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The regulation of cnidocyte discharge

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ABSTRACT

Because cnidocytes are exceedingly complex cells which can only be used once, their discharge is highly regulated by way of a variety of chemosensory, mechanosensory and endogenous pathways. The integration of these various inputs ultimately results in exocytosis and then discharge of the cnidocyte's diagnostic organelle, the cnidocyst. Here we review what is known about the sensory pathways that regulate cnidocytes, the electrical events that manifest in cnidocytes following sensory stimulation and the ionic mechanisms that underlie discharge.

1. Introduction

Cnidocytes, the diagnostic feature of members of the Phylum Cnidaria are, arguably, some of the most complex of all eukaryotic cells. They arise from multi-potent interstitial stem cells and their development (reviewed by Kass-Simon and Scappaticci, 2002) is characterized by the intra-cytoplasmic assembly of a single large, membrane-bound cyst and tubule, and the subsequent inversion and coiling of that tubule together with venom and other intra-capsular molecules into the lumen of that cyst. This is accompanied by the development of a complex ciliary apparatus at the apical end of the cell and the migration and dense packaging of the mature cells into a tentacle or other structure (Fig. 1).

A cnidocyte's structural complexity is mirrored by its functional complexity. Cnidocyte discharge is, in essence, an explosive event that results in the inverted tubule being extruded through the operculum at the apical end of the cyst with, in the case of penetrant cnidocytes, sufficient force to penetrate the cuticle or skin of prey, over a time period of less than 3 ms (Tardent and Holstein, 1982; Holstein and Tardent, 1984; Nuchter et al., 2006). The mechanisms underlying the generation of the necessary force for discharge are not fully understood, but are thought to include, either in isolation or in concert, an increase in hydrostatic pressure created by a significant osmotic pressure gradient, mechanical energy stored in the inverted tubule (for review see Kass-Simon and Scappaticci, 2002) and, suggested most recently, a significant proton gradient across the membrane that envelops the cyst (Berking and Herrman, 2006).

The energetic demands of creating such a complex cell are made all that more profound by the fact that cnidocytes can only be used once: discharge is irreversible and discharged cysts cannot be replaced. Thus, the cells are typically very tightly regulated to ensure that they discharge only under appropriate conditions. In the case of the cnidocytes in tentacles that are used for food capture, appropriate conditions would be defined as those where the probability that discharge would result in capture of prey is high.

Cnidocytes are used for a variety of functions, most notably prey capture, defense and locomotion. While the mechanism of discharge in each case is likely to be similar, the degree of regulation of that discharge may be very different. For example, cnidocytes involved in locomotion are likely to adhere to any structure they encounter during

Abbreviations: *Pp*$Ca_V\beta$, *Physalia physalis* voltage-gated Ca^{2+} channel beta subunit; *Pp*K_V1, *Physalia physalis* Type 1, voltage-gated K^+ channel.

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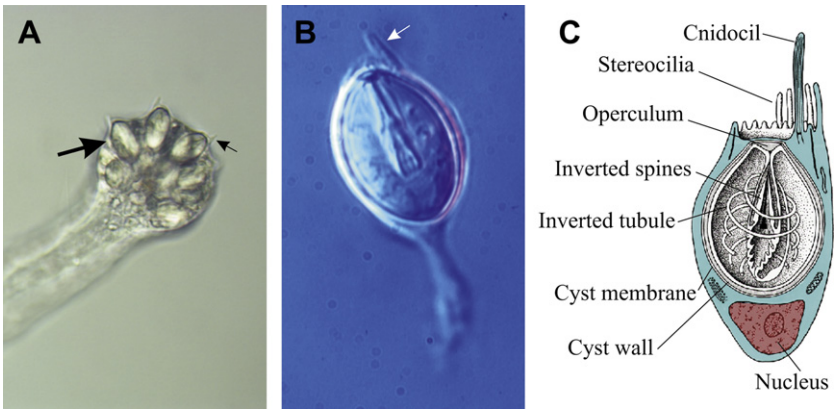

