ISJ 3: 25-39, 2006 ISSN 1824-307X

Review

The sea urchin immune system

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Accepted May 10, 2006

Abstract

Metchnikoff's use of sea star larvae to observe encapsulation and phagocytosis, which was followed much later by allograft rejection kinetics, revealed that echinoderms had an innate immune system that was lacking of adaptive attributes. Larval sea urchins mount defenses in response to contact with microbes, which are mediated by phagocytic blastocoelar cells and pigment cells. In the adult, the coelomocytes mediate immune responses through phagocytosis and encapsulation of foreign particles in addition to degranulation of antimicrobial molecules. Molecular analysis of immune functions in the sea urchin has demonstrated a complement system that appears to have multiple alternative pathways and several activators of the lectin pathway, but may be missing the terminal pathway. Other genes and proteins involved in the sea urchin immunity include expanded sets of lectins, proteins with scavenger receptor cysteine-rich repeats, Toll-like receptors and associated signalling proteins. A vast array of proteins belonging to the 185/333 family are expressed in coelomocytes in response to lipopolysaccharide and show a surprising level of diversity. The sea urchin innate immune system has a number of large gene families with unexpected complexities and elevated levels of diversification.

Key words: evolution; deuterostome; echinoderm; coelomocyte; innate immunity; diversification; complement; *185/333* genes

Introduction

For many years, it was thought that invertebrates did not possess a recognizable immune system, a viewpoint which ignored Metchnikoff's seminal research on the mechanisms of inflammation (Metchnikoff, 1893). He was the first to demonstrate cellular encapsulation of a foreign body inserted into an invertebrate, and employed the larval sea star, *Astropecten pentacanthus*. Metchnikoff extended this observation to A phylogenetically diverse array of animals and was able to establish that phagocytic cells are a basic characteristic of metazoan biology. These observations formed the basis of the emerging field of cellular immunity for which he was awarded the Nobel Prize in 1908. However, it was not until seventy years later that Hildemann and colleagues demonstrated the ability of several echinoderm species to differentiate self from non-self tissues through allograft rejection studies (Hildemann and Dix, 1972; Karp and Hildemann, 1976). These initial observations were extended in studies of sea urchins, *Lytechinus pictus* and *Strongylocentrotus purpuratus*, in which the non-specific nature of innate immune responses of the sea urchins was defined (Coffaro and Hinegardner, 1977; Coffaro, 1979, 1980; Smith and Davidson 1992). These and other studies identified the cells of the open circulatory system in adult echinoderms, the coelomocytes, as the main effectors of defense

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responses and as the primary mediators of allograft rejection (Hildemann and Dix, 1972; Coffaro and Hinegardner, 1977), response to injury or infection, and the clearance of foreign substances (Reinisch and Bang, 1971; Bertheussen, 1981; Yui and Bayne, 1983; Plytycz and Seljelid, 1993). Of the echinoderms, the genus *Strongylocentrotus* is one of the most comprehensively studied with respect to immunological defense capabilities, particularly in the adult (reviewed by Gross *et al.*, 1999). Yet, the embryonic and larval forms also must contend with microbes in marine waters (reviewed by Smith, 2005), and studies to understand the immune capabilities at those life stages are underway.

Larval Immunity

The life cycle of most sea urchin species begins with an egg in which fertilization and development are initiated after release into the water column. Although there is significant variation among species, the resulting ciliated embryo typically hatches from the fertilization envelope just prior to gastrulation and develops into a simple feeding larva that lives in the plankton and bears little resemblance to the adult form (Fig. 1). Pluteus larvae are bilateral with an internal calcite skeleton that supports the overall structure of the larva including the gut, the blastocoelar spaces, and the "arms" that extend out from the oral side of the organism. Cilia cover the animal but are concentrated in bands at the intersection of the oral and aboral ectoderm (Strathmann, 1975). These ciliated bands enable larvae to swim and remain within the plankton near the surface, as well as to sweep food particles into the mouth and down the esophagus. Planktonic larvae eat a variety of organisms including bacteria, single celled and multicellular eukaryotes. Both the embryos and the larvae live in environments teeming with potential microbial pathogens, including those that are introduced into the gut. Consequently, these animals must have mechanisms for protecting themselves against microbial colonization and invasion of the two major potential sites of pathogen entry; the ectodermal surfaces and along the internal surfaces of the gut (reviewed by Smith, 2005).

In a similar but more recent study than those by Metchnikoff, Silva (2000) demonstrated that embryonic mesenchyme cells were capable of phagocytosing yeast that had been injected into the blastocoelar cavity. Although the identity of these cells was left unclear, possible candidates for embryonic phagocytes include the blastocoelar cells that populate the blastocoelar spaces of the larva and pigment cells that are found within and near the ectoderm (Fig. 1) (Gibson and Burke, 1985, 1987). Both of these cell types originate from the secondary mesenchyme cells that are specified from a mesodermal ring in the gastrulating embryo. During gastrulation ~30 pigment cell precursors migrate to positions in the aboral ectoderm (Gibson and Burke, 1985, 1987), and after differentiation, they are filled with granules containing red pigment and express a set of enzymes required to produce echinochrome (Castellani *et al.*, 2003). Echinochrome A has been shown to have effective antibacterial properties (Service and Wardlaw, 1984; see below), suggesting that pigment cells may have protective functions within the larval ectoderm. The blastocoelar cells develop from the secondary mesenchyme cells that migrate from the tip of the extending archenteron at late gastrulation (Tamboline and Burke, 1992). Approximately 20 of these cells take up residence in the blastocoel, form long pseudopodia, and are particularly concentrated around the gut (Fig. 1). Recently, both of these cell types have been shown to be capable of bacterial recognition and phagocytosis (Hibino and Rast, unpublished). The combination of these two cell types thus may 'patrol and protect' the larval ectoderm and gut from microbial colonization and invasion.

Commonly, phagocytosis either requires, or is significantly augmented by, molecular interactions between the phagocyte membrane and foreign particles. This interaction may be mediated by either an opsonin deposited on the foreign particle for which the phagocyte has a receptor, or by the presence of a receptor on the phagocyte that directly recognizes a microbial molecular pattern. No information is available as to whether opsonins or specific receptors are required for microbial phagocytosis by blastocoelar cells or degranulation of echinochrome by pigment cells. However, Silva (2000) suggested that contact with blastocoelar fluid might be required for phagocytosis of yeast by cells in the embryos and larvae of the sea urchin, *Lytechinus variagatus*. It is therefore interesting that two homologues of sea urchin complement C3 (SpC3 and SpC3-2; see below for more details) are expressed in the embryos and larvae. Furthermore, the gene encoding SpC3 shows low expression in embryos, but is elevated in response to contact with bacteria just prior to gastrulation (Shah *et al.*, 2003). SpC3 is known to function as an opsonin in adult sea urchins (Clow *et al.*, 2004), and may have a similar function in the embryos and larvae. The second sea urchin C3 homologue, SpC3-2, is not well characterized but is expressed at higher levels in the gastrulating embryo and larvae, whereas the transcripts are much less prevalent in adult coelomocytes (Rast, unpublished). Because SpC3 is highly expressed in coelomocytes and SpC3-2 is more highly expressed in embryos and larvae, these two C3 homologues may represent the core of two alternative complement pathways that operate at two different developmental stages of the sea urchin (see below).

