells; two types of large phagocytes[24-27] and small phagocytes.[28,29] The discoidal-shaped phagocytes (Fig. 2) are large phagocytes that display rapid, actin-based retrograde/centripetal flow over their entire diameter, and contain a dense cortical actin meshwork rich in the Arp2/3 complex (Fig. 4C), radial actin bundles that connect the cortex to the perinuclear region, and a centralized distribution of myosin II (Fig. 4B).[25,27] The retrograde/centripetal flow activity in discoidal cells tends to restrict the distribution of microtubules (Fig. 4A), organelles (endoplasmic reticulum, mitochondria, lysosomes and granules) and

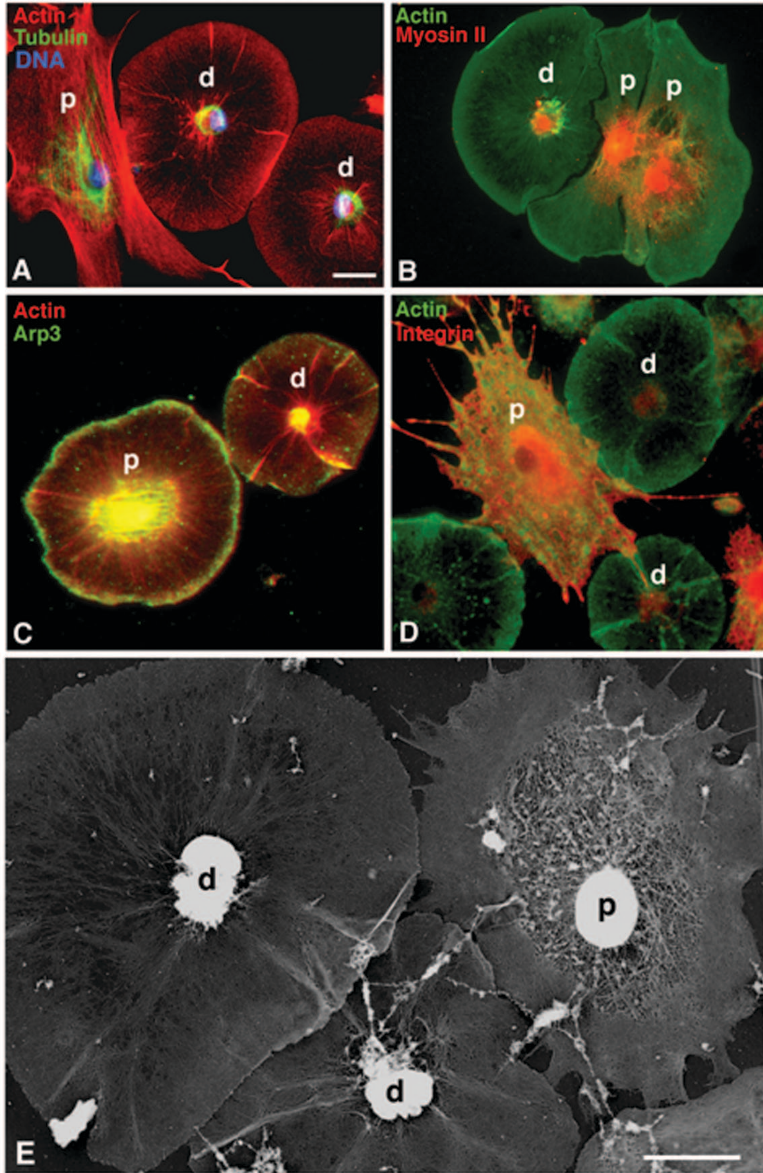


Figure 4. Large phagocytes of the discoidal (d) and polygonal (p) subpopulations can be differentiated based on cytoskeletal organization. Actin (panels A-D) in discoidal cells displays a broad cortical meshwork containing Arp3 (panel C) that feeds into a radial array of centralized bundles. The cortical actin and Arp3 meshwork is more limited in polygonal cells (panel C) and cells often contain prominent actin bundles oriented either radially in discoidal cells (panel C) or parallel to the long axis in polygonal cells (panels A,D). Both myosin II (panel B) and microtubules (panel A) are perinuclear in discoidal cells and more widespread in polygonal cells. β integrin expression is restricted to the polygonal cells (panel D). The significant difference between the structural organization of the cytoskeletons of the two large phagocytes is best appreciated in TEM images of critical point dried and rotary shadowed replicas of detergent extracted cells (panel E). Bar = 10 microns; magnifications of panels A-D are equivalent.

associated kinesin motor proteins to the perinuclear region.[26] The second type of large phagocyte is polygonal-shaped cells (Fig. 2, labeled 2), which display retrograde flow in a thin rim along the cell margin corresponding to more limited distribution of the Arp2/3 complex-containing actin meshwork (Fig. 4C). These large cells are integrin-positive (Fig. 4D) and the interior cytoplasm contains elongate actin bundles associated with myosin II (Fig. 4A-D),[25,27] as well as a broad array of microtubules (Fig. 4A) and associated organelles and kinesin (Fig. 4E).[26] Furthermore, both cell types undergo significant shape changes from a lamellipodial/petaloid to a filopodial form (Fig. 3B; see also ref. 20), inducible by the calcium-dependent clotting process, treatment with hypotonic shock[23] or calcium ionophore.[30]

Large phagocytes display phagocytic activity associated with clearance of bacteria, xenogeneic cells, foreign particles and proteins, both in vivo and in vitro (reviewed in ref. 18). Phagocytes have also been implicated in encapsulation and graft rejection responses, as well as cytolytic/cytotoxic reactions,[8,31] (reviewed in refs. 18,19). Phagocytes participate in the cellular clotting process, in which their filopodial form facilitates the cell-cell interactions leading to cellular clot formation and retraction.[16,23] Based on their size, preponderance in the CF and their phagocytic and encapsulation functions, the large phagocytes appear to be the major mediators of cellular immunity in echinoderms.

The third and least common form of phagocyte is the small phagocyte (Fig. 2, labeled 6)[28,29] of which some express the Sp185/333 family of highly diverse immune response-related proteins (see below). These cells are characterized by their small size relative to the large phagocytes, numerous small cytoplasmic granules and filopodial morphology (Fig. 5). The morphology of small phagocyte filopodia appears different from that of large phagocytes in that they are less numerous, thicker and often contain periodic knobs and microtubule bundles (Fig. 5D).[29]

Spherule Cells

Red spherule cells (Fig. 1B; Fig. 2, labeled 3; Table 1) have been called amoebocytes, spherulocytes, morula cells, pigment cells, granulocytes and eleocytes in the literature, and are small cells filled with spherical red granules containing echinochrome A, a naphthoquinone pigment with antibacterial properties. Red spherule cells have been reported to move towards bacteria to initiate an encapsulation response and to degranulate in the presence of bacteria.[32-35] They have been reported to migrate to and form a rim around the edge of wounds, infections and tissue grafts.[8,36,37] When settled onto a substrate, red spherule cells exhibit dynamic actin-based, amoeboid mobility, which may account for their ability to surround wounds and infections. Colorless spherule cells (Fig. 1C; Fig. 2, labeled 4; Table 1) contain granules and have therefore been referred to as morula cells (from Latin for 'mulberry'). Their properties and functions are not well known, however, a recent study suggests that these cells have potent cytolytic activity that is augmented by the presence of phagocytes.[38]

Vibratile Cells

Vibratile cells are round, highly motile, flagellated cells that contain large cytoplasmic granules (Fig. 1D; Fig. 2, labeled 5; Table 1). Exocytosis of these granules may trigger the initiation of the clotting reaction[32,39] (Sacchi and Smith, unpublished), however this hypothesis will require additional testing.

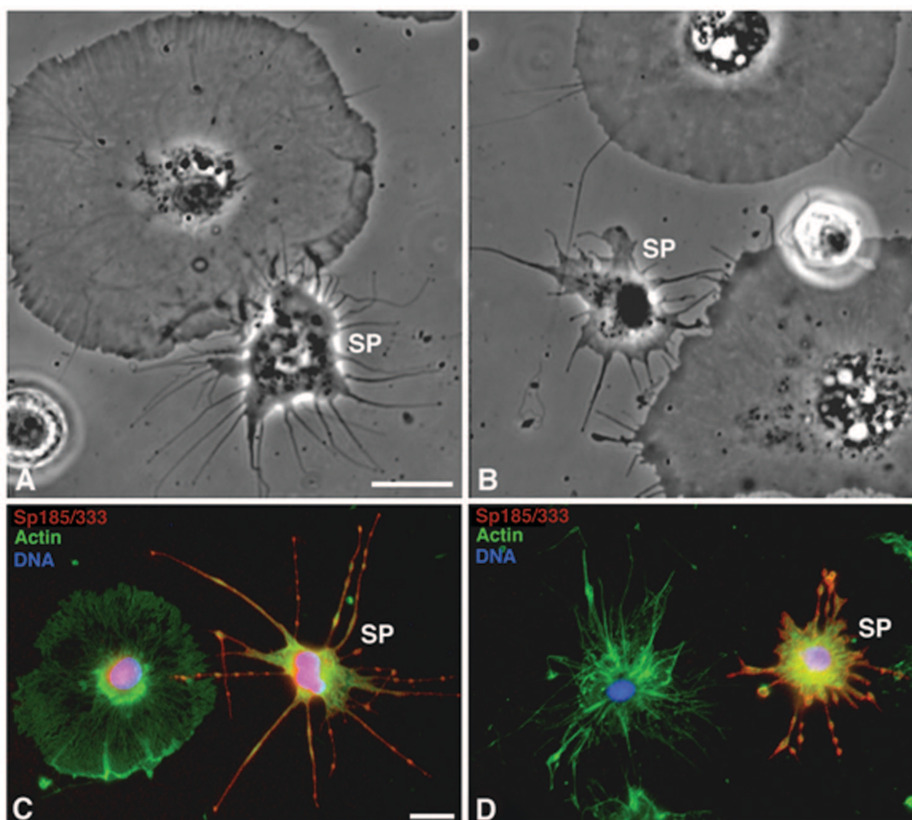


Figure 5. Small phagocytes in living preparations. Small phagocytes (SP) can be identified based on their relative small size, unusual filopodial morphology and granular cytoplasm (panels A,B). These cells are clearly labeled by antibodies against the Sp185/333 family of proteins (SP cell in panels C,D), whereas the petaloid and filopodial discoidal large phagocytes do not stain for Sp185/333. The Sp185/333 labeling of the small phagocytes highlights the atypical nature of their filopodia which are often thick and contain knobs, not common for filopodia of discoidal phagocytes (left-hand cell in panel D). Note that in some small phagocytes the filopodia become very elongate (panels A,C). Bar = 10 microns.