Fig. 1. (A) A micrograph of a capitate bulb at distal end of a tentacle in *Cladonema*. Individual cnidocytes are clearly visible (large arrow) as are the cnidocils on the apical end of each cnidocyte (small arrow). (B) A single stenotele cnidocyte isolated from *Cladonema*. The bulk of the cell is occupied by the membrane enclosed cyst, and the cnidocil and surrounding sterocilia are visible (arrow) at the apical end. (C) A drawing of a cnidocyte revealing the various anatomical components discussed in the text.

locomotion and, thus, are probably not regulated to the same extent as those involved in prey capture. Similarly, when grossly disturbed, many sea anemones extrude cnidocyte-covered acontia through pores in their body wall and those cnidocytes tend to discharge readily into or onto any structure they encounter, be it animate or inanimate. Here we will focus on those cnidocytes that likely express the highest degree of regulation, those involved in prey capture, and review what is known about the sensory modalities and mechanisms involved in their discharge and the ionic mechanisms that underlie that discharge.

It has long been known that optimal cnidocyte discharge requires a combination of chemical and mechanical stimulation. Pantin (1942) showed that chemical stimuli alone are insufficient to trigger discharge, that mechanical stimuli alone trigger only a baseline discharge, but that application of both stimuli, in close temporal proximity, produces maximal discharge. Over the years there have been many advances in our understanding of the pathways and mechanisms that underlie transduction of these two sensory modalities. One thing that has become particularly evident is that original classification of cnidocytes as "independent effectors" (Parker, 1919) is incorrect. Cnidocytes are, instead, proving to be exquisite integrators of chemosensory and mechanosensory information provided by both inter- and intracellular signaling pathways.

2. Organization of cnidocytes and associated sensory cells

Cnidocytes are found almost exclusively in the ectoderm of the animal in a variety of configurations that likely reflect their function. For example, the acontia of sea anemones, which are used exclusively for defense, are covered by a near continuous layer of cnidocytes separated by an occasional supportive cell (Salleo et al., 1996). Similarly, the cnidocytes that are scattered over the surface of the bells of scyphomedusae are almost certainly defensive in function and not known to form assemblies with other cell types.

In contrast, cnidocytes in the tentacles of cnidarians, which are used primarily for food capture, typically exist in cellular complexes that include sensory cells and other supporting cells. The level of organization of these complexes, together with their dimensions and other features shows considerable variation, ranging from discrete islets of cnidocytes and supportive cells to a rather uniform coverage over the full extent of a tentacle. Much of this variation tends to be Class-specific; discrete concentrations of cnidocytes tend to prevail in hydrozoans, the tentacles of anthozoans tend to have cnidocytes uniformly distributed, while those of scyphozoans and cubozoans are often intermediate between the two extremes. This variation has made it difficult to distinguish common features of the cnidocyte assemblies in different organisms from Class-specific variations, a problem has been compounded somewhat by the tendency among investigators to develop terminologies that are particular for their model of choice. For example, the tentacles of *Hydra* possess "battery cell complexes" (Hufnagel et al., 1985), which can best be described as discrete islands of cnidocytes which, together with sensory cells, and a subset of neurons called ganglion cells, are enveloped by modified myoepithelial cells called battery cells. Interestingly, the cnidocytes appear not to be enveloped by the battery cell but to somehow perforate it, such that the cnidocyte occupies an apically/basally oriented tunnel within battery cell, as has been described for mechanoreceptors in the filiform tentacles of capitate hydroids (Tardent and Schmid, 1972). In other hydroids such as *Cladonema* (Rees, 1979) and *Coryne* (Tardent and Schmid, 1972), cnidocytes and supporting cells are restricted to capitate bulbs at the ends of each tentacle, while in siphonophores, cnidocytes are found in a series of large bulbous protrusions of the tentacle, termed cnidosacs (Skaer, 1988), that give the tentacle the appearance of a string of beads. The different terminologies imply that the different assemblies are truly structurally different, beyond their relative sizes, as well as functionally different. Our current understanding of the field does not allow this assumption to be tested.