Metamorphosis and adult coelomocytes

The larvae of indirect-developing sea urchins feed in the plankton for weeks to months in a developmental process, which serves, disperse the larvae far distances from their benthic parents. After a period of feeding, the adult rudiment forms within left side of the larva (Okazaki, 1975) and upon full development, the larvae leave the plankton and metamorphose if they settle on an appropriate substrate. The adult rudiment is everted into

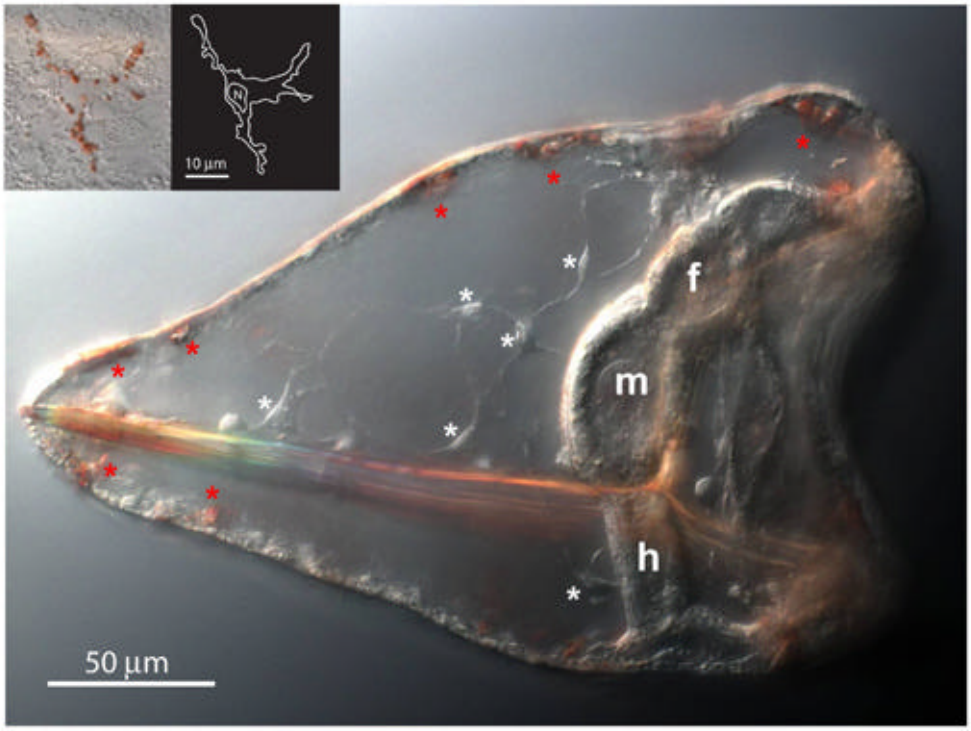

Fig. 1 Cells with immune function in the sea urchin pluteus larva. Blastocoelar cells with pseudopodia (some marked with white asterisks) occupy the blastocoel and surround the gut. Pigment cells (some marked with red asterisks) are usually in close apposition to the aboral ectoderm. A single pigment cell with vesicles is shown among cells of the aboral ectoderm (left inset). The same pigment cell is shown in outline in the right inset. Subdivisions of the gut are indicated for orientation. f, foregut; m midgut; h, hindgut. The spicular skeleton is birefringent in the preparation.

a juvenile, leaving a "turban" of larval tissues on the dorsal side of a tiny sea urchin that has five tube feet and five spines. This process transforms the animal from a bilateral larval form to a pentamerous juvenile. The anatomy of the fully metamorphosed sea urchin is essentially the same as the adult with Aristotle's lantern (mouth parts), gut, and gonad being the major organs in the coelomic cavity. Filling the spaces between the organs within the coelomic cavity is coelomic fluid that contains coelomocytes. There are a variety of coelomocyte types in *S. purpuratus* that can be differentiated based on their structural attributes (Johnson, 1969; Bertheussen and Seljelid, 1978; Edds, 1993; Gross *et al.*, 2000). Four categories are currently recognized; phagocytes, red spherule cells, colorless spherule cells and vibratile cells (Table 1; Fig. 2). The proportions of each type of coelomocyte in the coelomic fluid vary considerably (Table 1) at both the inter- and intra-individual level (Smith, unpublished). This variability may reflect the nutritional, immunological and homeostatic status of the individual sea urchin from which the coelomocyte samples were taken (Smith and Davidson, 1994; reviewed by Gross *et al.*, 1999).

The phagocytes constitute the majority of coelomocytes and have been reported to be involved in graft rejection, chemotaxis, phagocytosis, encapsulation, immune gene expression, agglutination (Gross *et al.*, 2000; Clow *et al.*, 2004; reviewed in Gross *et al.*, 1999) and clotting reactions (Hillier and Vacquier, 2003). Phagocytes also appear to be the major cell type to express immune genes including complement homologues, a C-type lectin and a homologue of NFkB (Fig. 3). Phagocytes can be sub-categorized into three types based on morphology. Type 1 phagocytes (Fig. 2A, B), also known as discoidal cells, can be readily differentiated from type 2 phagocytes (Fig. 2C, D), or polygonal cells, by their distinct cytoskeletal morphologies (Edds, 1993; Henson *et al.*, 1992; 1999). Discoidal cells have radially arranged actin filaments emanating from the central nuclear region of the cell, whereas polygonal cells have their actin filaments arranged laterally and lie along the length of the cell membrane forming irregular polygonal shapes (Edds, 1993). The discoidal cells are stationary *in vitro*, while the polygonal cells show greater motility (Henson *et al.,* 1992) and can translocate across glass like fibroblasts when in contact with coelomic fluid proteins (Edds *et al.*, 1983). Differences in these two cell types also include subcellular localization of kinesin, microtubules, and myosin II, in addition to positioning of the mitochondria (Henson *et al.*, 1999). The possible developmental relationships between these two subtypes of phagocytes have not been described. Small

Table 1 Coelomocyte cell types and functions in the purple sea urchin *Strongylocentrotus purpuratus.*

Cell type	% in coelomic fluid	Morphology	Function
Type 1 - Discoidal cell	40-80 % (Total phagocytes) (Edds 1993, Gross *et al.*, 2000 and Smith L.C., unpublished)	Dense nucleus with clear disc shaped cytoplasm, devoid of organelles with actin striations radiating from the center (Fig. 2A, G)	Immune function: encapsulation, opsonisation (Clow *et al*., 2004), chemotaxis and phagocytosis (reviewed by Gross *et al.,* 2000), graft rejection (reviewed by Edds 1993)
Type 2 - Polygonal cell		Clear nucleolus (granular nucleus) and obvious organelles in the surrounding cytoplasm. Forms large thin irregular shapes. Actin bundles run parallel to straight edges of cell (Fig. 2G, H)	Immune function: chemotaxis, opsonisation (Clow *et al.*, 2004), phagocytosis (reviewed by Gross *et al.*, 2000), antibacterial activity due to lysozyme (Gerardi *et al.*, 1990), clotting and encapsulation, graft rejection (reviewed by Edds 1993)
Type 3 - Small phagocyte		Smaller than both type 1 and type 2 with little cytoplasm (fig. 2C)	unknown
Red spherule	7-40 % (Gross *et al.*, 2000 and Smith L.C., unpublished)	Amoeboid cells with red spherical inclusions, (Fig. 2D)	Antibacterial activity not due to lysozyme (Gerardi *et al.*, 1990) contains echinochrome A (Service and Wardlaw 1984)
Colorless spherule	3.7-25 % (Gross *et al.*, 2000 and Smith L.C., unpublished)	Colorless amoeboid cells with spherical inclusions; may be several types in other echinoderms (Fig. 2E)	unknown
Vibratile (Boolootian and Geise 1958, Johnson 1969, Karp and Coffaro 1980, Smith 1981)	11.9-20 % (Gross *et al.*, 2000 and Smith L.C., unpublished)	Round colorless cells with a single flagellum (Fig. 2F)	Movement or agitation of coelomic fluid? Associated with clotting (Bertheussen and Seljelid, 1978)

phagocytes (Fig. 2E), a third type of phagocyte identified by Gross *et al.* (2000), are smaller with less cytoplasm than either of the other two cell types. They have not been reported previously, perhaps because of their small size and low numbers. To date, the function of these cells is unknown.