Coelomocytes in Other Echinoderms

Asteroids clear their coelomic cavities efficiently despite having only two major cell types, phagocytes and colorless spherule cells.[22,40] Rapid activation and differentiation of the phagocytes into multiple functional cell types upon bacterial infection is likely responsible for efficient microbial clearance.[41] The phagocytes in the sea star, *Asterias rubens*, are present in a continuum of small to large cells that change relative abundance upon immune stimulation. Larger cells become more numerous and exhibit strong phagocytic reactions to bacteria and the total number of phagocytes triple 24 hrs after challenge.[41] Increased numbers of total coelomocytes have also been noted in response to wounding and hypoxic conditions.[42] Swift responses, immune challenges, and the possible differentiation of phagocytes into cells with augmented phagocytic activity is an interesting approach to infection in sea stars that have fewer morphotypes of coelomocytes compared to echinoids.

Box 1. Larval immune cells.

While most investigations of echinoderm immunity have been carried out in the adult, the larvae of indirectly-developing species possess specialized mesodermal cells derived from embryonic blastocoelar cells that display a variety of immune behaviors. The immune activities of the larval immunocytes were recognized at the inception of cellular immunity by Metchnikoff and his infamous experiment of encapsulation of a rose prickle by blastodermal cells of a sea star larva.[3] This was the first demonstration of phagocytosis and encapsulation and lead to a nobel prize. More recently, recognition and phagocytic behavior of larval immune cells responding to bacteria or yeast injected into the blastocoel has been demonstrated.[218,219] The immune cells in the embryo and larvae are the blastocoelar cells located in the blastocoel[220] and the pigment cells in the ectoderm[19] that produce echinochrome A.[221] Embryonic and larval immune cells are also known to express immune factors, including the complement homologue SpC3[92] and the immune response genes *Sp185/333*.[10,70,92] Besides immune effector functions, these cells carry out a number of immune functions including surveillance-like behavior, wound healing and the expression of a complex suite of immune recognition, regulatory and effector genes[10] (Rast, unpublished). As more data are accumulated about this simple stage of the sea urchin life cycle, postgastrula embryos and larvae are becoming an increasingly valuable model system in which to investigate immunity.

Holothurians have lymphocytes, morula cells, amoebocytes, crystal cells, fusiform cells and vibratile cells.[22,40] In addition to phagocytosis, holothurian coelomocytes exhibit brown body formation in response to multicellular parasites. These are pigmented aggregates of phagocytes and spherule cells that encapsulate parasites that are too large to be phagocytized.[43] Brown bodies in the sea cucumbers, *Holothuria polii* and *H. tubulosa*, are tissue nodules containing entrapped parasites that are first surrounded by a fibrous, electron-dense, noncellular layer—probably melanin—and then covered by layers of specialized cells[44,45] including subsets of elongated phagocytes[44] and spherule cells.[46] Spherule cells likely degranulate to chemically kill and/or degrade the invader,[46] while other cells within the brown bodies possess active phenoloxidase resulting in melanization.[44] Brown bodies are progressively eliminated from the animal through coelo-rectal canaliculi.[44,45,47] Essentially, coelomocytes appear to have two means for clearing microbes and parasites; phagocytosis and encapsulation.

Crinoids have phagocytes, red spherule cells and colorless spherule cells (or morula cells), whereas ophiuroids have phagocytes, colorless spherule cells and possibly crystal and vibratile cells.[22,40] Because little work has been done on these classes of echinoderms, little is known about the functions of coelomocytes in these groups.

CLOT FORMATION

Clotting is mediated by a variety of agglutination factors. It is an important response to injury in echinoderms functioning to block loss of CF resulting from wounds, and to sequester pathogens and prevent their invasion throughout the body. A 220 kDa agglutinating factor thought to be involved with coagulation is secreted from coelomocytes of the sea cucumber *Holothuria polii*.[48] Coelomocytes from the purple sea urchin express amassin-1, a 75 kDa, multidomain protein with an olfactomedin domain that is stabilized by multiple disulfide

Table 2. ESTs from the purple sea urchin and a sea cucumber

Category	Examples
Defense	Sp185/333, DD104, DD186, Complement C3, Complement factor B, Galectin, SpEchinoidin and other C-type lectins, Thrombospondin, Amassin, Integrin-βC, Ferritin, Melanotransferrin, Annexin, Serum amyloid A, Echinonectin, Fibrinogen-like protein, Ficolin-like protein, Kazal-type serine proteinase inhibitors
Cytoskeleton	Actin, Gelsolin, Cofilin, Thymosin β, Protein tyrosine kinase-9, Fascin, Tubulin, Microtubule-associated protein
Signal Transduction	SpTie1/2, Steroid hormone receptor, LPS-induced TNFα, Protein tyrosine phosphatase receptor, Adenylyl cyclase, Receptor for activated protein kinase C, Guanine nucleotide binding protein β, Rho-GTPase, Rho-GDP dissociation inhibitor, GTPase-activating protein, FK Binding Protein-12, GF14/14-3-3
Nuclear Activities, Splicing	RNA/DNA binding protein, DNA methyl transferase-associated protein, Nonhistone nucleic acid-binding protein, Splicing factor 30, Paraspeckle protein, ET putative translation product, Heterogeneous nuclear ribonucleoprotein R, PolyA binding protein
Transcription factors	Steroid hormone receptor, LPS-induced tumor necrosis factor α, Immediate early-response protein, SpRunt, SpNFkB
Protein Metabolism, Synthesis, Degradation	Translation elongation factor 1α,1γ, 2, Translation initiation factor, Peptide chain release factor, Signal sequence receptor, Protein disulfide isomerase, Binding Protein, Heat shock protein, gp96, Presenilin, Proteosome subunit, Ubiquitin
Molecular Transport	Dynein, Kinesin, Vesicle trafficking protein, Coated vesicle membrane protein, ER transport protein, Vacuolar protein sorting protein, Rab7, Rab5-interacting protein, Sec22, vSNARE, Mannose-6 phosphate receptor
Endosomal System	Vacuolar H^+-ATPase, Cathepsins, Lysozyme, Arylsulfatase
Proliferation, Apoptosis	Polo-like kinase, Bax inhibitor-1, Allograft inflammatory factor-1
Metabolism	ATP synthase, Cytochrome C oxidase subunit 1, 4, Cytochrome b, NADH dehydrogenase subunit 2, 4, 5, Fatty acid desaturase, ATP/ADP translocase, Vacuolar ATP synthase subunit, Na^+/phosphate cotransporter, Na^+K^+-transporter, Citrate synthase, Malate dehydrogenase

From references 49, 52, 53, 88 and 165.

bonds (Table 2; Table 3;[49] Dheilly, Raftos and Nair, unpublished). Amasin-1 functions in cellular clot formation through homooligomerization and cross-linking coelomocytes.[50] A search of the sea urchin genome identified a putative receptor for amassin-1, colmedin, which also has an olfactomedin domain and is expressed in coelomocytes.[51]

Other molecules involved in clotting mechanisms, including transglutaminase homologues, were identified during an expressed sequence tag (EST) study from gut tissue of the sea cucumber *Holothuria glaberrima*.[52,53] Many of the genes referenced from

Table 3. Proteomic analysis of whole coelomic fluid from *Strongylocentrotus purpuratus*

Classification	Examples
Immune Response	Complement components (SpC3, SpBf), Scavenger receptors cysteine-rich, Sp185/333
Pathogen Destruction	Arylsulfatase and other lysosomal enzymes, α-2-macroglobulin
Clotting	Amassin, Annexin V, Von Willebrand factor
Metal binding	Major yolk protein, Transferrin, Ferritin, Ceruloplasmin
Cytoskeleton	Actin, Profilin, Fascin, Cofilin, Gelsolin, Myosin, Microtubule-associated protein, Arp 2/3 complex proteins, Coronin, Tubulin, α-actinin, Tetraspannin, Talin, Vinculin, Rab
Cell Adhesion	Integrin, NCAM, Selectin, Cadherin, Fibronectin
Signalling	Ras
Cytoplasmic Enzymes	Oxidative enzymes

From Dheilly, Raftos and Nair, unpublished.

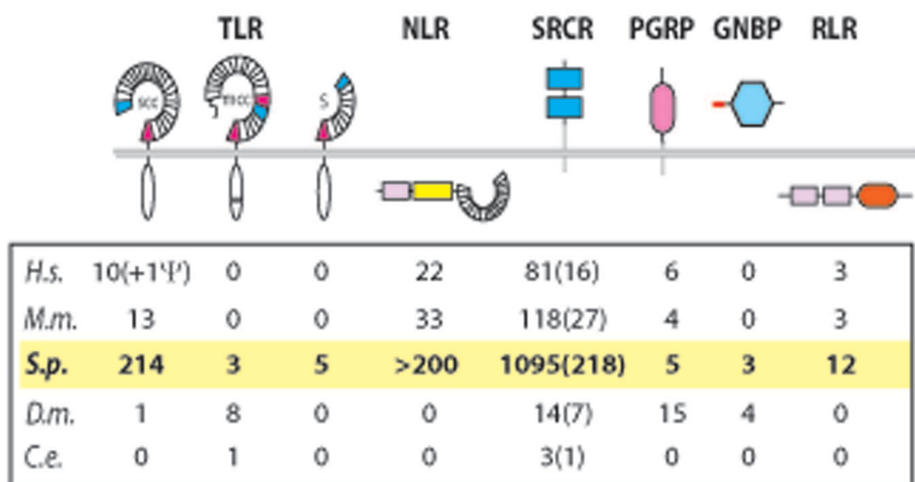
these EST studies were originally studied in the context of the intestinal regeneration that occurs after sea cucumbers expel their internal organs as part of an antipredation response, and then regenerate the entire digestive tract in 30 days. As such, evisceration and regeneration exposes the coelomic cavity to microorganisms from the surrounding seawater and the animal's own enteric microflora. Thus, genes related to immune function and tissue regeneration are upregulated in intestinal tissues undergoing regeneration and are likely essential for the animal to survive the process.[52-55]