3. Sensory regulation of cnidocyte discharge

As noted earlier, the optimal discharge of cnidocytes in the tentacles of cnidarians requires application of

two stimulus modalities: chemical and mechanical. The receptors for these modalities have been localized to both the cnidocytes themselves, and to various supportive cells.

The work of Thurm et al. (Holtmann and Thurm, 2001a,b; for review see Thurm et al., 2004) have provided a good understanding of the composition, organization and physiology of the capitate bulbs in hydroids. These structures consist of cnidocytes and four types of putative sensory cells, all of which are separated by supporting cells. The sensory cells are characterized by a central cilium, of variable length, that may, or may not be surrounded by microvilli. One class of putative sensory cells, the vesicle-rich cell, may serve a chemosensory function, while the others, which include hair cells, are believed to be mechanosensory (Holtmann and Thurm, 2001b). All sensory cells are surrounded by supporting cells and are both pre-synaptic and post-synaptic to elements of the underlying nerve net (Holtmann and Thurm, 2001a). The cnidocytes, in turn, are pre- and post-synaptic to elements of the underlying nerve net as well as the putative sensory cells (Fig. 2).

The complexity of the synaptic architecture revealed by ultra structural studies is borne out by physiological studies (Oliver and Thurm, 1996; Brinkmann et al., 1996; reviewed by Thurm et al., 2004). Mechanical deflections of the cilium of a hair cell, or cnidocil of a cnidocyte trigger electrical events in other cnidocytes, indicating that there is afferent mechanosensory input from hair cells to cnidocytes, and between cnidocytes.

In hydrozoans, cnidocytes receive chemosensory input from both contact and non-contact chemoreceptors. When probes coated with water-insoluble compounds, most notably phospholipids and glycolipids, touch the distal

