



SYMPOSIUM

Sponge Behavior and the Chemical Basis of Responses: A Post-Genomic View

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Synopsis Sponges perceive and respond to a range of stimuli. How they do this is still difficult to pin down despite now having transcriptomes and genomes of an array of species. Here we evaluate the current understanding of sponge behavior and present new observations on sponge activity *in situ*. We also explore biosynthesis pathways available to sponges from data in genomes/transcriptomes of sponges and other non-bilaterians with a focus on exploring the role of chemical signaling pathways mediating sponge behavior and how such chemical signal pathways may have evolved. Sponge larvae respond to light but opsins are not used, nor is there a common photoreceptor molecule or mechanism used across sponge groups. Other cues are gravity and chemicals. *In situ* recordings of behavior show that both shallow and deep-water sponges move a lot over minutes and hours, and correlation of behavior with temperature, pressure, oxygen, and water movement suggests that at least one sponge responds to changes in atmospheric pressure. The sensors for these cues as far as we know are individual cells and, except in the case of electrical signaling in Hexactinellida, these most likely act as independent effectors, generating a whole-body reaction by the global reach of the stimulus to all parts of the animal. We found no evidence for use of conventional neurotransmitters such as serotonin and dopamine. Intriguingly, some chemicals synthesized by symbiotic microbes could mean other more complex signaling occurs, but how that interplay might happen is not understood. Our review suggests chemical signaling pathways found in sponges do not reflect loss of a more complex set.

Introduction

Behavior fascinates us, both in its complexity (e.g., cooperative hunting by killer whales) and its apparent simplicity (e.g., closing of the Venus flytrap around an insect). How animals came to have complex behaviors involving learning and motor coordination from the simple actions available to the unicellular ancestor is a challenging problem that has attracted philosophers and scientists for

centuries. In the postgenomic era, in theory all the building blocks are known, and yet we still don't understand how complex chemical and neural signaling arose. Why not?

One answer is that most studies are still focused on vertebrates and even more so on mammals because of the goal of curing disorders of the human nervous system. Far fewer studies focus on non-conventional model animal systems (Russell et al.

2017; Renard et al. 2018). There are many genomes of dogs, and you can have your own exome sequenced for less than a thousand dollars (NIH 2019), however it still costs a lot more to sequence a shrimp or a sponge. More relevant though is the fact that a large proportion of genes in animals like sponges have no ortholog in the bioinformatics' databases, and a similar fraction lack an identifiable PFAM domain (Ryan et al. 2013; Moroz et al. 2014; Fernandez-Valverde et al. 2015). Typically, the proportion of unique proteins found in sponges is roughly 40% (e.g., Fernandez-Valverde et al. 2015; Guzman and Conaco 2016). Fernandez-Valverde et al. (2015) found that approximately 43% of the genes in the demosponge *Amphimedon queenslandica* neither possessed a conserved PFAM domain nor produced a significant BLAST hit.

Two other handicaps are even bigger. One is that we still have a very poor understanding of how behavior works in non-bilaterian animals. A survey of chemical molecules in cnidarians concluded that "the diversity of chemical transmitter systems in sea anemones could not have been anticipated on the basis of the small range of behaviors and effector activities available to these animals" (Ancill 2009). Even less is known of sponge behavior. The second major challenge today is the still undecided phylogenetic relationship of the four non-bilaterian groups: Porifera, Ctenophora, Cnidaria, and Placozoa (Dunn et al. 2008; Philippe et al. 2009; Ryan et al. 2013; Moroz et al. 2014; Feuda et al. 2017; Pett et al. 2019). Understanding branching order is crucial to understanding events leading to the emergence of the bilaterian nervous system.

The two groups that are proposed as sister to all other metazoans, sponges (Porifera), and ctenophores (Ctenophora), have radically different morphology and behavioral complexity. Ctenophores develop through gastrulation by epiboly to form a through-gut with anal pores through which wastes are excreted (Martindale and Henry 1999; Presnell et al. 2016). They have nervous systems and although they lack obvious eyes they have many opsins and other well-developed sensory organs (Schnitzler et al. 2012; Moroz et al. 2014; Tamm 2014; Moroz 2015) that allow them to be agile swimmers and active predators (Haddock 2007; Tiselius and Møller 2017). Sponges, in contrast, develop through a range of cellular migrations to form bilayered larvae whose tissues reorganize at metamorphosis to form a sessile filter feeding animal (Maldonado and Bergquist 2002; Leys and Ereskovsky 2006; and references therein). Sponges do not have a conventional nervous system or muscle. Their larvae are short-lived,

millimeter-long, ciliated propagules, which because they are motile, have the most overt behavior (Leys and Degnan 2001; Maldonado et al. 2003). But adult sponges are remarkably sensitive to their surroundings. Their responses are simply in a different time frame than ours (Nickel 2004; Elliott and Leys 2007). In adult sponges, the sensory cells known so far are specialized epithelia with tiny hair cells that strategically line the filtration system, and in particular, the osculum (Nickel 2010; Hammel and Nickel 2014; Ludeman et al. 2014).

There are several good reviews and new work that cover sponge larval ecology and behavior (Maldonado 2006; Wahab et al. 2014; Ueda et al. 2016), and so in this paper we focus in particular on the chemical basis of behavior in larvae and adult sponges.