KEY MOLECULES IN ECHINODERM IMMUNITY

Proteins with Leucine Rich Repeats

Toll-Like Receptors

The Toll-like receptors (TLRs) are best characterized in insect and mammalian systems, in which they form small gene families of ~10 genes.[56,57] These receptors have an N-terminal solenoid-like leucine-rich repeat (LRR) ectodomain, a transmembrane region and a cytoplasmic Toll/interleukin 1 receptor (TIR) in the C-terminus.[58] The genes are often encoded in a single exon. There are more than 200 TLRs in the *Strongylocentrotus purpuratus* genome, far more than has been identified in any other species to date.[10] These genes fall into three categories: a large family of more than 200 genes with vertebrate-like structure; a set of three genes with structure similar to *Drosophila* Toll (which differs from that of the vertebrate TLRs and *Drosophila* Toll-9[59]) and a family of five genes with a short truncated ectodomain structure (Fig. 6). In vertebrates, the ectodomain consists of canonical leucine-rich repeats flanked by specialized, cysteine-rich domains (designated single cysteine cluster, sccTLR). In



	TLR			NLR	SRCR	PGRP	GNBP	RLR
H.s.	10(+1Ψ)	0	0	22	81(16)	6	0	3
M.m.	13	0	0	33	118(27)	4	0	3
S.p.	**214**	**3**	**5**	**>200**	**1095(218)**	**5**	**3**	**12**
D.m.	1	8	0	0	14(7)	15	4	0
C.e.	0	1	0	0	3(1)	0	0	0

Figure 6. Pattern recognition receptor gene families are significantly expanded in the purple sea urchin with respect to other model organisms. The numbers of gene models encoding Toll-like receptors (TLR), Nod-like receptors (NLR), scavenger-receptor cysteine-rich (SRCR), peptidoglycan recognition proteins (PGRP), Gram negative binding proteins (GNBP) and RIG-I-like receptors (RLR) for human (*H.s.*), mouse (*M.m.*), purple sea urchin *S. purpuratus* (*S.p.*), fruit fly *Drosophila melanogaster* (*D.m.*) and nematode *Caenorhabditis elegans* (*C.e.*)[212] are shown. Given the complexity associated with identifying SRCR gene models accurately, the number of SRCR domains is shown, with the number of gene models that contain multiple SRCR domains indicated parenthetically. In the electronic version of this chapter, colors are used to illustrate protein domains and to emphasize the sea urchin gene model numbers. A color version of this image is available at www.landesbioscience.com/curie.

contrast, the *Drosophila* Toll has specialized domains that are located in the center of the ectodomain (multiple cysteine cluster; mccTLR).[58] The mccTLRs may be the ancestral form,[10] as mccTLR genes are present throughout eumetazoans, with the exception of the vertebrate lineage, in which it has apparently been lost. The sea urchin short TLRs have distant similarity within the TIR domain to the ancient Toll genes.[60]

The large family of sea urchin TLRs is unusual in both its multiplicity and the apparent rapid diversification of some of its subfamilies. The ~210 genes in this family can be divided into seven subfamilies based on phylogenetic analysis of the TIR domain. Some of these subfamilies are composed of many members that differ primarily within their ectodomains while the TIR domains show greater conservation. Divergence within the leucine-rich repeats takes a number of forms, including point mutations, insertion-deletions between LRRs and insertion-deletions of whole LRR units.[10] The extensive diversity of this class of sea urchin TLRs, along with a relatively large proportion of pseudogenes, differs from the more conserved picture seen in vertebrate TLR evolution[61] and suggests that this complex family of receptors function fundamentally differently.[60]

Many of the sea urchin TLR families are expressed most highly in coelomocytes in addition to gut tissue.[10] Expression of sea urchin TLRs is not detected in the embryo but many families are expressed in the feeding larvae. Thus, expression patterns and diversity of sea urchin TLRs are consistent with an immune rather than developmental function. Consistent with this is the fact that extensive investigations of vertebrate TLRs only show immune functions.

NOD-Like Receptors

The second family of expanded immune receptors in the sea urchin is the NOD-like receptors (NLRs).[10] NLRs are cytoplasmic pattern recognition receptors (PRRs) that recognize a variety of microbial signatures, including LPS, peptidoglycan, dsRNA and flagellin.[62] Structurally, NLRs are composed of C-terminal LRRs, a central NACHT domain and one of several N-terminal domains that function in protein-protein interactions. NLRs in mammals function in the immune response by serving as scaffolding proteins to assemble protein complexes that lead to the activation of the NFkB and MAPK signalling pathways. NLRs have also been shown to activate inflammatory caspases, including caspase-1, which is responsible for the processing of pro-interleukin-1β.[63] There is a significant expansion of the NLR gene family in the *S. purpuratus* genome, which has over 200 NLR gene models compared to vertebrates that typically have ~20 NLR genes (Fig. 6).[10] NLRs appear to be restricted to the deuterostome lineage and have not been identified in any of the sequenced protostome genomes, including *Drosophila* or *Caenorhabditis elegans*. As in vertebrates, the sea urchin NLR genes contain LRRs, a central NACHT domain and an N-terminal protein-protein interaction domain. However, unlike in the vertebrate system, this N-terminal domain is most commonly a DEATH domain, another member of the death-domain superfamily. A small number of sea urchin NLRs also contain CARD domains at the N-terminus, while the PYD domain is absent outside of the vertebrate lineage. Although many of the NLR gene models encode C-terminal LRR domains, these domains are lacking from some sea urchin gene models, which may be due to problems with accurate computational prediction of gene structure. The sea urchin NLR family appears to be the result of a sea urchin-specific gene expansion, which is seemingly more diverse than the similarly expanded sea urchin TLR family. The extent of this diversity is likely underrepresented given the incompleteness of the gene models, particularly within the LRR region. Although the function of the sea urchin NLRs is unknown, it is notable that they are most highly expressed in the gut,[10] and therefore may be involved in managing gut microflora. This mimics the role of the NLR NOD2 protein in mammalian systems, which when mutated, results in inappropriate inflammatory reactions in the gut tissue and leads to Crohn's disease.[64] The LRR gene families in the sea urchin are greatly expanded compared to families in vertebrates and insects and it is noteworthy that the LRR families in amphioxus are also expanded,[65] suggesting that these receptors play an important role in the innate immune functions of these deuterostome invertebrates.

Sp185/333—A DIVERSE FAMILY OF GENES AND PROTEINS EXPRESSED IN RESPONSE TO IMMUNE CHALLENGE

The *Sp185/333* Gene Family

The initial discovery of the *Sp185/333* family was the result of an EST analysis of transcripts that are upregulated in response to LPS challenge (see Box 2).[49] The diversity observed among the *Sp185/333* transcripts is intriguing for a putative immune response repertoire and resulted, in part, from an extraordinarily diverse gene family. The *Sp185/333* genes are small with two exons, of which the first is short and encodes a hydrophobic leader sequence, while the second encodes the remainder of the highly variable protein. The *Sp185/333* genes are atypical for *S. purpuratus* in three respects: (1) the second exon

ranges in size from 771-1431 base pairs (bp), which is notably larger than the average exon length (100-115 bp) as characterized from all gene models in the genome; (2) the intron is smaller than average (~400 bp, compared to an average intron size of ~750 bp); and (3) the average *S. purpuratus* gene has 8.3 exons.[11,66] The most interesting aspect of the *Sp185/333* genes is the structure of the second exon. It is composed of contiguous blocks of sequence called *elements* that are defined from sequence alignments that require the insertions of large gaps (Fig. 7).[49,66,67] Elements are variably present or absent in different genes (and transcripts, see Box 2) in recognizable mosaic combinations that have been called *element patterns*. Each element is actually a set of sequences that are similar but not necessarily identical—elements in the genes and transcripts differ by single nucleotide polymorphisms (SNPs) and small insertions or deletions (indels). Much of the sequence diversity among the gene results from the element patterns in the second exon. Although there are only six *Sp185/333* genes assembled in the v. 2.1 build of the *S. purpuratus* genome, three independent lines of evidence suggest that the gene family is composed of 40 to 60 paralogous loci: quantitative PCR (qPCR) of genomic DNA,[68] statistical estimates based on the frequency with which unique genes were cloned,[69] and an estimate based on results from screening two BAC libraries for *Sp185/333* genes combined with assumptions about gene linkage.[70]

The *Sp185/333* genes have six types of repeats (Types 1-6)[69] in addition to elements (Fig. 7). Individual genes contain between two and four Type 1 repeats, which are located tandemly at the 5′ end of the second exon. In contrast, repeat types 2-6 are arranged as mixed, interspersed groups in the 3′ half of the second exon. The complexity of these repeats facilitates multiple sequence alignments, two of which have been analyzed in detail.[66] The “cDNA-based alignment” (Fig. 7A) was generated using the location of gaps in the *Sp185/333* transcript sequences as a guide.[49] Alternatively, because of the repeats within the sequences, genes (and transcripts) can also be aligned according to the boundaries of the repeats, which results in the “repeat-based alignment” (Fig. 7B). Regardless of the criteria used to align the gene sequences, they are characterized by a similar diversity in element pattern variation as is observed among the transcripts (see Box 2). To date, 171 genes have been isolated from four animals, of which 121 have unique coding sequences and have 33 different element patterns.[66] Furthermore, identical sequences have not been isolated from more than one individual, suggesting a level of diversity that exceeds the estimated 4-5% sequence divergence between *S. purpuratus* individuals.[71] Large, diverse gene families are a common theme in purple sea urchin immunity (see above) and understanding the mechanisms by which this diversity is generated and maintained is an important aspect of investigations of sea urchin immunity.