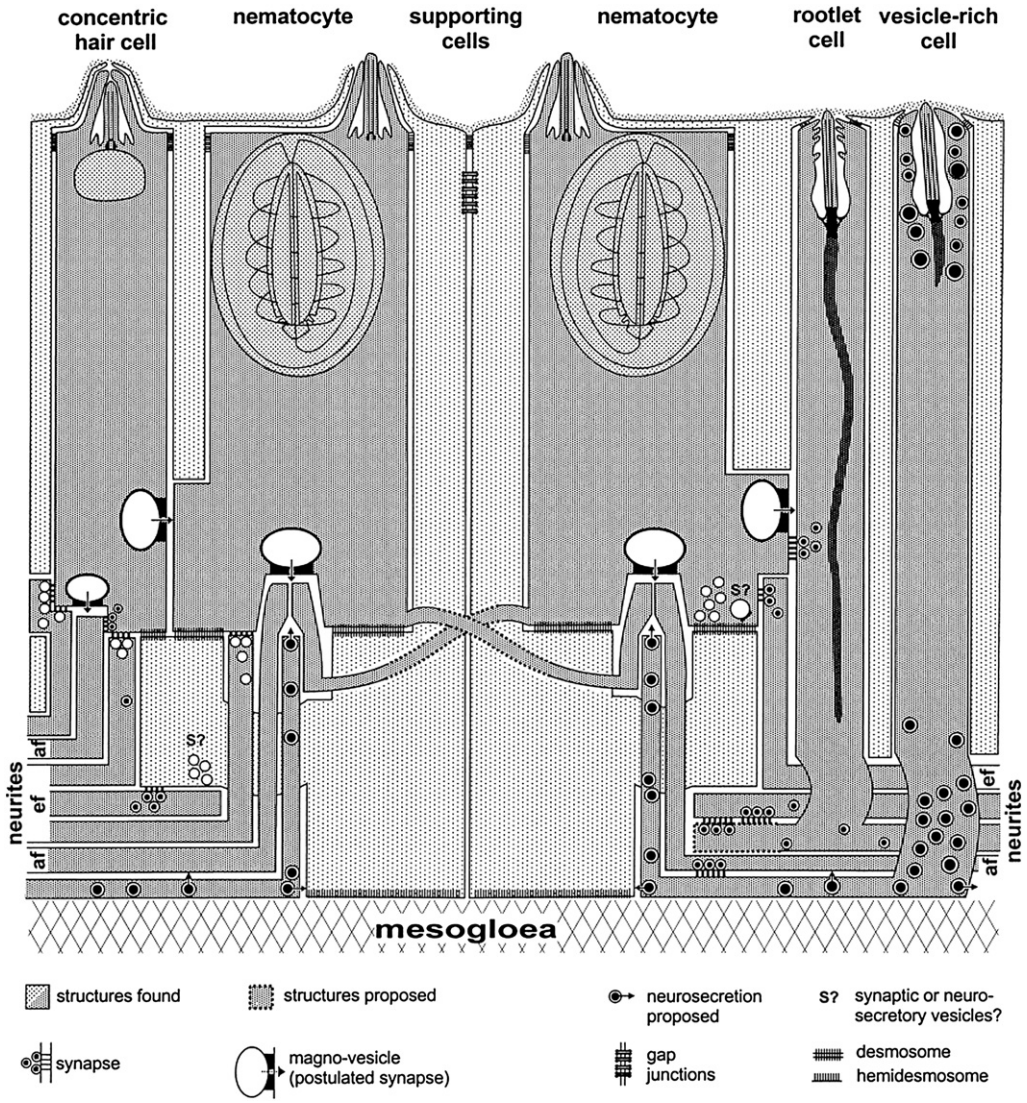

Fig. 2. The organization of the capitate bulb of the hydroid *Coryne*, showing that cnidocytes contain both pre- and post-synaptic elements, and interact synaptically with neurons, sensory cells and, potentially, other cnidocytes. From Holtmann and Thurm (2001a). Reprinted with permission of John Wiley & Sons, Inc.

region of the cnidocil of cnidocytes in *Hydra* (Lawonn and Thurm, 1992) and the hydroid *Stauridiosarsia* (Brinkmann et al., 1994; Thurm et al., 1998), the probability of discharge and the probability and strength of synaptic activity received by other cnidocytes (i.e. afferent input) are greatly increased, in comparison to the effect evoked by application of clean probes (Thurm et al., 2004). The fact that water-insoluble compounds are effective when applied directly to the cnidocil indicates that contact chemoreceptors are present on the cnidocil itself.

At the same time, the introduction of an aqueous extract of the prey of *Cladonema* (brine shrimp) or *Physalia* (fish mucus) into a stream of water flowing over that animal's tentacle triggers a burst of complex electrical activity in cnidocytes (Purcell and Anderson, 1995; Price and Anderson, 2006), including synaptically driven action potentials (Fig. 3). This effect is abolished in Ca^{2+}-free seawater. Moreover, the "odor-evoked" activity occurs synchronously in multiple cnidocytes, presumably a reflection of a common synaptic input, but also due, in part, to the presence of electrical coupling between cnidocytes (Price and Anderson, 2006). While the presence of non-contact chemoreceptors on the cnidocytes themselves cannot be excluded, these findings indicate that water-soluble, prey-specific chemicals activate cnidocytes by way of the animal's nervous system. Neuro-cnidocyte synapses have been