There are two types of spherule cells, those with red spherules, also called morula cells (Fig. 2F), and those with colorless spherules (Fig. 2G). They are similar in size and are both amoeboid (Matranga *et al.,* 2006), however the red spherule cells are significantly more dense than the colorless type, enabling separation by density centrifugation (Bertheussen and Seljelid, 1978; Gross *et al.*, 2000). The red spherules contain echinochrome A, a naphthoquinone which gives the cells their characteristic red color. Echinochrome A is degranulated in the presence of bacteria (Johnson, 1969) and has antimicrobial properties against both Gram positive and Gram negative bacteria (Service and Wardlaw, 1984; Gerardi *et al.*, 1990; Haug *et al.*, 2002). Red spherule cells accumulate around injuries and sites of infection (Johnson, 1970; Coffaro and Hinegardner, 1977), suggesting that these cells and echinochrome A play a role in the immune response in adult sea urchins. The functions of the colorless spherule cells have yet to be identified. Vibratile cells (Fig. 2H) are spherical and show no amoeboid movement but have a single flagellum, which may propel them through the coelomic fluid. They have been associated with clotting reactions (Bertheussen and Seljelid, 1978), which is an important response to injury.

Upon immediate removal from a sea urchin, the phagocytes appear as bladder amoebocytes (Bertheussen and Seljelid, 1978) or petaloid phagocytes (Edds, 1979; 1983), which is a description of lamellipodia that extend from the cell body in all dimensions. This may be the normal morphology of phagocytes while in the coelomic cavity, but upon settling on glass, the petaloid

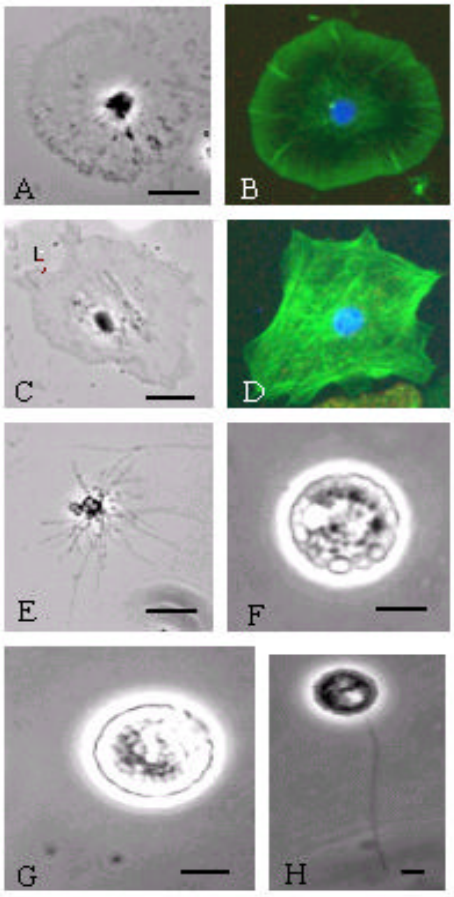

Fig. 2 Coelomocyte types in the sea urchin, *S. purpuratus*. Phase contrast images of live cells are shown for type 1 discoidal phagocyte (A), type 2 polygonal phagocyte (C), small phagocyte (E), red spherule cell (F), colorless spherule cell (G) and vibratile cell (H). Fluorescent images of a type 1 discoidal cell (B) and a type 2 polygonal cell (D) were obtained after settling the cells on poly-L-lysine (0.1 g/ml) coated coverslips for 5 min in calcium - magnesium-free seawater with 30 mM EDTA, and then in coelomocyte culture media (Henson *et al*., 1999) for 30 min. The cells were fixed and stained with a monoclonal anti-actin antibody (ICN) followed by goat anti-mouse Ig labeled with fluorescein and counterstained with Hoescht 33258 (Molecular Probes) nuclear stain. All images were collected using a Zeiss Axioplan fluorescence microscope. Scale bar in A and C is 15 μM. Scale bar in E, F and G is 12 μM. Scale bar in H is 10 μM.

lamellipodia transform into discoidal or polygonal morphology and may show ruffling (Henson *et al*., 1992; Edds, 1993). This cellular behaviour has been considered akin to encapsulation, because the glass slide is perceived as foreign (Edds, 1993). Upon exposure to hypotonic media, lamellipodia of both discoidal and polygonal cell types transform into filopodia starting with the formation of serrations at the lamellipodial edge followed by cytoplasmic retraction and filopodial extension (Edds, 1980). The filopodia have been described as forming physical links among several phagocytes, or filopodial intertwining, to result in a cellular clot, which is retracted through filopodial shortening (Edds, 1977; Smith, 1981).

A complement system in sea urchins

Coelomocytes are the central mediators of immune responses in sea urchins. Besides graft rejection, encapsulation, and cellular clot formation (Smith and Davidson, 1994; reviewed by Gross *et al*., 1999), coelomocyte activities include the efficient clearance of a broad array of foreign cells or particles injected into the coelomic cavity, including bacteria (Bertheussen, 1981; Yui and Bayne, 1983; Plytycz and Seljelid, 1993), xenogeneic cells (Reinisch and Bang, 1971), latex beads and yeast (Bertheussen, 1981), bacteriophage (Coffaro, 1979), and red blood cells (Kaplan and Bertheussen, 1977; Bertheussen, 1981; for reviews see Smith and Davidson, 1994; Gross *et al*., 1999).

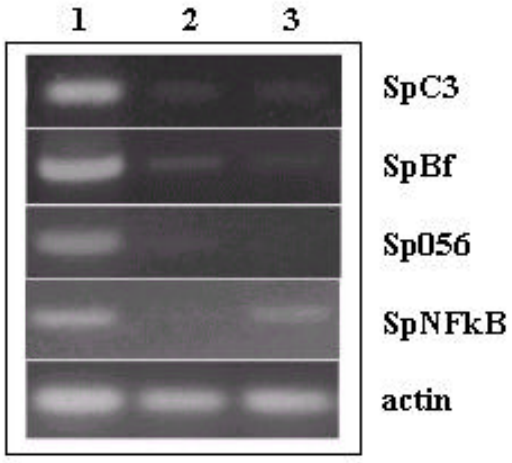

Fig. 3 Phagocytes are the major expressors of immune response genes. Expression of several immune genes were analyzed in fractions of coelomocytes by reverse transcriptase – PCR. The genes analyzed encode complement proteins SpC3 (Clow *et al*., 2000) and SpBf (Smith *et al*., 1998; Terwilliger *et al*., 2004), a C-type lectin, Sp056, called SpEchinoidin (GenBank accession number; AAR02404) and the transcription factor SpNFkB (Pancer *et al*., 1999). Actin expression was used as the control. Phagocytes show the strongest expression of all genes while the red spherule cells also express SpNFkB. Total RNA was isolated from density separated coelomocytes (see Gross *et al*., 2000), reverse transcribed with a random hexamer and employed in PCR with primers specific for the genes listed to the right of the image. Lane 1, phagocytes; lane 2, mix of vitratile cells and colorless spherule cells; lane 3, red spherule cells. Lanes 2 & 3 contain $\leq$ 2.1% phagocytes, and may account for the minor bands in those lanes.