Gene-Level Diversification through Recombination

Despite the striking number of unique genes that have been isolated to date, the *Sp185/333* sequences are surprisingly similar. Overall, the genes share >88% pairwise identity. The number of different versions of each element is relatively low.[72] The complexity of the *Sp185/333* gene family, therefore, is the result of a mosaic pattern of these few element sequences distributed among the genes. Analysis of the evolutionary histories of five of the elements that were present in all of the genes revealed that each element evolved independently. This level of incongruence suggests that the extant *Sp185/333* gene family is the result of rapid and recent diversification events.[69] It is peculiar, given this high rate of diversification that pseudogenes have yet to be identified.

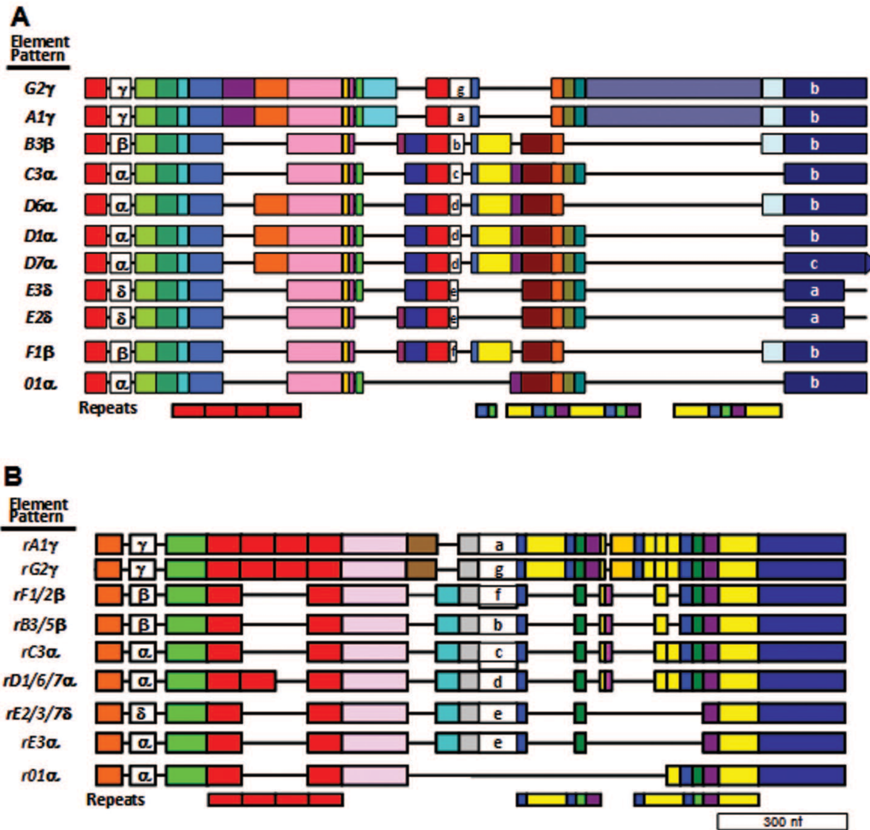


Figure 7. Two optimal alignments of the *Sp185/333* genes. The sequence complexity and repeats within the *Sp185/333* genes facilitate multiple alignments, two of which have been analyzed in detail.[66] The alignments differ in the pattern of elements in the second exon; the first exon and intron sequence do not have elements. Phylogenetic analysis of the intron sequences define five major types[66] (designated α-ε). The type of intron associated with each element pattern is indicated. A) The "cDNA-based alignment" results from the gaps used to optimize alignments of the *Sp185/333* ESTs and cDNAs.[49,68] This alignment divides the second exon into 25 elements. The terminal element is differentiated (designated a-c) based on the position of the first of three possible stop codons. The locations of each of the repeat types is indicated by the colored boxes under the alignments. B) An alternative alignment, the "repeat-based alignment", results from inserting gaps so that the repeats and elements correspond as much as possible.[66] This alignment divides the second exon into 27 elements based on the locations of gaps, as well as the locations of the repeats. The elements in this alignment that correspond to the repeats are color coded accordingly. The electronic version of this chapter shows the elements and repeats in color. A color version of this image is available at www.landesbioscience.com/curie.

With one exception, all but one of the cloned genes are predicted to encode intact open reading frames.[66] This may reflect a bias, however, in the primers that were designed in the untranslated regions of expressed genes,[49] so results do not confirm with certainty to a true lack of pseudogenes within the genome. It should be noted that, extensive computational analysis of elements and regions of the intron suggest that element boundaries do not necessarily serve as "recombination hotspots", but rather, that recombination appears to occur throughout the length of the genes.

The mechanisms that promote this frequent recombination are unknown. However, there are a few characteristics of the *Sp185/333* gene family that may contribute to its rapid diversification. Amplification of the regions between genes and preliminary analysis of a sequenced BAC insert with six *Sp185/333* genes show that many genes are closely linked (~3 kb). In addition to the six types of repeats found within the coding regions, the genes are flanked on either side by stretches of di- and trinucleotide microsatellites[66] (Miller, Buckley and Smith, unpublished). These repeats are closely associated with the boundaries of two types of large segmental duplications that include the *Sp185/333* genes (Miller, Buckley, Easley and Smith, unpublished). Microsatellites have been associated with genomic instability and increased recombination frequency[73,74] and have been implicated in mediating recombination of the variable surface glycoprotein (VSG) genes from *Trypanosma brucei*[75] and plant *R* genes.[76] The genomic organization, high sequence similarity and repeats within and surrounding the genes likely promote the diversification of the *Sp185/333* gene family through frequent recombination and thereby contributing to the complex protein repertoire.

Sp185/333 Transcript Editing

Given the diversity of the *Sp185/333* gene family, comparison of the gene and message sequences from individual animals yielded the surprising result that the two sets of sequences are very different.[72] Specifically, 148 messages and 53 genes were isolated from a single animal, of which only five of the sequences matched identically to another. Similarly, there was little or no overlap in gene and message sequence in two other animals. Furthermore, the gene and message repertoires were generally characterized by different element patterns, such that the predominantly expressed element pattern following immune challenge was *E2*, whereas the most common gene element pattern was *D1*. Although about half of the messages isolated from immunoquiescent animals had a truncated *E2* element pattern, called *E2.1*, the SNP that introduced an early stop codon was never found among the *Sp185/333* genes. In fact, no genes were identified with premature stop codons or indels resulting in frame shifts, which were both common features of *Sp185/333* transcripts.[67] When genes and message from individual animals were compared, the large majority of messages expressed both before and after immune challenge were the likely product of a few genes.[72] Conversely, most of the genes that comprise the large *Sp185/333* gene family were not transcribed. Notably, the pattern of nucleotide substitutions between the messages and the genes from which they were most likely transcribed indicated a bias towards transitions, specifically a uridine in the message at a position in which the gene contained a cytidine. This pattern of nucleotide substitution is consistent with cytidine deaminase activity. A number of cytidine deaminase-like molecules have been annotated within the sea urchin genome, but phylogenetic analysis of these sequences fails to identify homologues of activation-induced cytidine deaminase (AID).[10] AID, which has only been identified in vertebrates, is involved in class switch recombination and somatic hypermutation of immunoglobulins in B cells.[77] Alternatively, it is possible that the observed differences between *Sp185/333* gene and message sequences result from low-fidelity polymerase activity and the sea urchin genome does contain a homologue of terminal deoxytransferase and polymerase μ (Tdt/Polμ).[10,78] In higher vertebrates, this enzyme is also involved in immunoglobulin diversification, as well as low-fidelity DNA replication.[79,80]

Two Levels of Diversity

The *Sp185/333* gene family is an intriguingly diverse facet of the sea urchin immune response.[66] In response to immune challenge, this gene family is highly expressed and produces a diverse message repertoire.[49,67,68] The genes are believed to diversify through frequent recombination that does not appear to be limited to element boundaries and may be mediated by repeats within and flanking the coding sequences.[69] Given the diversity within the gene family, it is surprising that a second tier of diversification appears to affect the message sequences. That the majority of the messages appear to be derived from a few genes suggests that many of the *Sp185/333* genes may be nonexpressed pseudogenes and serve as a source of sequence diversification to the expressed genes. Alternatively, it may be that these genes are simply not expressed under the limited immunological challenges with which the animals have been presented.[67,68] Thus, the complex *Sp185/333* gene family represents a novel form of invertebrate immunological diversification both at the genomic and, also, possibly, at the posttranscriptional level.

In addition to diversity that appears to be generated by gene recombination, duplication, deletion, conversion,[69] and mRNA editing,[72] the array of Sp185/333 proteins show unexpectedly greater structural complexity than predicted from the genes and messages,[81] Not only are the arrays of Sp185/333 proteins different among different individual sea urchins, but the majority of sizes are at least twice as large as predicted and up to ≥ 200 kDa (Fig. 8).[29] The pI values range from 3-10 although the majority of isoforms have a pI more acidic than predicted. There are up to 260 discrete isoforms in

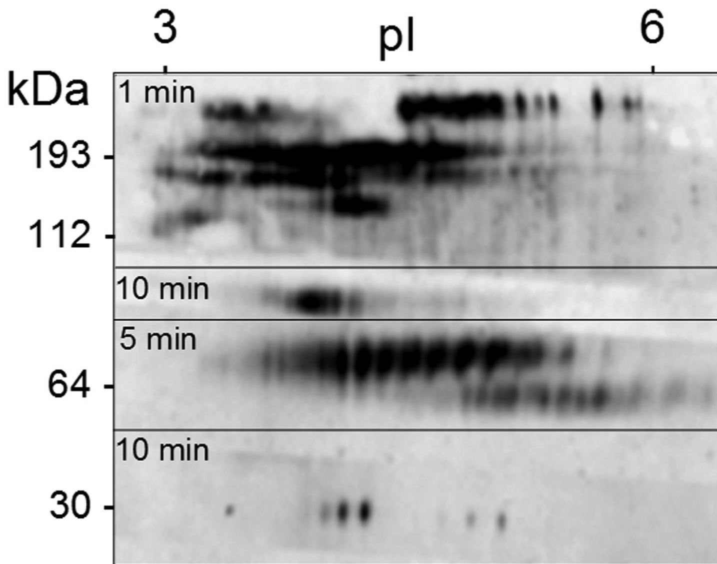


Figure 8. Over 260 spots for Sp185/333 proteins are present in CF from a single sea urchin. Total proteins from CF were separated by two dimensional gel electrophoresis and analyzed by Western blot with anti-185 antisera. The image is a composite of different regions of the blot that received different exposure times to optimize the spot intensities. pI units are shown at the top and molecular masses (kDa) are shown to the left. Reproduced from reference 81 with permission, ©2009, The American Association of Immunologists, Inc.

individual sea urchin coelomocytes and many of the expressed proteins are truncated, likely a result of mRNA editing.[67,81] Finally, the arrays of Sp185/333 proteins change in response to different pathogen-associated molecular patterns (PAMPs), which may result from a combination of variations in gene expression, mRNA editing and posttranslational processing of the proteins. How these several levels of diversification are coordinated and perhaps directed towards different types or species of pathogens will be the focus of future research.