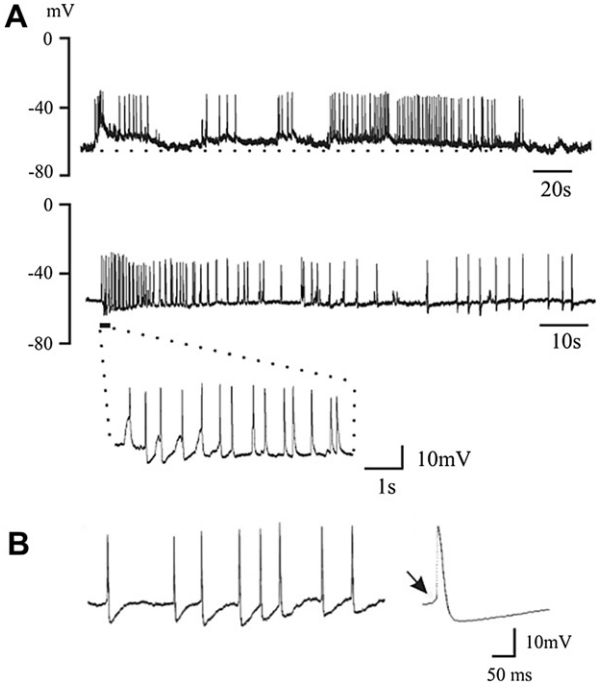

Fig. 3. (A) Intracellular recordings from stenotele cnidocytes in tentacles of *Cladonema*. Introduction of an extract of *Artemia* into the seawater flowing over the tentacle evokes a burst of electrical activity. This activity consists of large numbers of depolarizing events (inset). (B) Part of an "odor" evoked burst of activity, at high sweep speed. The activity consists of fast depolarizing events that resemble action potentials. The single event at the right is the average of 12 events from the same burst. This is preceded by a small depolarization that resembles a synaptic potential (modified from Price and Anderson, 2006).

described (Yu et al., 1985) and various immuno- and histochemical studies have revealed the presence of various putative transmitters in neurons associated with cnidocytes and supportive cells. These include neuropeptides (Anderson et al., 2004), glutamatergic, GABAnergic (Kass-Simon and Scappaticci, 2004; Scappaticci et al., 2004), and catecholaminergic (Westfall et al., 2000) pathways.

The organization of the sensory apparatus underlying cnidocyte discharge in anthozoans is quite different from that in hydroids and, so far as we know, cubozoans (Rifkin and Endean, 1988) and scyphozoans. In anthozoans, the supporting cells contribute to the ciliary apparatus of both the cnidocytes and sensory cells. Sea anemone tentacles bear two distinct classes of ciliated sensory structures that are likely to be involved in the regulation of cnidocyte discharge. The first class of sensory structure, which is closely associated with cnidocytes, is the cnidocyte/supporting cell (CSC) complex (Mariscal et al., 1978; Thorington and Hessinger, 1988b). This is a ciliary cone structure that is formed by stereocilia that project from the supporting cells and surround the stereocilia of the cnidocyte which, in turn, surround the cnidocil of the cnidocyte (Westfall, 1965). These structures likely contain the contact-sensitive mechanoreceptors that trigger cnidocyte discharge (Thorington and Hessinger, 1988b).

The second is the sensory cell/supporting cell (SCSC) complex (for review, see Watson and Mire, 2000, 2004) which is structurally similar to the CSC complex, except that the inner ring of stereocilia and the central cilium (or kinocilium) project from a central sensory cell rather than a cnidocyte (Peteya, 1975; Mire-Thibodeaux and Watson, 1994a). Various types of linkages connect the kinocilium of the SCSC complex with the surrounding stereocilia, and "tip-links" connect the smaller diameter sterocilia contributed by the supporting cells (Watson et al., 1997) in a manner that is very reminiscent of the situation in vertebrate vestibular hair cells where it is thought to serve as an external gating spring for mechanotransduction (Hamill and Martinac, 2001). Although no synapses have been identified between the sensory cells of the SCSC complexes and neural elements, their basal processes do project to the underlying nerve net (Watson and Mire, 2000), suggesting that they connect to the nerve net and do impart some degree of regulation.