Phagocytosis of sheep RBCs (SRBCs) by coelomocytes was not efficient until the SRBCs were opsonized with human IgM and C5-depleted serum (which blocked the lytic pathway) (Kaplan and Bertheussen, 1977; Bertheussen, 1981) and led to further investigations. Using isolated human complement components for controlled SRBC opsonization also augmented phagocytosis of SRBCs and suggested that both C3b and the inactivated form, C3bi, were involved in phagocytosis (Bertheussen, 1982). These results inferred that type 3 complement receptors were present on the phagocytes and lead to the speculation that sea urchins might have a complement system (Bertheussen, 1983).

Molecular identification of sea urchin complement components

An early molecular investigation of genes expressed in sea urchin coelomocytes showed that profilin transcripts were up-regulated in response to injections of lipopolysaccharide (LPS) into the coelomic cavity (Smith *et al.*, 1992, 1995). The implication was that because profilin functions in cytoskeletal modifications (Paavilainen *et al.*, 2004), its elevated expression indicated immune activation in coelomocytes as reflected by increased amoeboid movement, phagocytosis and secretion, all of which requires changes in cell shape. Consequently profilin was used as a marker for cell activation, and pools of coelomocytes were employed in an expressed sequence tag (EST) study to generate a snapshot of gene expression in the coelomocytes responding to LPS and to provide a basic understanding of the molecular immunological functions of these cells (Smith *et al.*, 1996). This study yielded 307 ESTs, of which 89 matched to known sequences derived from 55 distinct genes. Of those ESTs, two matched to mammalian complement proteins as had been predicted by Bertheussen (1982).

SpC3 identification

EST064 was a new member of the thioester-containing complement protein family that includes C3, C4, and C5 (Smith *et al.*, 1996). Alignments of the full length sequences showed that the deduced sea urchin protein matched most closely to other C3 proteins and was therefore called SpC3 (Al-Sharif *et al.*, 1998). The SpC3 protein contained a conserved α/β cleavage site to produce a mature protein with two chains and a conserved thioester site in the α chain. Sequence analysis of SpC3 identified N-linked glycosylation sites, cleavage sites for factor I and binding sites for factor H and factor B, leading to speculation that SpC3 functioned similarly to other C3 homologues (Al-Sharif *et al.*, 1998; Smith *et al.*, 1999, 2001; Smith, 2001). Immunoquiescent sea urchins have either undetectable or very low levels of SpC3 in the coelomic fluid (Gross *et al.*, 1999). Upon immune challenge with LPS, however, SpC3 protein appears in the coelomic fluid within 15 minutes (Clow *et al.*, 2000). Furthermore, SpC3 is produced by a subset of phagocytes that appear to maintain the protein within small cytoplasmic vesicles (Fig. 4). In addition to the SpC3 secretion response, there is also an increase in number of phagocytes containing SpC3 after challenge (Gross *et al.*, 2000). SpC3 was the first complement component identified in an invertebrate, which galvanized others to search for and to find complement systems in a wide variety of animals.

Phylogenetic analysis of the thioester-containing protein family placed SpC3 basal to the chordate complement proteins and therefore identified it as a homologue of the common ancestor of the C3, C4 and C5 family in deuterostomes (Al-Sharif *et al.*, 1998). C3 homologues have also been identified in the tunicates, *Halocynthia roretzi*, *Styela plicata* and *Ciona intestinalis* (Nonaka and Azumi, 1999; Marino *et al.*, 2002; Raftos *et al.*, 2002) and until recently, only deuterostomes were thought to contain complement-like proteins. However, C3 homologues have been found in the horseshoe crab, *Carcinoscorpius rotundicauda* (Zhu *et al.*, 2005) and in the gorgonian coral, *Swiftia exerta* (Dishaw *et al.*, 2005), indicating that C3 appeared prior to the protostome-deuterostome split. Thioester-containing proteins have also been identified in both *Drosophila* (Lagueux *et al.*, 2000) and the mosquito, *Anopheles gambiae* (Levashina *et al.*, 2001), but phylogenetic analysis of these proteins indicates that they fall into a different clade than either the complement clade or the α_2-macroglobulin clade proteins (Blandin and Levashina, 2004).

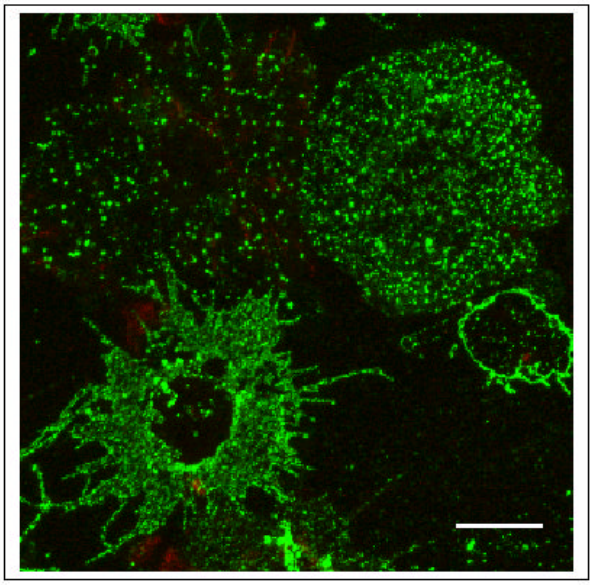

Fig 4. SpC3 is localized in small vesicles in phagocytes. Confocal fluorescent micrographs of SpC3 localization in the type 1 and type 2 phagocytes of *S. purpuratus*. Density separated phagocytes were centrifuged onto slides, fixed and stained with SpC3-anti-peptide antiserum followed by goat anti rabbit Ig conjugated to alexa (GáRIg-A, Pierce). Images were captured using a Bio Rad MRC 1024 Confocal Laser Scanning System attached to an Olympus IMT2-RFC inverted microscope (for details, see Gross *et al.*, 2000). Scale bar is 15 μM.

The identification of thioester containing proteins, which appear to be present throughout the animal kingdom, suggests that this mechanism for recognizing, binding to, and eliminating pathogens is very ancient.

SpC3 function

Sequence analysis had indicated that SpC3 was a C3 homologue, but it was important to determine whether the function of SpC3 was conserved. Based on the structure of C3 and C4, the active thioester site is recessed in a pocket and partially protected from deactivation (reviewed in Sim and Sim, 1983). However, when activated C3 proteins are denatured and heated under alkaline conditions, thioester sites undergo autolysis causing α chain cleavage (Sim and Sim, 1981, 1983). Autolysis does not occur when the thioester has either not been activated or has been deactivated, and therefore the chemical reaction can be used to assess the fraction of proteins containing active thioester sites. Alternatively, thioester function can also be demonstrated by binding small nucleophiles such as methyl amine or hydroxylamine. When these approaches were used to analyze SpC3, autolysis was found to occur in a fraction of the proteins that varied among individual sea urchins (Smith, 2002). Furthermore, SpC3 bound both methylamine and yeast, which blocked autolysis, and suggested that SpC3 could function as an opsonin. Consequently, when yeast were opsonized using coelomic fluid from immune activated sea urchins, phagocytosis was augmented (Clow *et al.*, 2004). Overall, these data indicate that the thioester site is functional on SpC3 and that it is a major opsonin in sea urchin coelomic fluid (Smith, 2001, 2002).