COMPLEMENT IN ECHINODERMS

The complement system is a system of central importance in immunity for vertebrates and comprises over 30 known humoral and cell surface proteins.[82] Complement activation occurs by three major pathways: the classical pathway that is activated by antigen-antibody interaction, the lectin pathway that is activated by mannose binding lectin (MBL) or ficolins and the alternative pathway that is initiated through C3 autohydrolysis (reviewed in ref. 83). C3, a thioester containing protein, is the central component of the cascade and is activated by all three initiation pathways. It acts to coat the surfaces of pathogens, functioning as the initiator of the terminal pathway, an adjuvant for activating the adaptive immune response[84] and as an opsonin for direct pathogen recognition, phagocytosis and killing.

Investigations of opsonization and phagocytosis by coelomocytes from the green sea urchin *Strongylocentrotus droebachiensis* show that opsonizing target cells with mammalian C3 enhances the coelomocyte response,[39,85-87] suggesting that coelomocytes have receptors for mammalian C3 and consequently, sea urchins themselves may express C3-like proteins. The identification of two expressed sequence tags (ESTs) from the purple sea urchin, *S. purpuratus* with sequence homology to C3 and to factor B (Bf) called SpC3 and SpBf[88-90] was the first evidence that invertebrates have a complement system. Gene models annotated from the sea urchin genome reports several gene models encoding thioester proteins, including a second C3 homologue, Sp-C3-2, several factor B genes, MBL and ficolins (Table 4) (Rast, unpublished).[10]

SpC3

SpC3 has several conserved functional regions that are also present in other members of the thioester protein family, including a conserved thioester site, a histidine that regulates binding specificity, a putative C3-convertase site, a two-chain structure putatively disulfide bonded via cysteines in conserved positions, putative factor I cleavage sites and other conserved regions.[90] Immunoquiescent sea urchins responding to LPS generally show dramatic increases in the amounts of SpC3 in the CF[91] with localization to small transport vesicles in subpopulations of discoidal phagocytes and polygonal phagocytes.[28] Expression of *Sp064*, the gene which encodes SpC3, responds to immune challenge in adult animals and also in embryos cultured in the presence of heat-killed bacteria.[92] The function of SpC3 could be predicted from the deduced amino acid sequence and through comparison to mammalian C3 functions. The characteristics of the thioester, which forms covalent bonds with target molecules that lack protection against complement attack (i.e., microbial surfaces), suggests that SpC3 functions as an opsonin. Classic assays demonstrate that SpC3 binds methylamine, a small nucleophile that interacts covalently with thioester.[93] Furthermore, not only does SpC3 undergo autolysis, a reaction that, under appropriate

Table 4. Complement proteins in the sea urchin, *Strongylocentrotus purpuratus*

Gene Model or cDNA	Encoded Protein	Predicted Pathway
Sp-064	SpC3	Alternative
Sp-C3-2	SpC3-2	Alternative?
Sp-TCP1, Sp-TCP2	Thioester containing proteins	?
Sp-thioester containing protein-1, -2, -3/4	Thioester containing proteins	?
Sp-factor B	SpBf	Alternative
Sp-factor B-2, -3	SpBf-2, SpBf-3	Alternative?
SpSM30-F	Mannose-binding protein	Lectin
Sp-C1q-like (4*)	SpC1q	Lectin
Sp-MACPF (21*)	Perforin-like proteins	?
Sp-CD59, Sca2-like1, 2	CD59	Regulatory
Sp5	SpCRL	Regulatory?
Sp5013	SpCRS	Regulatory?

*Numbers of gene models.
From references 10 and 124.

conditions, results in the cleavage of the peptide bond between glutamic acid and glutamine within the thioester (reviewed in ref. 94), but methylamine binding blocks SpC3 autolysis.[93] When CF containing SpC3 is incubated with yeast, SpC3 can be detected on the yeast surface and augmented phagocytosis of the yeast by coelomocytes is inhibited by addition of anti-SpC3 antibody.[95]

SpBf

The deduced amino acid sequence and domain structure of SpBf show significant similarity to the vertebrate Bf/C2 family of proteins.[89] SpBf is a mosaic protein with short consensus repeat (SCR) domains, a Von Willebrand Factor (vWF) domain and a serine protease domain. SpBf has a conserved cleavage site for a putative factor D that is conserved compared to cleavage sites in other Bf/C2 proteins. Members of the Bf/C2 family are mosaic proteins and most have three SCRs, although some have more than three[96] including SpBf, which has five.[89] Sequence analysis of these small domains shows that the first two may be the result of a duplication event and that SCR4 may be the result of a recombination between SCR3 and SCR5.[89] Furthermore, alternative splicing produces some mRNAs with three or four SCRs.[97] The results suggest redundancies of SCRs in the SpBf protein and that the predicted function may be conserved in SpBf, even though additional SCRs are present, as the relative order of the SCRs in SpBf is maintained.[89] The gene encoding SpBf, *Sp152*, is expressed in the phagocyte fraction of coelomocytes with low levels detected in ovary, testes, gut and esophagus,[97] although it is not clear whether expression in tissues is actually due to coelomocytes present in the tissues rather than the tissue cells themselves. Unlike *Sp064* expression, *Sp152* expression is not induced by LPS and appears to be constitutive.

Complement Phylogeny and Evolution

The identification of a C3 homologue in the purple sea urchin[88] inspired searches for complement components in a wide range of invertebrates. Phylogenetic analysis of thioester proteins including homologues of C3 with α-2-macroglobulin homologues as the outgroup shows that vertebrate C3, C4 and C5 cluster into three well supported clades (Fig. 9). The invertebrate C3 homologues cluster in three paraphyletic clades at the base of the vertebrate complement clades. SpC3 clusters with other invertebrate C3 sequences, while the tunicate sequences form a chordate cluster with the vertebrate sequences (see also ref. 98). The structure of the tree suggests that the thioester complement proteins diverged after the separation of the major phyla. Phylogenetic analyses of members of the Bf/C2 family show that the sea urchin homologue, SpBf, is positioned near the base of the tree[89] with cnidarian Bf/C2 being more ancient.[98] A number of analyses of the complement family of proteins have suggested that they evolved from a restricted set of primordial genes[99,100] (reviewed in refs. 101, 102). Conserved sequence motifs, such as the thioester site (GCGEQ) and similar organization of domains suggest that the ancestral complement system may have included a thioester protein, a Bf/C2 protein and a mannose binding lectin. These are all present in a number of invertebrates including the purple sea urchin (Table 4).

LECTINS

Lectins are a large and heterogeneous group of proteins and glycoproteins present in plants, microorganisms and animals, that function to bind mono- and disaccharides.[103,104] Lectins can be soluble proteins or integral membrane proteins that often exist as oligomers, contain at least two carbohydrate recognition domains (CRDs) and are capable of agglutinating cells and/or precipitating glycoconjugates.[105] In immunity, lectins are key molecules that function in cell-cell interactions, self/nonself discrimination and interactions between cells and the extracellular matrix (ECM), among other functions.[106] Lectins in invertebrates fall into four major groups based on similarity of structure to vertebrate lectins. C-type lectins are the most common type in various invertebrates[107-110] and consist of both soluble and integral membrane proteins that require divalent cations (Ca^{2+}) to maintain the CRD structure to bind carbohydrates. A second major group of invertebrate lectins are S-type lectins that specifically bind β-galactosyl residues,[111,112] S-type lectins are predominantly intracellular and employ free thiols for binding carbohydrates. A third major group of invertebrate lectins resemble vertebrate pentraxins and share properties with C-reactive protein and serum amyloid protein.[113-115] Finally, a fourth group of invertebrate lectins includes all those that cannot be otherwise categorized based on lack of information on their primary structure.

Innate immune functions performed by lectins include recognition and specific binding of microbial surface carbohydrates through the CRDs,[116] which exhibit seven different structural folding patterns for binding to different carbohydrate motifs.[117] Both mannose-binding lectin (MBL) and ficolins have been identified in the sea urchin genome[10] and a homologue of MBL has been characterized in a sea cucumber, *Apostichopus japonicus*.[118] It is speculated that these homologues may initiate the lectin pathway of complement in echinoderms (Table 4). MBLs and ficolins selectively bind mannose,

Figure 9. Please see the figure legend on the following page.