The morphology of the hair bundles of the SCSC complexes changes in response to chemosensory stimulation by N-acetylated sugars, a constituent of glycoproteins, mucins, chitin and certain mucopolysaccharides found at the surface of a prey's body. These sugars trigger actin polymerization-dependent lengthening of the large diameter stereocilia of the sensory cell (Watson and Roberts, 1995), and twisting of the entire bundle around the cilium (Mire and Nasse, 2002). These structural modifications alter the mechanical properties of the hair bundles, and tune them to the frequencies of vibrations emitted by swimming prey, resulting in an increase in the baseline discharge of cnidocytes when the anemone touches the prey (Thorington and Hessinger, 1988b; Mire-Thibodeaux and Watson, 1994a; Watson and Roberts, 1995).

This effect is counteracted by proline, which is present in the hemolymph of crustaceans and, therefore, likely to

be released by injured organisms. While proline alone has no effect on the rate of discharge from tentacles stimulated with vibrating probes, in presence of N-acetylated sugar proline triggers an actin-depolymerization-dependent shortening of the stereocilia (Watson and Roberts, 1994, 1995) and, consequently, shifts the maximal cnidocyte discharge of the vibrating probe to frequencies and amplitudes that correspond to the swimming frequency produced by wounded prey (Watson and Hudson, 1994; Watson and Hessinger, 1994).

To date, two classes of chemoreceptors (Thorington and Hessinger, 1988a) have been localized to supporting cells of sea anemones. Receptors for N-acetylated sugars have been localized to the apical surfaces of the supporting cells of these SCSC and CSC complexes (Watson and Hessinger, 1986, 1987). Binding of the ligand to the sugar receptor is transduced by way of a G-protein-mediated cascade similar to those present in invertebrate (Krieger and Breer, 1999) and vertebrate (Restrepo et al., 1996) olfactory systems. Pharmacological studies with the sea anemones *Haliplanella luciae* and *Aiptasia pallida* show that the binding of sugar to the receptor activates a GTP-binding protein, which in turn stimulates adenylate cyclase to increase cAMP production in the tentacular ectoderm (Watson and Hessinger, 1992; Ozacmak et al., 2001). The elevated cAMP activates protein kinase A to initiate actin polymerization and elongation of the large diameter stereocilia of the sensory cells in the SCSC complexes, in preparation for any subsequent mechanical stimuli (Mire-Thibodeaux and Watson, 1994b). Proline receptors are also present on the apical surfaces of the supporting cells (Watson and Roberts, 1994). They appear to operate by way of an IP_3-dependent second-messenger pathway (Russell and Watson, 1995) but the exact details of the pathway are still lacking. It is clear, however, that multiple signal transduction pathways influence the discharge of cnidocytes.

Based on these overall findings, it appears that the organization of the sensory structures associated with cnidocytes in anthozoans is quite different from that of hydrozoans and, very probably cubozoans and scyphozoans. Only in anthozoans do supportive cells contribute to the sensory structure both from the perspective of chemical and mechanical sensitivity. If stereocilia were absent from the ciliary complex of anthozoan cnidocytes, as earlier reports (Mariscal et al., 1978; Pantin, 1942; Weill, 1934) indicated, then cilia from the supportive cells would be required to enable the push-pull mechanism of mechanoreceptors transduction thought to be present in cnidocytes. However, a more recent study (Westfall et al., 1998) has shown that stereocilia are present on the apical surfaces of anthozoan cnidocytes, making them structurally similar to cnidocytes from the other three classes. Thus, the need for stereocilia from supporting cells is unclear. Moreover, in hydrozoans, the cnidocil bears contact chemoreceptors but the only chemoreceptors currently known to be associated with anthozoan cnidocytes are present on the supportive cells and not the cnidocil. These differences are very profound and speak strongly to the phylogenetic distance between anthozoans and the other cnidarian classes.