SpBf identification

Factor B (Bf), which has been found throughout the deuterostomes (Ishiguro *et al.*, 1992; Nakao *et al.*, 1998; Smith *et al.*, 1998; Nonaka and Azumi, 1999; Azumi *et al.*, 2003), is the second component of the alternative pathway, and the second complement homologue identified in the sea urchin (Smith *et al.*, 1996, 1998). Bf is a mosaic protein containing several short consensus repeats (SCRs), a von Willebrand factor (vWF) domain and a serine protease domain. Most Bf proteins have three SCRs, however, exceptions include the carp Bf homologue with four SCRs (Nakao *et al.,* 1998), and the sea urchin Bf protein (SpBf) with five SCRs (Smith *et al.*, 1998). The Bf protein in the tunicate, *C. intestinalis*, is quite different and has three SCRs and two CUB (C1r, u-epidermal growth factor, bone morphogenic protein) domains (Azumi *et al.*, 2003). Expression of the gene encoding SpBf (*Sp152*) was constitutive and not affected by challenge with LPS (Terwilliger *et al.*, 2004). Along with the full-length *Sp152* transcript containing five SCRs, some *Sp152* transcripts were alternatively spliced into remove the first and/or the fourth SCR (Terwilliger *et al.*, 2004). However, only those messages that were missing the fourth SCR encoded an in-frame protein. Messages in which the first SCR was deleted had a frame shift which resulted in an early stop codon and a truncated protein. Therefore, it appears that in most higher deuterostomes, Bf proteins have three SCRs, while in lower deuterostomes, including *S. purpuratus* and the tunicate *C. intestinalis*, Bf proteins have four or five SCRs (Azumi *et al.*, 2003; Terwilliger *et al.*, 2004). It is not clear from the phylogenetic analyses of Bf proteins whether the evolutionary process has selected for the loss of SCRs in higher deuterostomes from an ancestral protein structure containing five SCRs, or if the lower deuterostomes have undergone domain duplication more recently to result in Bf proteins with five SCRs (Terwilliger *et al.*, 2004).

Complement systems in lower deuterostomes

In higher vertebrates, the complement system is composed of about 35 serum and cell surface proteins (Volanakis, 1998) and is categorized into three activating pathways, called classical, alternative and lectin, which unite to trigger the terminal pathway (Dodds and Law, 1998) (Fig. 5). In addition, there are a number of regulatory proteins located in both the serum and on cell surfaces, and complement receptors located on many cell types including phagocytes. Analysis of the *C. intestinalis* genome has shown preliminary identification of many complement components in this invertebrate including duplications of proteins in the alternative and lectin pathways, and several matches to C6 in the terminal pathway (Azumi *et al.*, 2003; reviewed in Nonaka and Yoshizaki, 2004). Although cloning and sequencing have identified C3 and Bf homologues in the sea urchin, searches of the first build of the sea urchin genome (7/18/05 assembly) have uncovered additional gene models that match to complement homologues. These include four members of the C3/4/5 family, three members of the Bf/C2 family, mannose binding lectin (MBL), and several matches to C1q (Fig. 5) (unpublished). In addition, a number of matches were identified to variant forms of MBL-associated serine proteases (MASPs) and to proteins with a perforin-membrane attack complex (MACPF) domain. With three gene models that encode C3 homologues and three that encode Bf homologues, the sea urchin complement system appears as an expansion of the alternative pathway, perhaps with activation through an expanded lectin pathway using MBL, C1q and MASPs, plus a possible terminal pathway. However, without clear identification of members of the terminal pathway, the major function of this system may be opsonization.

A complement system consisting of the alternative and lectin pathways and functioning in opsonization can be of significant value in combating microbial invasion. The alternative pathway in higher vertebrates has been considered to be the core of the complement system, and the current characterization of the sea urchin system emphasizes this notion. The positive feedback loop significantly augments the rate at which foreign particles can be opsonized and is therefore more effective and efficient than individual opsonins such as simple lectins that rely on diffusion to bind to the target. The presence of a C3-convertase in the sea urchin, composed of a complex of SpC3 and SpBf, and the presence of a positive feedback loop has been suggested previously

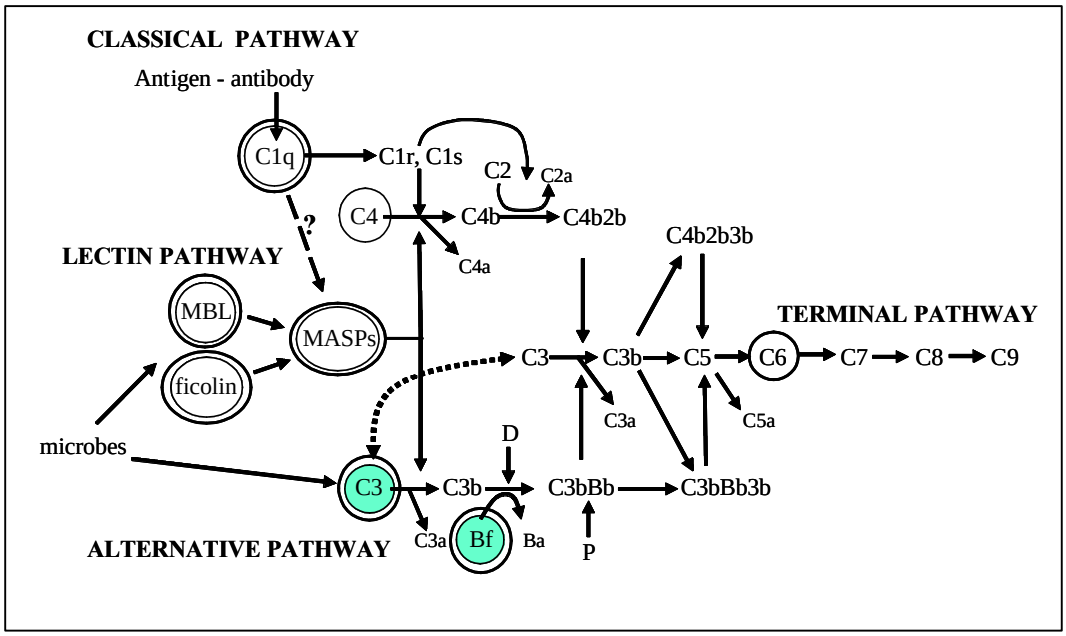

Fig. 5 The Complement Cascade. The mammalian complement cascade is shown with the known (green circles) and predicted (green striped circles) sea urchin complement proteins mapped onto it. Complement proteins known in *Ciona intestinalis* are circled with a bold line. The dotted line represents the positive feedback loop in the alternative pathway. The dashed line suggests the possible activation of MASPs by C1q homologues in both sea urchins and tunicates.

and might be of significant benefit to the species (Fig. 5) (Smith *et al.*, 1999, 2001; Smith, 2001). The importance of the complement system within the immune response of the sea urchin is reflected by multiple alternative pathways that may be activated through MBL or C1q. These results infer the importance of opsonization followed by phagocytosis by coelomocytes for removing and destroying invading microbes.

Regulatory proteins and receptors in the complement system

The amount of functional C3 in body fluids depends on the rate of thioester activation vs. deactivation either by the formation of covalent bonds with a target or by hydrolysis. The thioester site is an intra-chain bond between the side groups of cysteine and a nearby glutamic acid (Dodds and Law, 1998) and when activated, becomes available for ester or amide bond formation with nearby substrate molecules. Normally, if covalent bonds are not formed, the reactive thioester undergoes hydrolysis due to the abundance of surrounding water, thereby limiting the spread of reactive thioester-containing molecules (Dodds and Law, 1998). The short time frame of thioester reactivity helps to ensure that covalent bond formation occurs near where complement activation was initiated, usually on the surface of a pathogen rather than in the plasma or on host cell surfaces. However, autoactivation of C3 and augmented C3 activation by C3-convertase functioning within the positive feedback loop can lead to large amounts of activated C3 and inappropriate deposition of C3 fragments on host cells. Consequently, to protect self tissues from autologous complement attack, a number of regulatory proteins are required both in body fluids and on cell surfaces. Many of the regulatory proteins and complement receptors in higher vertebrates are constructed of short consensus repeats (SCRs) or complement control protein (CCP) modules. Searches of the sea urchin genome reveal 247 proteins with SCR domains (7/18/05 assembly). It is feasible that some of these gene models will be characterized in the future to encode complement receptors and regulatory proteins that function to protect self. Two examples of expressed genes have been identified that encode proteins with putative complement regulatory function or may be members of a primitive terminal pathway (Multerer and Smith, 2004). They are SpCRL (*S. purpuratus* complement related protein, long form) and SpCRS (short form) and both have multiple SCRs and a FIMAC (factor I-membrane attack complex) domain. Both share domains with factor H and factor I, which have regulatory functions, and C6 and C7, which are members of the terminal pathway.