Figure 9, viewed on previous page. The phylogenetic relationships among members of the thioester protein family. Amino acid sequences for the thioester family were obtained from GenBank. The alignment was done in T-coffee[213] and manual editing was done with Mesquite.[214] A consensus tree was constructed in PAUP*[215] using maximum parsimony. Bootstrap support was generated with 10,000 iterations. Similar cladogram results were obtained by the maximum parsimony method in PAUP*, Neighbour-joining distance method in PAUP* and the Bayesian method in Mr. Bayes.[216] The Bayesian method was used with default priors and the GTR+G+I as the nucleotide substitution model suggested by jModelTest.[217] The α2macroglobulin sequence from horseshoe crab (*Limulus polyphemus*) was chosen as the outgroup. Sp-C3, *Strongylocentrotus purpuratus*, NP_999686; Hs-C3, *Homo sapiens*, AAR89906; Rn-C3, *Rattus norvegicus*, NP_058690.2; Me-C3, *Macropus eugenii*, AAW69835; Gg-C3, *Gallus gallus*, NP_990736; Xl-C3, X*enopus laevis*, AAB60608; Lj-C3, *Lethenteron japonicum*, AAR13241; Ej-C3, *Entosphenus japonicus,* Q00685; Bb-C3, *Branchiostoma belcheri,* BAB47146; Cr-C3, *Carcinoscorpius rotundicauda*, AAQ08323; Se-C3, *Swiftia exserta*, AAN86548; Hr-C3, *Halocynthia roretzi,* BAA75069; Ci-C3, *Ciona intestinalis*, Q8WPD8; Eb-C3, *Eptatretus burgeri,* CAA77677; Om-C3, *Oncorhynchus mykiss*, I51339; Cc-C3, *Cyprinus carpio*, BAA36618; Es-C3, *Euprymna scolopes*, ACF04700; Vd-C3, *Venerupis decussates*, ACN37845; Gg-C4, *Gallus gallus*, T28153; Xl-C4, *Xenopus laevis*, BAA11188, Hs-C4, *Homo sapiens*, AAB67980; Cc-C4, *Cyprinus carpio*, BAB03284; Mm-C4, *Mus musculus,* CAA28936; Ts-C4, *Triakis scyllium*, BAC82347; Hs-C5, *Homo sapiens*, AAI13739; Mm-C5, *Mus musculus*, P06684 ; Gg-C5, *Gallus gallus*, XP_415405; Om-C5, *Oncorhynchus mykiss*, AAK82852 ; Lp-A2M, *Limulus polyphemus*, BAA19844; Lj-A2M, *Lethenteron japonicum*, BAA02762; Pt-A2M, *Pan troglodytes*, XP_001139559; Xl-A2M, *Xenopus laevis*, AAY98517; Cc-A2M, *Cyprinus carpio*, BAA85038; Cf-α2M, *Chlamys farreri*, AAR39412; Ci-A2M, *Ciona intestinalis*, NP_001027688; Hs-α2M, *Homo sapiens*, P01023.

fucose and other specific carbohydrates present exclusively on the surface of pathogens resulting in the direct activation of complement.[119-122]

Lectins were first demonstrated in echinoderms in the early 1980s.[123] Lectins have been identified in asteroids, echinoids and holothurians and characterized essentially from a functional and biochemical point of view. With improved molecular methods greater numbers of lectins and proteins with putative lectin function have been identified. Preliminary analysis of the sea urchin genome shows gene models encoding more than 100 small C-type lectins, over 400 mosaic proteins with lectin domains, 34 galectins, in addition to a few pentraxins and fucolectins[124] (Cohen and Smith, unpublished). This indicates that lectins in general are likely to have a variety of important functions in echinoderms, including recognition of foreign cells. Many, but not all of the lectins that have been identified in echinoderms have been C-type lectins (Table 5). Many show opsonin and agglutinin functions with the capability of binding carbohydrates on the surface of pathogens. It is generally accepted that lectins in echinoderms play an important role in the immune system, functioning as key molecules in immune responsiveness and to augment coelomocyte functions in host defense.

SCAVENGER RECEPTORS

Another expanded gene family in the sea urchin genome encodes a large repertoire of scavenger receptors containing multiple scavenger-receptor cysteine-rich (SRCR) domains that are both membrane-bound and secreted proteins.[125,126] Receptors of this structure are found throughout the animal kingdom but the sea urchin genome encodes more than 1000 SRCR domains in ~180 gene models, which greatly exceeds the multiplicity of these genes in other characterized species (Fig. 6).[10] These proteins are known to act as phagocytic receptors and some family members in mammals have been shown to bind bacteria.[127] These receptors are highly polymorphic in the population of purple sea urchins and show

Table 5. Echinoderm lectins

Class	Species	Name	Structure	Specificity	Agglutination	Reference
Asteroidea	*Asterina pectinifera*	(-)	C-type lectin	α-N-acetyl-galactosamine	yes	224
	Oreaster reticulatus	(-)	C-type lectin	galactosyl	(-)	225
Echinoidea	*Strongylocentrotus purpuratus*	SpEchinoidin	C-type lectin	galactose and derivates	(-)	88,124
	Anthocidaris crassispina	Echinoidin	C-type lectin	N-acetyl-galactosamine	yes	108
		SUEL	unique	D-galactoside	(-)	123
	Lytechinus variegatus	Echinonectin	(-)	galactoside	(-)	226
	Paracentrotus lividus	(-)	C-type lectin	(-)	yes	146
		*Pl*SL	(-)	D-glucose, L-rhamnose D-arabinose, L-fucose N-acetyl-D-glucosamine	yes	227
	Toxopneustes pileolus	SUL-I	(-)	D-galactose D-fucose	(-)	228
		SUL-II	(-)	D-galactose	(-)	228
	Tripneustes gratilla	TGL-I	C-type lectin	(-)	(-)	228

continued on next page

Table 5. Continued

Class	Species	Name	Structure	Specificity	Agglutination	Reference
Holothuroidea	*Apostichopus japonicus*	SJL-I	(-)	N-acetyl-D-galac-tosamine	yes	229
		SJL-II	(-)	simple carbohydrates	yes	229
		SPL-I	C-type lectin	D-glucuronic acid, D-galacturonic acid	(-)	230
		SPL-II	C-type lectin	D-galactosamine, D-galactose	(-)	230
		MLB-AJ	C-type lectin	α-D-mannans	yes	118
	Cucumaria echinata	CEL-I	C-type lectin	N-acetyl-galactosamine	yes	231
		CEL-II	C-type lectin	(-)	no	232
		CEL-III	C-type lectin	(-)	yes	231
		CEL-IV	C-type lectin	N-acetyl-galactosamine, α-galactose	yes	232
	Cucumaria japonica	(-)	(-)	branched α-D-mannans	yes	233
	Holoturia scabra	HSL	(-)	galctose derivates, T-antigen	yes	234

(-), not known, not done, not named.

pronounced variability in coelomocyte expression among individual animals suggesting a complex expression control system.[125] It is notable that representatives of all three of the most expanded families of sea urchin receptors (TLR, NLR and SRCR receptors) form a coregulated immune circuit that, in mammals, functions in gut immunity.[128]

ANTIMICROBIAL PEPTIDES

Antimicrobial peptides (AMPs) have been identified in a wide variety of species including bacteria, fungi, plants, insects, tunicates, amphibians, birds, fish and mammals.[129-132] Since the discovery of cecropins in insects[133] and defensins in mammals,[134] more than 1,200 different eukaryotic AMPs have been characterized.[135] In eukaryotes they form the first line of host defense against pathogenic infections and are a key component of the innate immune system. AMPs have an enormous variety of sequences and structures, but certain features are common. Most have a net positive charge and are 12-50 amino acids long, of which approximately half are hydrophobic.[136,137] However, a few peptides of up to 100 residues are also recognized as AMPs.[138]

Early work to document antimicrobial activities of crude extracts from echinoderms showed a wide range of activities against bacterial and fungal isolates[45,139] of which some functioned as antifoulants to deter the settlement of barnacle and bryozoan larvae in addition to bacterial colonization.[140] More recently, a variety of molecules with antimicrobial properties have been isolated from echinoderms, including steroidal glycosides,[141-143] polyhydroxylated sterols,[141] naphthoquinone pigments such as echinochrome A,[33,144] and complement homologues.[89,90] Lysozymes with antibacterial activity have also been detected,[145-147] and concentrations up to 15 μg/ml have been found in red spherule cells of the sea urchin *Paracentrotus lividus*.[34] Lysozyme and other antibacterial factors may act synergistically to provide effective defense against bacterial infections. In the Strongylocentrotids, the antimicrobial pigment echinochrome A is present in vesicles of red spherule cells and is bound to uncharacterized coelomocyte proteins.[148] A semi-purified coelomocyte fraction from *Paracentrotus lividus*, included fragments of beta-thymosin that were proposed to have antibacterial activity.[149] In extracts from coelomocytes of the sea star *Asterias rubens*, a number of partial peptide sequences were obtained and identified as fragments of actin, histone H2A and filamin A.[150,151] Antibacterial activity was detected in extracts of several tissues from the green sea urchin *Strongylocentrotus droebachiensis*, the common sea star *Asterias rubens*, and the sea cucumber *Cucumaria frondosa*,[152] with most activity in coelomocytes and body wall.

Strongylocins

Scans of the gene models in the purple sea urchin genome did not detect any sequences encoding recognizable AMPs.[10] This may be due to short exons that are difficult to recognize computationally, but may also be due to the divergent nature of these small proteins. However, two cysteine-rich AMPs, called strongylocins, have recently been isolated and characterized from the green sea urchin, *S. droebachiensis*.[153] Homologues are also present in the sister species *S. purpuratus*, called SpStrongylocins.[154] The strongylocin peptides are members of the cysteine-rich AMP family, which have six cysteines with three disulfide bonds involved in peptide conformation, stabilization

and resistance to proteases,[155] and which are crucial for the antimicrobial activity.[156,157] The strongylocins have a novel cysteine pattern (Table 6) suggesting a different conformation than the other members of the group, perhaps to resist proteolysis within the coelomocytes and in the CF. Both the native and recombinant peptides show antibacterial activity against both Gram-positive and Gram-negative bacteria (Table 7). In addition, other peptides have been isolated and characterized from *S. droebachiensis* that appear as heterodimers and have strong activity against Gram-positive and Gram-negative bacteria (Stensvåg, unpublished). The heavy chain from one of the heterodimeric peptides also has strong activity against fungi and yeast.