4. Electrical activity and ionic currents in cnidocytes

Despite the fact that the cyst occupies so much of the volume of any given cnidocyte, it has proved relatively easy to obtain intracellular recordings from cnidocytes *in situ* using sharp electrodes (Purcell and Anderson, 1995; Brinkmann et al., 1996; Price and Anderson, 2006). Such recordings, which have typically been obtained in conjunction with chemical and/or mechanical stimulation of cnidocytes, reveal that cnidocytes can be very active electrically. As noted above, the introduction of an aqueous, prey-specific extract into the stream of seawater flowing over a tentacle of *Cladonema* or *Physalia* evokes a burst of electrical activity (Fig. 3). These have been most closely examined in *Cladonema* (Price and Anderson, 2006) and have been shown to consist of synaptic events and synaptically driven action potentials. Action potentials have also been recorded from cnidocytes from *Stauridiosarsia* (Brinkmann et al., 1996), another hydroid. Moreover, recordings from pairs of cnidocytes in the capitate bulbs of *Cladonema* reveals that the pattern of activity in both cnidocytes is identical and that current injected into one cnidocyte spreads to the other, indicating that they are electrically coupled (Price and Anderson, 2006).

Electrical coupling may also be involved in the regulation of cnidocyte discharge in anthozoans but the evidence is still less direct. Mire et al. (2000) describe dye coupling between cells in the tentacles of the sea anemone *Haliplanella*, provide evidence that coupling is inhibited by gap junction uncouplers, and show that the extent of that dye coupling is somehow modulated by deflections of sensory/supporting cell complexes. They also provide electrophysiological evidence that uncoupling agents alter the spread of vibration induced electrical activity within the tentacle. The role of electrical coupling in regulating cnidocyte discharge in anthozoans is, however, complicated by the fact that gap junctions, the structural manifestations of electrical coupling which are so prevalent in hydrozoans, have never been described in either anthozoans or scyphozoans.

Gap junctions represent areas of well organized, close membrane apposition that are created by the tight packing of large numbers of intercellular pore-forming proteins. There is, however, no *a priori* reason why functional electrical and dye coupling could not be achieved by way of dispersed pore-forming entities. If such entities were composed of only a small number of pore-forming structures they might not be obvious at the ultra structural level, at least to the degree of the classical gap junctions that are so common in hydrozoans. Indeed, Germain and Anctil (1996) describe small discrete areas of membrane apposition between cells in the anthozoan *Renilla* and speculate that these might represent areas of coupling that previous studies focused on classical gap junctions (Mackie et al., 1984) have missed.

The presence of pore-forming entities, dispersed or otherwise, in anthozoans, is, however, further confused by the reports of several authors (Germain and Anctil, 1996; Mire et al., 2000) that sea anemones display immunoreactivity to connexin, the pore-forming proteins that form the basis of gap junction in higher animals. This evidence,

which was obtained using both immunohistochemistry and Western blotting analysis, is difficult to reconcile with the fact that *in silico* analyses of the genomes of various animals, including the sea urchin, a deuterostome (Burke et al., 2006), *Hydra* and the sea anemone *Nematostella* (Sullivan et al., 2006), indicate that connexins are not present in invertebrates but, instead, are found only in protochordates and chordates (Alexopoulos et al., 2004; Panchin, 2005). Moreover, it appears that the invertebrate equivalents of connexins are the innexins (Phelan and Starich, 2001), which are completely different proteins. Given that the small size of anthozoan cells makes intracellular recordings extremely difficult, definitive proof of the role of electrical coupling in the regulation of cnidocyte discharge in sea anemones will obviously benefit from the cloning of any intercellular pore-forming proteins from these animals and their subsequent localization at small discrete areas of close membrane apposition, of the type described by Germain and Anctil (1996).