Other genes expressed in coelomocytes

Lectins

In addition to the complement system, C-type lectins may also be of significant importance in immune defense

in the sea urchin. C-type lectins are carbohydrate binding proteins that require Ca^{2+} for proper conformation and function of the carbohydrate recognition domain (CRD). An EST that matched to a C-type lectin, shows significant sequence similarity to echinoidin (Smith *et al.*, 1998), which was previously characterized in the sea urchin, *Anthocidaris crassispina* (Giga *et al.*, 1987). SpEchinoidin (echinoidin from *S. purpuratus*; GenBank accession AY336600) has six cysteines in conserved positions and a seventh that suggests that it may form a homodimer (Smith *et al.*, unpublished). The carbohydrate binding motif composed of three amino acids within the CRD, predicts that SpEchinoidin may bind galactose or its derivatives. The gene encoding SpEchinoidin, *Sp056*, is expressed exclusively in the phagocyte class of coelomocytes and only after immune challenge (Multerer and Smith, 2004; Terwilliger *et al.*, 2004; Smith *et al.*, unpublished). Analyses of small lectins in the coelomic fluid of *S. purpuratus* suggest that there is vast array that have differing carbohydrate binding specificities and are expressed in response to immune challenge (Smith *et al.*, unpublished). This is in agreement with searches of the sea urchin genome (7/18/05 assembly) in which a large number of gene models matched to single domain C-type lectins and have motifs for binding an array of carbohydrates.

SRCRs

Coelomocytes express a large, complex family of transcripts that contain domains called scavenger receptor cysteine-rich (SRCR) repeats (Pancer *et al.*, 1999; Pancer, 2000) and comprise a protein superfamily in metazoans. SRCR domains are 110 amino acids in length and exhibit a conserved spacing of six to eight cysteine residues, which is important for intradomain disulfide bonds (Freeman *et al.*, 1990). SRCR-containing proteins have been identified in a wide variety of animals (Sarrias *et al.*, 2004) and in invertebrates, have been shown to function as an aggregation receptor in a marine sponge (Blumbach *et al.*, 1998), or a sperm activation receptor in a sea urchin (Dangott *et al.*, 1989). In vertebrates, however, SRCR-containing proteins play a critical role in the regulation and development of the immune response. SRCRs are expressed in both hematopoietic and non-hematopoietic vertebrate immune cells (Sarrias *et al.*, 2004), and down-regulate antigen receptor signalling on T and B cells (Perez-Villar *et al.*, 1999), down-regulate host response to endotoxin (Trahey and Weissman, 1999), regulate apoptosis (Miyazaki *et al.*, 1999), inhibit endocytosis (Takito *et al.*, 1996), and bind pulmonary lectins (Holmskov *et al.*, 1999). Relevant functions of SRCR proteins within innate immunity include binding repetitive polyanionic structures such as modified lipoproteins, bacterial lipids and certain nucleotide aggregates (Sarrias *et al.*, 2004). In the sea urchin, a diverse set of SRCR-containing transcripts was isolated from coelomocytes, revealing six types of SRCR molecules (Pancer *et al.*, 1999; Pancer, 2000). The proteins have between 2 and 20 SRCR domains plus a variety of other domains, including a von Willebrand factor (vWF) domain, epidermal growth factor (EGF) domains, SCRs, and an extracellular-matrix-like domain (ECM). The SRCR transcripts are derived from a large gene family in the sea urchin and genome blots revealed complex hybridization patterns suggesting that SRCR genes may exist in multiple forms and may be clustered. Furthermore, the diversity of SRCR genes is high both within individuals and within the population, which is supported by a preliminary analysis of the sea urchin genome (7/18/05 assembly), which indicates that there are 228 SRCR gene models present.

The SRCR transcript repertoire of individual sea urchins is highly transient in unchallenged animals and is greatly altered after injury and bacterial or fungal challenge (Pancer, 2000). Unchallenged animals display changes in SRCR expression of 20- to 30-fold over a period of three months. Animals challenged with pathogens also display variations in transcript expression of this magnitude, although there is no specific expression pattern observed among the animals. Although the regulation of these genes is unknown, a high degree of conservation in the 5' flanking region suggested possible coordinated regulation of SRCR gene transcription. Many of the SRCR-containing genes in the sea urchin may have immunological relevance, although the specific functions of the proteins encoded by the individual genes are unknown.

Toll receptors and transcription factors

Animal Toll receptors are characterized by extracellular leucine rich repeats (LRR), which function to recognize molecular pathogen signatures (Pasare and Medzhitov, 2005), and cytoplasmic TIR (Toll-IL1 receptor) domains, which are important for signalling. *Drosophila* has eight Toll genes and humans have eleven, however, plant genomes have hundreds of R genes which have LRRs. Similar to the plant system, the purple sea urchin has a very large array of predicted Toll genes (Pancer and Cooper, 2006). Although expression of most of these genes is currently unknown, a few are expressed in coelomocytes (see GenBank accessions AAK25761, AAK25762). In correlation with the expansion of the Toll genes in the sea urchin genome, there is a moderate expansion of genes encoding proteins that initiate the signalling pathways that are activated by Toll (Rast, unpublished). These include MyD88 homologues, which are cytoplasmic proteins that interact with the TIR domains.

The end of the Toll signalling pathway results in the release of Rel transcription factors from their cytoplasmic inhibitors, followed by their translocation into the nucleus. *SpNFkB* (*S. purpuratus* Nuclear Factor kappa B) was the first Rel protein described in the sea urchin and is homologous to *Drosophila* Relish and vertebrate p105 and p65 proteins (Pancer *et al.*, 1999). Other Rel proteins have been identified in the sea urchin genome including a second NFkB homologue and one NFAT homologue (unpublished). *SpNFkB* and *SpRunt* expression in non-activated coelomocytes is undetectable, but both transcription factors are highly expressed 6 to 12 hrs after bacterial challenge or injury (Pancer *et al.*, 1999). On the other hand, *SpGATAc* has

the opposite expression pattern in coelomocytes. Its expression in non-activated coelomocytes is down-regulated in response to bacteria and injury. Based on the expression patterns in coelomocytes, SpNFkB may be transcription activator for immune genes while SpGATAc may be a repressor (Pancer *et al.*, 1999). In embryos, *SpRunt* expression is associated with proliferating cells (Robertson *et al.*, 2002) and consequently in coelomocytes it may be involved in proliferative responses to immune challenge.

Other LPS-responsive ESTs

In an effort to identify transcripts that appear in coelomocytes in response to LPS challenge, an EST study employed probes produced from subtractive suppressive hybridization using coelomocyte mRNA from immunoquiescent sea urchins and the same sea urchins after LPS challenge (Nair *et al.*, 2005). Screens of arrayed coelomocyte cDNA libraries identified ~6000 clones (of 92,160 clones in a library) representing putative LPS-responsive genes (Nair *et al.*, 2005). EST analysis of 1247 clones led to the identification of numerous novel genes expressed in *S. purpuratus* coelomocytes during LPS challenge and included proteins that function in host defense, as cell surface receptors, signalling molecules, cytoskeletal and cytoskeleton modifying molecules, proteases, RNA splicing, protein synthesis, protein processing, protein degradation, cell proliferation, and apoptosis.