The strongylocins are composed of three regions: a signal peptide, a prosequence and the mature peptide, and the strongylocin 1 peptides show high amino acid sequence similarity throughout (Fig. 10). However, SpStrongylocin 2 shares an identical signal peptide with strongylocin 1, instead of strongylocin 2. The prosequences are negatively charged, which may act to neutralize and stabilize the positive charge of the mature peptide[153,154] and presumably function as an intracellular steric chaperone during folding.[158-160] The peptides become active after the prosequences are cleaved during maturation. The first amino acid in the mature peptide of strongylocin 2 from *S. droebachiensis* is a tryptophan which is likely brominated. Although the recombinant SpStrongylocin 2 is not brominated, it shows equivalent antimicrobial activity to native peptides. Therefore, the posttranslational modification of tryptophan may affect the properties of the peptides by enhancing stability rather than mediating antimicrobial activity. The site of strongylocin activity is likely to be intracellular based on membrane integrity assays.[154]

Nonechinoids also synthesize a range of AMPs, of which many have been characterized. For example, the antibacterial activity in the CF of the orange-footed sea cucumber, *Cucumaria frondosa,* has been traced to small peptides ($\leq$6 kDa) that appear to be active at low pH (5.0-6.5) and which may be similar to the clavanins found in solitary tunicates.[161] Other immune-active chemical compounds with roles in maintaining antiseptic environments in nonechinoids include saponins and saponin-like compounds in sea stars and brittle stars, which are active against some Gram-positive bacteria.[141] Sea stars and brittle stars in particular express steroidal glycosides that exhibit antifungal activity and toxicity against brine shrimp.[162-164] It is likely that multitudes of molecules

Table 6. Cysteine patterns in AMPs containing six cysteines

Peptide Family	Cysteine Pattern[a]	Group of Organism
Strongylocins	C – C – C – **CC** – C	Echinoderms
Beta-defensins	C – C – C – C – **CC**	Mammals, birds
Alpha-defensins	C – C – C – C – **CC**	Mammals
Tachystatins	C – C – **CC** – C – C	Horseshoe crab
Knottin-type AMPs	C – C – **CC** – C – C	Plants
Thionins Type III and IV AMPs	**CC** – C – C – C – C	Plants
Insect defensins	C – C – C – C – C – C	Insects
Mytilus defensin	C – C – C – C – C – C	Molluscs

[a]Adjacent double cysteine residues are highlighted. Information regarding cysteine arrangements in the different peptides was obtained from the *Antimicrobial Peptide Database*.[135]

Table 7. Susceptibility of bacteria to strongylocins, recombinant SpStrongylocins and dimeric peptides from *Strongylocentrotus droebachiensis* and *S. purpuratus*

	Minimal Inhibitory Concentration (μM)					
Peptide	*Listonella anguillarum*	*Escherichia coli*	*Corynebacterium glutamicum*	*Staphylococcus aureus*	*Penicillium rogveforti*	*Candida albicans*
Strongylocin 1[a]	2.5	5.0	2.5	2.5	Nt[c]	Nt
Strongylocin 2[a]	1.3	5.0	2.5	2.5	Nt	Nt
Recombinant SpStrongylocin 1[b]	15.0	7.5	7.5	15.0	Nt	Nt
Recombinant SpStrongylocin 2[b]	15.0	7.5	3.8	15.0	Nt	Nt
Dimeric peptide 1[a]	2.5	1.3	1.3	2.5	6.25[d]	6.25[d]
Dimeric peptide 2[a]	2.5	2.5	1.3	5.0	Nt	Nt

[a]Minimal inhibitory concentration was determined as the lowest concentration of peptide causing an optical density less than 50% of the growth control without any peptide present.
[b]Minimal inhibitory concentration was determined as the lowest concentration of peptide causing 100% growth inhibition of the test organism compared to the growth control.
[c]Nt = Not tested.
[d]The peptide is the heavy chain of dimeric peptide 1. Growth inhibition was determined microscopically after 48 hrs of incubation.

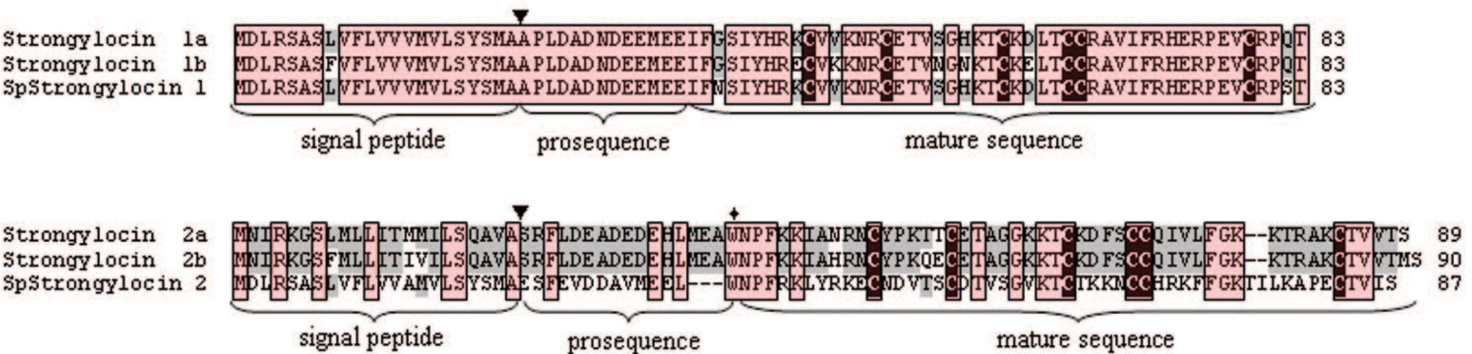


Figure 10. Alignment of strongylocins from *S. droebachiensis* and SpStrongylocins from *S. purpuratus*. The predicted cleavage site between the signal peptide and the proregion is indicated with a ▼. The first amino acid in active strongylocin 2 and SpStrongylocin 2 are likely a modified tryptophan, as indicated with a ♦. Identical amino acids are shown in boxes, similar amino acids are shown in gray and cysteines are highlighted in black. A color version of this image is available at www.landesbioscience.com/curie.

with activity against all types of pathogens function efficiently in echinoderms and have central importance for immune functions in this group.

GENE EXPRESSION IN SEA URCHIN COELOMOCYTES

Before it was possible to scan for gene models encoding proteins with putative immune function in the sea urchin genome, the only feasible approach for understanding the echinoderm molecular immunology was through EST profiling of messages in coelomocytes under challenged vs nonchallenged conditions.[49,88,165] ESTs encoding proteins with putative immune function have also been reported for the sea cucumber *Holothuria glaberrima*, undergoing gut regeneration after evisceration.[52,53] A variety of categories of proteins are expressed in coelomocytes that illustrate the activities of these cells in an assortment of functions (Table 2). The main focus of these studies was to identify immune response genes (see Box 2), which included protein matches suggesting several mechanisms for opsonization, clotting, iron sequestration plus the activity of a variety of signaling pathways. Matches were found to proteins with functions in the endosomal system (lysosomes) that may be involved in killing phagocytosed pathogens. Coelomocytes express a number of genes encoding proteins involved in splicing transcripts and translating proteins. They process, package, transport and secrete proteins as suggested from the transcripts encoding proteins involved in trafficking transport vesicles. Phagocytes have extensive cytoskeletons (as illustrated in Figs. 1-5), which is borne out by the number of ESTs matching proteins that function as cytoskeletal elements and others that act to modulate the cytoskeleton. One of the benefits of generating

Box 2. EST studies identify immune-related transcripts; discovery of the *Sp185/333* sequences.

Differential characterization of ESTs of coelomocytes from *Strongylocentrotus purpuratus* before and after immune challenge provide a picture of global changes in transcriptional activity. Genes encoding complement homologues,[88-90] transcription factors,[126] and a lectin,[67,88] among many others are induced by immune challenge (Table 6). Significantly upregulated transcripts include a large and diverse set of novel transcripts designated *Sp185/333* that were first discovered with the use of differential display[165] and subtracted probes followed by EST analysis.[49] Probes representing transcripts from LPS-activated coelomocytes were used to screen a high-density arrayed, conventional cDNA library made from bacterially-activated coelomocytes. About 4.5% of the clones in the library were positive and about 60% of clones selected for EST analysis showed significant sequence similarity to two previously uncharacterized cDNAs: *DD185* and *EST333*.[70,88,126] Given the considerable number of related *Sp185/333* sequences, the coding regions of the ESTs could be aligned. However, optimization of the alignment required the insertion of multiple, large artificial gaps[49] that defined 25 to 27 blocks of shared sequence called *elements*, which are variably present or absent in *element patterns* (Fig. 7).[66,67] The extraordinary diversity of the *Sp185/333* transcripts is based on the mosaic element patterns in addition to extensive single nucleotide polymorphisms (SNPs). The induction of *Sp185/333* gene expression in response to LPS and other pathogen-associated molecular patterns (PAMPs)[67,165] plus the diversity of the sequences suggest immune-related functions for the encoded proteins in *S.purpuratus*.

ESTs is that the level of expression rather than the level of match to known sequences can sometimes provide clues to putative immune function for unknown sequences. This has been the case for the *Sp185/333* sequences that are not known outside of the echinoids, but for which there is strong evidence of immune function.[70]

PROTEIN EXPRESSION PROFILE OF SEA URCHIN COELOMOCYTES

In addition to genome scans and EST analyses, high throughput methods in proteomics are being applied to analyzing proteins in echinoderms. In response to LPS challenge, 319 proteins were identified in the wCF of the purple sea urchin, *Strongylocentrotus purpuratus*, of which 284 were encoded by gene models in the genome and 48 were encoded by hypothetical open reading frames (Table 3) (Dheilly, Raftos and Nair, unpublished). The majority of the proteins were involved in modulating the cytoskeleton and linkage between the cytoskeleton and cell adhesion molecules, all of which are essential for intracellular transport and behaviors such as locomotion and phagocytosis. Cell adhesion molecules and intracellular signaling proteins were also identified, suggesting that coelomocytes respond to LPS with large-scale alterations to the cytoskeleton. Furthermore, the response to immune challenge implies the secretion of cellular products, as well as the endocytosis of extracellular fluids and phagocytosis of pathogens. Proteins involved in clotting and coagulation suggest another mechanism to sequester pathogens and to clear them from the CF, in addition to the prevention of CF loss after trauma. A number of proteins involved in opsonization are present in wCF including complement homologues. Metal-binding proteins are present, including ferritin, which has been identified in several echinoderm species under various conditions and using different analytical approaches.[49,52,88,166] Consequently, sequestration of iron must be an important mechanism for controlling the proliferation of invading microbes.