5. Ionic currents underlying discharge

The organelle that is the hallmark of the cnidocyte, the cnidocyst, is enclosed within a membrane and, thus, is an intracellular vesicle, albeit an exceedingly large one that envelops an unusually rigid structure. Given that it is a vesicle and that discharge involves releasing the contents of that vesicle into the extracellular medium, it has long been accepted that discharge is an exocytotic event (Skaer, 1973) whereby the membrane of the cnidocyst vesicle fuses with the apical membrane of the cell. This model, which is supported by the observation that the membrane potential of a cnidocyte is not lost during discharge (Thurm et al., 2004) and that cnidocyst discharge is voltage- and Ca^{2+}-dependent (Gitter et al., 1994), would imply that the cnidocyte contains much of the same machinery that is present in other exocytotic structures, most notably the terminals of chemical synapses.

Exocytosis in synaptic terminals and adrenal chromaffin cells, two of the best studied examples, is mediated by voltage-gated Ca^{2+} currents that trigger a series of molecular interactions that culminate in exocytosis of the vesicle (for review see Brunger, 2005). This would imply that voltage-gated Ca^{2+} channels must also be present at in the apical membrane of the cnidocyte, the site of fusion between the cell membrane and the vesicular membrane enveloping the cnidocyst.

Recordings have been obtained from cnidocytes isolated from the hydrozoan *Cladonema* and the scyphozoan *Chrysaora* (Anderson and McKay, 1987). Only those from *Cladonema* produced action potentials which were not, in and of themselves, able to trigger discharge. Voltage clamp recordings reveal that cnidocytes from both species display delayed rectifier or steady state K^+ currents, and that *Cladonema* cnidocytes also produce voltage-dependent Na^+ currents, presumably a reflection of their ability to produce action potentials. Those recordings did not, however, reveal the presence of any voltage-gated Ca^{2+} currents (Anderson and McKay, 1987). This may, however, simply be a consequence of the recording method. The whole-cell configuration of the patch clamp technique used with cnidocytes is notorious for "washing out" essential components of Ca^{2+} currents, with the result that Ca^{2+} currents can be undetectable.

Molecular cloning strategies, using an amplified cDNA library prepared from cnidocytes from *Physalia physalis* (Bouchard et al., 2006) have revealed the presence of a variety of ion channels in these cells. These include Ca^{2+} channel α_1 and β (*Pp*$Ca_V\beta$) subunits, and a voltage-gated K^+ channel (*Pp* K_V1). The $Ca_V\beta$ and K_V1 channels have been expressed (Bouchard et al., 2006) and found to be completely functional. Because cnidocytes have been shown to be pre-synaptic to elements of the nervous system, including sensory cells (Holtmann and Thurm, 2001a) (Fig. 2), the presence of voltage-gated Ca^{2+} channels is not unexpected. However, *Pp*$Ca_V\beta$ has been localized to the apical end of the cnidocyte (unpublished data), consistent with its presumed role in exocytosis.

It is important to realize, however, that cnidocyte discharge may be regulated more tightly than the exocytotic machinery at a chemical synaptic terminal. Depolarization of cnidocytes by intracellular current injection, by way of microelectrodes rather than patch electrodes, in the presence of extracellular calcium, an action that would be sufficient to trigger exocytosis of neurotransmitter from nerve terminals, does not itself evoke discharge (Anderson and McKay, 1987). Indeed, action potentials can be evoked in single isolated cnidocytes, without triggering discharge, and synaptically driven action potentials do not trigger discharge *in vivo* (Price and Anderson, 2006). Thus, one must assume that the mechanosensory and chemosensory input to the cnidocytes from the various pathways discussed above must have some effect, above and beyond merely evoking, in the case of hydroid at least, action potentials. One could well envisage a biochemical cascade that is a pre-requisite for activation of the exocytotic machinery that underlies discharge. The cnidocyte-specific amplified cDNA libraries that are now available hold the promise of allowing components of such pathways to be identified.

Conflict of interest

The authors declare that there are no conflicts of interest.

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