One of the largest categories of ESTs identified from both of the EST studies (Smith *et al.*, 1996; Nair *et al.*, 2005) matched to cytoskeletal proteins including α and β-tubulin, dynein heavy chain, kinesin light chain, actin, gelsolin, fascin, and thymosin-β. Additional matches include the mena neural variant protein, a receptor for activated protein kinase C (RACK), integrin βC, protein tyrosine phosphatase receptor type F, protein tyrosine kinase 9-like protein, Rho, Rho-GDP dissociation inhibitor, cofilin, avena, and microtubule associated protein. This set of genes, which are up-regulated in response to LPS and encode both cytoskeletal proteins and cytoskeleton-regulating proteins, suggest that active and extensive remodelling of the cytoskeleton is a direct response of coelomocytes to immune challenge. This is in agreement with the initial identification of profilin up-regulation in coelomocytes responding to LPS (Smith *et al.*, 1992).

Changes in nuclear activities were inferred from matches to proteins involved in DNA transcription and mRNA splicing. Alternative splicing of defense-related transcripts has been observed for three complement components, SpBf (Terwilliger *et al.*, 2004), SpCRL and SpCRS (Multerer and Smith, 2004), and the number of EST matches with putative splicing function suggests that alternative splicing may be common. Increases in messages encoding proteins involved in the synthesis, processing and degradation of proteins corresponds with coelomocyte responses to immune challenge. Furthermore, matches to proteins that function in sorting (e.g. a p24 homologue which regulates vesicular traffic between the ER and the Golgi apparatus) and vesicular trafficking within the endosomal system (e.g. Rab5-interacting protein and the mannose-6-phosphate receptor homologues). Indeed, previous studies have inferred that SpC3 is synthesized and transported in cytoplasmic vesicles prior to secretion (Clow *et al.*, 2000; Gross *et al.*, 2000; Smith, 2001). These results suggest that coelomocytes are involved in a significant level of protein production, transport and secretion beyond what is known about the complement components.

EST matches also indicate that coelomocytes express genes encoding proteins involved in stimulating cell proliferation (e.g. a polo-like kinase and allograft inflammatory factor), while other genes encode proteins that inhibit apoptosis (e.g. Bax inhibitor-1). The activities of both these groups of proteins (i.e. proliferative and anti-apoptotic) may serve to enhance coelomocyte numbers during immune challenges. Such changes have been noted as increases in the numbers of cells expressing SpC3 that appear in the coelomic fluid after immune challenge (Clow *et al.*, 2000). This suggests changes in cell numbers may be due to proliferation or reduced turnover rather than the release of emarginated coelomocytes into the coelomic fluid.

Aggressive clotting reactions are essential for protecting sea urchins from injuries and damage to the body wall because these animals have a ridged skeleton, or test, that cannot be contracted to close wounds. Clotting of the coelomic fluid is a response to injuries and is a complex reaction involving both coelomocytes and coelomic fluid components. A recent study characterizing the clotting reaction in *S. purpuratus* showed that the coelomic fluid protein amassin is a prime mediator of the clotting reaction (Hillier and Vacquier, 2003) by employing disulfide bond formation; Bertheussen and Seljelid, 1978). This protein, which was also found to be expressed by LPS-activated coelomocytes (Nair *et al.*, 2005), contains an olfactomedin domain and mediates the intercellular adhesion of coelomocytes during cellular clot formation. Current analysis of the sea urchin genome (7/18/05 assembly) reveals four additional gene models with olfactomedin domains, although their functions are unknown (unpublished). Although the cell surface receptor for amassin has not been identified, it may function in clotting by binding to integrins. It is noteworthy that a match to integrin βC was identified (Nair *et al.*, 2005), which was reported previously from sea urchin embryos (Murray *et al.*, 2000; Burke *et al.*, 2004). Both the βC and βL integrins can be detected serologically on the surface of coelomocytes (R Burke, personal communication), and a number of integrins have been identified in the sea urchin genome (unpublished).

The *185/333* gene family

Message structure

Screens of the bacterially activated arrayed cDNA library using subtracted probes showed that about 60 % of the positive clones matched to an unknown sequence that encoded a family of proteins (Nair *et al.*, 2005). Re-screens showed that approximately 6.5 % of

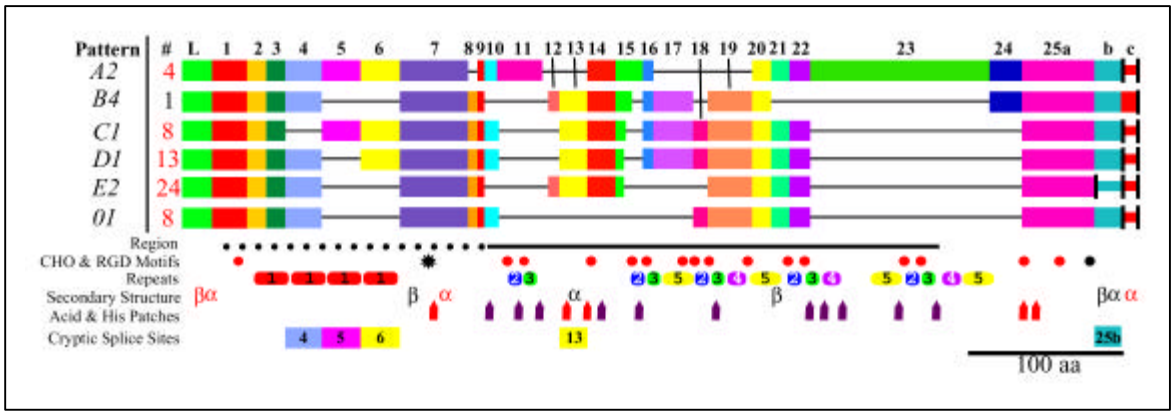

Fig. 6 Full-length sequences from 81 cDNA clones from the arrayed library were manually aligned and gaps (represented by horizontal lines) were inserted to optimize the alignment. The gaps separated blocks of sequence or elements, which are represented by colored boxes (numbered at the top). The figure shows a representative set of element patterns, which were established on the presence and type of element 15 (shown as varying sizes). An example of each group of cDNA is represented here. Group 1 is defined by element 15a (*A2*), group 2 by element 15b (*B4*), group 3 by element 15c (*C1*), group 4 by element 15d (*D1*), group 5 by element 15e *(E2*), and groups 6 (01) and 7 (not shown) do not have element 15. Element 25 was divided into three sub-elements, 25a, b and c, based on the location of the stop codon (black vertical lines). The deduced protein shows little secondary structure. It is separated into a glycine-rich region (dotted horizontal line) and histidine-rich region (solid horizontal line). Symbols indicate the presence of an RGD motif in element 7 (black star); conserved N-linked glycosylation sites (red circles) and O-link glycosylation sites (black circle); five types of repeats shown as numbered colored ovals (type 1 = red; type 2 = blue, type 3 = green; type 4 = purple; type 5 = yellow); secondary structure predictions (either á-helices or â-strands); short stretches of acidic amino acids (red vertical bars); histidine patches (purple vertical bars); and the five elements which are surrounded by putative cryptic splice signals. The scale bar is located at the lower right. (modified from Terwilliger *et al.*, 2006).