Proteomic analyses of coelomocytes from a second sea urchin species, *Heliocidaris erythrogramma*, demonstrated different expression profiles over time in response to sterile injections compared to injection of microbes (Dheilly, Raftos and Nair, unpublished). Results from shotgun proteomics showed two different profiles of proteomic changes. The proteins identified in the response to bacterial challenge were different from those identified in the controls. The proteomic profile changed in the coelomocytes from bacterially challenged animals within the first 6 hrs, largely due to the increased abundance of some proteins involved in cytoskeletal dynamics, while others decreased such as F-actin capping protein, advillin and α-actinin. Other cytoskeletal proteins that were absent in the controls, such as actins and annexin A7, were present in the challenged coelomocytes. Other proteins that appeared in the challenged coelomocytes included the complement homologue, C3 and SRCRs. The protein expression profile in sea urchin coelomocytes indicates that these cells show dynamic responses to wounding and immune challenge. Temporal analysis of proteomic changes in coelomocytes indicates that cellular responses to wounding and infection are biphasic. The initial phase, occurring within the first 24 hrs after treatment, appears to be a generalized response common to both types of insult. This phase involves reactions including CF coagulation and coelomocyte cytoskeletal remodelling, which resolves by 48 hrs to control levels. The second phase, which peaks at 48 hrs after injection, appears to be specific to microbial infections and the proteins expressed in this phase

are involved in pathogen recognition and opsonization as well as the destruction of the invading microbes. A number of attendant sub-cellular pathways involved in signal transduction, endocytosis and exocytosis are also enhanced during this phase. These two phases thereby function in an integrated manner to repair wounds and to neutralize microbial infections.

REGENERATION

Many classes of echinoderms are remarkably plastic in their abilities for repair and regeneration resulting from both proliferation and transdifferentiation of circulating cells of mesenchymal origin (see Microscopy Research and Technique vol 55 no. 6, 2001). New evidence suggests that cells required for regeneration originate from coelomocytes.[167-169] Unfortunately, sea urchins have limited regenerative capabilities[170] compared to sea stars and crinoids, which have received more attention.[168] Sea stars are generally known for arm regeneration and levels of heat shock protein 70 (Hsp70) increase in the common sea star *Asterias rubens*, following arm tip amputation.[169] Furthermore, manganese treatment induces proliferation of coelomic epithelial cells that is coupled to traumatic stress responses including increased expression of Hsp70.[171] Red spherule cells (or amoebocytes) and polygonal phagocytes appear to be involved in regeneration.[169] These findings suggest a role for increased coelomocyte numbers and the expression of classic stress molecules in the early repair phase of tissue damage and regeneration.

Toposome, which is also referred to as the major yolk protein (MYP), is the most abundant protein in the CF (Table 3).[172] A monoclonal antibody to embryonic toposome also recognizes sea star coelomocytes and the coelomic epithelium.[169,173,174] Although previous efforts in cloning the MYP gene in *Strongylocentrotus purpuratus* suggested its relationship to vitellogenin,[175] protein fragments were too short for unambiguous identification. The cDNA sequences encoding MYP from the sea urchins, *Pseudocentrotus depressus*, *Paracentrotus lividus* and *Tripneustus gratilla*, show that they are members of the transferrin family, lack iron-binding sites, and are not homologous to vertebrate vitellogenins.[173,176] The toposome precursor, which has been postulated to serve multiple functions, is synthesized exclusively in the gut of the adult animal as a 180-190 kDa glycoprotein and the mature protein is found in the CF. The amount of toposome protein increases in response to traumatic stresses in agreement with increased gene expression as deduced from ESTs matching toposome in sea cucumbers regenerating gut tissue.[177] Toposome is expressed with other genes encoding proteins that function in wound healing, cell proliferation, differentiation, morphological plasticity, cell survival, stress response, immune challenge and neoplastic transformation.[55] It is emerging that circulating coelomocytes from some echinoderms originate from coelomic epithelia and are able to differentiate into a few tissue types, including nerve and muscle cells.[178] Accordingly, research on stem cells in marine organisms is becoming important for both comparative studies and for future applications.

ECOTOXICOLOGY AND THE ECHINODERM IMMUNE SYSTEM

Because coelomocytes are sensitive, stress-activated effectors of the echinoderm immune response,[19,179] they are good candidate biosensors for monitoring environmental stress in an environmental management context. Several examples of using coelomocytes as indicators employ both analyses of specific proteins and of cell function. Unfractionated coelomocytes from the sea urchin *Paracentrotus lividu*s cultured at stress-inducing temperatures, (4°C or 35°C), or exposed to acidic pH, or high levels of cadmium, express high levels of Hsp70,[180,181] a well-recognized stress marker induced in response to a wide range of biological and physicochemical stresses.[106,182] In addition to serving as molecular chaperones, secreted and membrane-bound heat shock proteins (particularly Hsp60, Hsp70, Hsp90 and gp96) are potent activators of the innate immune system capable of inducing the production of proinflammatory cytokines by the monocyte-macrophage system.[183] Sp-gp96, which is expressed in sea urchin coelomocytes[88] is present on the surface of coelomocytes.[184] High levels of Hsp70 are also observed in coelomocytes obtained from sea urchins collected from waters heavily polluted with urban run-off and industrial waste[181] and, more recently, with the explosive 2,4,6-trinitrotoluene (TNT) from conventional weapons dumped at sea at the end of World War II.[185] Increased levels of Hsp70 levels in coelomocytes from specimens of the sea star *Asterias rubens*, collected along a transect from inland waters to the open sea along the Norwegian fiords correlate with a natural concentration gradient of heavy metals.[186] Increased production of reactive oxygen species (ROS) by coelomocytes from *A. rubens* is also observed after cadmium exposure in a dose-dependent manner.[187-189] Cadmium, on the other hand, leads to reduced phagocytic activity by coelomocytes.[190] Other heavy metals, including cadmium, as well as UV light can also result in single strand DNA breaks in coelomocytes.[20,191,192] Exposure to lead increases the phagocytic activity of sea star coelomocytes, though another pollutant, polychlorinated biphenyls (PCBs), do not have this effect.[190] Responses to temperature stress levels can also be detected in coelomocytes as increases in acetylcholine esterase (AChE) activity.[193] Overall, there are several approaches for monitoring protein levels and enzyme activities that can be employed as signs of environmental stress both in field and in laboratory studies.

Another approach for employing echinoderms as biosensors has been to evaluate the numbers of coelomocytes in the CF, which can be altered by environmental stressors. For example, the percentage of red spherule cells increase from 5% to 40% of total coelomocytes in animals collected from polluted seawaters or that are subjected to accidental injury.[181,185] Similarly, the numbers of total coelomocytes increase in immunoquiescent sea urchins after simple immune challenge in the lab.[29] Therefore, levels of red spherule cells and perhaps total coelomocytes may be used as a practical marker of environmental stress in animals collected in costal surveys for marine management.

CONCLUSION

Echinoderms are important members of marine ecosystems and are required for the stable maintenance of habitats. This has been illustrated by the aftermath of the disappearance of the long-spined black sea urchin, *Diadema antillarum*, the top herbivore

on Caribbean coral reefs. The population crash in 1983-1984, with repeat crashes in 1985 and 1991-1992, reduced the population in the Caribbean and Western Pacific by 95-99%.[194,195,196] General destabilization of the coral reef ecology resulting from herbivore release that was exacerbated by hurricane damage, lead to a change in the trophic cascade resulting in a swift and sustained switch to an alternative ecological state; reef cover of mostly coral switched to mostly soft algae.[197-200] The effect of the disappearance of *D. antillarum* was predicted by Sammarco[201] and modeling these population changes show the same outcome.[202]

The population crash of *D. antillarum* progressed from west to east in the Caribbean and was speculated to have been due to a pathogen[198] that may also have been a commensal.[203] Disease outbreaks and mass mortalities have also been noted in the green sea urchin, *S. droebachiensis*, along the Atlantic coast of Nova Scotia.[204] Little is known of the bacterial pathogens of sea urchins[205-209] and much less is known of the fungal and viral pathogens of any class of echinoderm. This general lack of knowledge regarding pathogenesis in echinoderms is an important problem that will require coordinated efforts of both ecologists and immunologists. Understanding the population dynamics in a complex ecosystem such as coral reefs should include investigations of the host-pathogen interactions that are involved in the stability of the ecosystem or lack thereof.

Until recently, workers interested in the immune system of echinoderms were hobbled by identifying and analyzing one gene or protein at a time. Cross-phylum searches for genes encoding proteins involved in microbial recognition and immune effector functions were complicated by the rapid pace of immune gene evolution. However, the availability of the purple sea urchin genome[11] has greatly increased the sensitivity with which immune gene homologues can be identified, in addition to the efficient characterization of complex multigene families. Immune transcription regulators are generally very well conserved and BLAST type sequence identity searches are typically adequate to identify these factors. In the case of many immune receptors and effectors, primary sequence is poorly conserved but domain structure can be used as a unique identifier and combinatorial domain profile searches can be useful. A final class of immune mediators is encoded by genes that have novel structure relative to other phyla. These are typically identified in experimental surveys, but in some cases multiplicity of domains that are common to proteins with immune functions can be identified in purely bioinformatic surveys for candidates of novel immune mediators.

Analysis of the purple sea urchin genome has revealed a complex repertoire of immune receptors, regulators and effectors unlike those known in other phyla.[10,60,78,210] In addition to the LRR-containing proteins and the *Sp185/333* gene family described above, the genome encodes a virtually complete set of homologues of (i) vertebrate hematopoietic and immune transcription factors, (ii) candidate effector proteins and (iii) genes with distant homology to key adaptive immune mediators of the jawed vertebrates.[10] Transcription regulators of haematopoiesis include a nearly complete set with respect to the vertebrate homologues and include representatives of some subfamilies such as the PU.1/SpiB/SpiC Ets factors that are important regulators of vertebrate myeloid and lymphoid immunocyte development not found outside of deuterostomes.[211] Transcription factors involved in the regulation immune response genes include GATA1/2/3, SCL and NFkB have also been identified.

Future work on echinoderm immunity will focus on the purple sea urchin, but additional echinoderm genomes are needed. Preliminary 1X sequence coverage of the genomes for

S. franciscanus and *Allocentrotus fragilis*, sister species of *S. purpuratus* are available (http://www.hgsc.bcm.tmc.edu/project-species-o-Strongylocentrotus%20purpuratus.hgsc?pageLocation = Strongylocentrotus purpuratus) and preliminary analyses suggest a similar repertoire of immune genes. Additional genomes from species from other classes are needed and will not only promote phylogenetic and comparative evolutionary genomics, but will enable the characterization of the immune gene repertoire for a different echinoderm species. This will be of particular interest given that the immune genes and the mechanisms of immune gene diversification are dictated by the life history, pathogens and habitat of individual species.

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