the clones in the bacterially activated coelomocyte library were positive, compared to only 0.0087 % of the clones in the non-activated coelomocyte cDNA library. This was in agreement with Northern blots that revealed significant up-regulation in expression of this message in response to bacterial challenge (Rast *et al.*, 2000). Originally identified as EST333 (Smith *et al.*, 1996), later called DD185 (Rast *et al.*, 2000), and now referred to as *185/333* (Nair *et al.*, 2005), these transcripts are unusual because they display an elevated level of sequence diversity that is unexpected for an invertebrate. Comparisons among 81 cDNAs identified 67 different sequences encoding 64 different proteins (Terwilliger *et al.*, 2006) (Fig. 6). The variability in the messages is based on the presence or absence of 25 blocks of sequence called elements, which resulted in 22 different element patterns (some of those patterns are shown in Fig. 6) (Terwilliger *et al.*, 2006). Besides the variations in element patterns, the *185/333* cDNA sequences also show significant sequence diversity within the elements resulting from non-random single nucleotide polymorphisms (SNPs) and small insertions and deletions (indels). One effect of the extensive number of SNPs is to alter the position of the stop codon in element 25 (Fig. 6). An initial analysis of sequence diversity for 42 unique ESTs and subsequent analysis of 81 full length cDNAs found that synonymous/non-synonymous (dn/ds) ratios of the leader and first element are under positive selection for diversification, although most of the diversity is located within the first element and not the leader (Nair *et al.*, 2005; Terwilliger *et al.*, 2006).

The *185/333* cDNAs can be categorized into seven groups based on the presence or absence and length of element 15 (Fig. 6), which is shared by most of the sequences, and has a large number of indels. Notably, within these groups, the presence or absence of elements 13-25a (histidine-rich region and part of the terminal region) is identical, suggesting that certain suites of elements tend to appear together (Terwilliger *et al.*, 2006). Analysis of the messages reveals elevated dn/ds ratios which suggest that the genes are undergoing selection for diversification, possibly as a result of pathogen pressure. Initial analyses of *185/333* expression in individual sea urchins responding to bacterial and fungal molecular patterns show that the element patterns in messages present prior to challenge are generally different from those present after challenge (Terwilliger *et al.*, unpublished). Furthermore, the sequence diversity of the messages present in coelomocytes after challenge is elevated compared to messages isolated prior to challenge. Changes in the expression patterns of the various *185/333* transcripts in response to immune challenges suggests that expression

may be under some type of transcriptional or post-transcriptional control.

The 185/333 proteins

The 185/333 proteins are not similar to any known protein and showed little discernible secondary structure. All have a leader, all lacked cysteines, and have three distinct regions; a glycine-rich region, a histidine-rich region and a terminal region (Fig. 6) (Terwilliger ***et al.***, 2006). Five types of repeats, 11 histidine patches, and six acidic patches are also present. A number of conserved sites for post-translational modification are predicted throughout the deduced proteins, the most interesting of which is an RGD motif in element 7 suggesting that these proteins may bind to integrins. Recent cytological analyses has shown that the 185/333 proteins are expressed by a subset of phagocytes and that they appear as diverse bands on Western blots (Brockton, et al.; unpublished). Although the functions of the 185/333 proteins are not known, based on the timing of expression of the *185/333* genes in response to immune challenge (Rast ***et al.***, 2000; Nair ***et al.***, 2005), they may have an important immunological function.

185/333 gene structure and diversity

The numerous element patterns initially identified from the transcripts suggested a significant level of alternative splicing (Nair ***et al.***, 2005). This was thought to imply long primary transcripts from a very large gene that were spliced to produce all possible mature mRNAs, a diversification mechanism similar to that found for *DSCAM* transcripts in *Drosophila* (Schmucker ***et al.***, 2000; Watson ***et al.***, 2005). However, the two *185/333* genes identified in an early version of the sea urchin genome are not as predicted, and instead have two exons and a single, small intron (Terwilliger ***et al.***, 2006). The first exon encodes the leader, while the second exon encodes the rest of the open reading frame, i.e., elements 1 through 25 (Fig. 6). Analysis of genomic DNA by quantitative PCR suggests that the *185/333* gene family consisted of ~100 alleles, or 50 loci per genome (Terwilliger ***et al.***, 2006). Analysis of a later sea urchin genome assembly (7/18/05) shows two contigs with two or three *185/333* genes, which are spaced approximately 3 kb apart. Based on the estimated number of genes per individual and the diversity levels of these genes, it is reasonable to conclude that the observed *185/333* transcript diversity may result from many small, closely linked genes that are members of a large family. This characteristic of clustered immune genes has been observed repeatedly in both animals and plants, and may be integral to gene diversification.

Sequences of genes cloned from two individuals show that element patterns mimic those found in the transcripts. The diversity observed from analyses of gene sequences corresponds to transcript diversity with respect to percent of unique nucleotide sequences, single nucleotide and amino acid polymorphisms, and diversity of sequences within sets of genes that have the same element pattern. These data suggest that the immune system of the sea urchin has an unknown mechanism of generating immune diversity in the *185/333* gene family. This is a shift from current paradigms, which assume that innate immunity is mediated by germline encoded proteins that have broad recognition specificities for identifying conserved molecular patterns in large classes of microbes.

Conclusions

As we currently understand the sea urchin immune system, its most important functions appear to be opsonization and phagocytosis. The immune cells in the embryo, larva, and adult that mediate immune functions are amoeboid phagocytes. The adult coelomocytes are found in high concentrations in the coelomic fluid and are present in or on all tissues and organs in the animal. Their mobility, phagocytosis and secretion functions are reflected in the large numbers of ESTs that match to cytoskeletal proteins and proteins involved in cytoskeletal modifications. Opsonization to augment phagocytosis (or encapsulation) may be the general molecular response of sea urchins to foreign pathogens. A variety of types of opsonins are present in the sea urchin, including lectins, which bind oligosaccharides, SRCRs, which bind many types of molecules including modified lipids, and the thioester-containing proteins of the complement system, which covalently bind to hydroxyl and amine groups. Although the functions of the 185/333 proteins are not known, the expression of the genes in response to challenge from bacteria or LPS plus their diversity supports the hypothesis that these proteins are somehow involved in coelomocyte interactions with microbes. The arrays of lectins, SRCR proteins, 185/333 proteins, in addition to the expanded complement activation pathways that lead to opsonization, demonstrate the molecular diversity that is part of the sea urchin immune system. Furthermore, the putative recognition system, as exemplified by the large numbers of TLRs and other LLR-containing proteins in the sea urchin genome, illustrates that both the detection and effector systems appear to be expanded and diverse. It is likely that diversification of innate immune systems in both invertebrates and plants may be driven by pathogen pressure. Diversification of innate immune systems is a recent change in the previously accepted paradigm that innate immune systems are undiversified and static. The new paradigm results from recent data on sea urchins reviewed here and from analyses of other invertebrates.

The importance of understanding the sea urchin system is based on the phylogenetic position of the echinoderms at the base of the deuterostome lineage and as a sister phylum to the chordates. Determining how the sea urchin immune system functions and defining how it might be similar to other invertebrate and vertebrate systems will be of interest with respect to understanding the evolution of immune functions within the deuterostomes. The on-going analysis of the sea urchin genome is expected to provide additional surprises and insights into this invertebrate immune system and to accelerate the rate at which experimental data is

generated to test predictions from the genome. Initial efforts will be centered on understanding how large families of duplicated genes are generated and maintained, and understanding the underlying mechanisms of innate immune diversification.

Acknowledgements

Writing this review was supported by the National Science Foundation (LCS), the Canadian Institutes of Health Research and the National Sciences and Engineering Research Council of Canada (JPR), and the Australian Research Council (SVN).

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