Behavior in sponges

Larval behavior

There is a range of sponge larval types and with this a range of behaviors (Maldonado and Bergquist 2002; Maldonado 2006). Most sponge larvae are generally short-lived, settling within days of release, although a few can live up to a month (Maldonado 2006). Some larvae can respond to gravity (Warburton 1966), and chemical and light cues play a role in settlement and metamorphosis (Bergquist and Sinclair 1968; Leys and Degnan 2001; Jackson et al. 2002; Leys et al. 2002; Maldonado 2006; Jindrich et al. 2017). Specializations for settlement in the right spot must be highly selected for as explained by Bidder (1937) who estimated that in the calcareous sponge *Leuconia* on average only two of some 5 million larvae would survive, and prosaically concluded, "this elimination to the millionth ensures that of young sponges, side by side . . . only something approaching the fittest larval form shall survive."

Many larvae are polarized with either unciliated poles, or with a tuft of longer cilia at one pole, and these are commonly either negatively or positively phototactic, with the light response changing over the larval life as the larva begins metamorphosis (Leys and Degnan 2001; Maldonado et al. 2003; Collin et al. 2010). In *A. queenslandica* the rapid straightening and bending of the posterior cilia is a shadow response that acts as a rudder, giving the larvae the ability to turn quickly (Fig. 1A). Most metazoan photoreceptors are based on opsins, but so far no opsins have been found in sponge genomes or transcriptomes (Srivastava et al. 2010; Fortunato et al. 2012; Leininger et al. 2014). Instead spectral

sensitivity curves for *A. queenslandica* larvae hinted at a flavonoid or cryptochrome (Leys et al. 2002) and Rivera et al. (2012) identified two cryptochromes, one of which (Cry2) was expressed at the posterior pole of the larva, and showed sensitivity at the same wavelength as the larval response, roughly 440 nm. We also now know Cry2 is upregulated in dark conditions in both larva and adult (Jindrich et al. 2017).

The amphiblastula of *Sycon coactum* is a tiny polarized larva that is negatively phototactic (Elliott et al. 2004). The photoreceptor is probably involved with curious cross cells, four cells placed in quadrants of the tiny larva. Bidder (1937) suggested the light goes through the anterior cells as an eye and probably stimulates more active beating of parts of the larva to keep it oriented upward (Fig. 1B). Cross cells do express multiple sensory–neural markers (Fortunato et al. 2012), however since every sensory–neural marker expressed by cross cells is also expressed in oocytes (Mah and Leys 2017), it is impossible to draw conclusions regarding photoreceptor function from genes expressed. So far the photoreceptor has not been identified and neither the *S. coactum* transcriptome nor *S. ciliatum* genome have opsin or cryptochrome genes (Fortunato et al. 2014), so the photoreceptor of *Calcarea* remains a mystery.

Behavior in adult sponges

Sponges are quite active in a slow time-frame (minutes), and while a small sponge can contract its whole body, it is most often the canals and especially the osculum—the excurrent chimney which vents all the water filtered by the sponge—that are most responsive in larger animals (Parker 1910; McNair 1923; Nickel 2004; Elliott and Leys 2007; Ludeman et al. 2014; Kumala et al. 2017; Ludeman et al. 2017; Strehlow et al. 2017). Early work gives a good description of the types of responses of the osculum. Parker (1910) found that the osculum of *Hymeniacidon* (*Stylotella*) *heliophila*, a marine demosponge, contracted in 3 min when it touched air as the water level dropped with the outgoing tide; it expanded again as water covered it, but that took 10 min (Fig. 1C). McNair (1923) found only the very tip of the osculum of *Ephydatia fluvialilis*, a freshwater demosponge, was sensitive to touch; the osculum contracted, also in about 3 min. However, the rest of the osculum was fairly insensitive to most contact, except that a sharp blow caused it to “shrinkle up immediately” for 20–30 min.

Many researchers have since recorded contraction rates and studied what the contractile cells are by morphology and histochemical staining, studied responses of ostia, oscula, and the whole sponge to a range of chemical and mechanical stimuli (Ellwanger and Nickel 2006; Elliott and Leys 2007; Nickel et al. 2011; Ludeman et al. 2014, 2017; Kumala et al. 2017). From these studies we know that most demosponges are sensitive to mechanical stimuli, responding with slow contractions (rates of mm s^{-1}) and that many chemicals tested on sponges cause contraction (reviewed in Nickel 2010; Leys 2015). Oxygen depletion also causes sponges to contract and reduce excurrent flow (Parker 1910; Leys and Kahn 2018), and inversely contraction causes reduction in oxygen in the sponge (Leys and Kahn 2018). Calcareous sponges and homoscleromorphs also contract, and all three cellular sponge classes have spontaneous contractions every few hours (Nickel 2010). Contractions include “twitches” (a highly localized contraction on one part of the sponge), “ripples” (a set of sequential contractions that run over a portion of a sponge), and “cringes” (contractions of the whole sponge body) (Elliott and Leys 2007). Some sponges have pacemaker-like rhythmic contractions, while others have daily contractions (Reiswig 1971; Nickel 2004). Often encrusting forms close ostial pore fields (Reiswig 1979), but much larger barrel- or vase-shaped sponges close atrial openings (Reiswig 1971; Fig. 1D). Nothing is known of specific triggers but recent work implicates a role of photoreceptor and potentially clock genes in *Amphimedon* adult and larval behavior (Jindrich et al. 2017), and so these may also be activated in other sponges and it would be especially interesting to investigate their role in sponges with rhythmic contractions.

A number of studies have concluded that sponge contractions do not mean that the choanocyte pumps must stop (Parker 1910; Reiswig 1971; Kumala et al. 2017). Parker (1910) watched the incurrent and excurrent flow at the ostia and osculum and concluded that the pump cells—the choanocytes—never stopped beating but that instead the sponge controls flow through its body by constricting the smooth muscle-like cells on the epithelia, such as ostia, sphincters in canals, or the osculum. He calculated that the choanocytes only generated pressure of 3.5–4 mm H₂O (3.5–4 kg m^{−2}), a third less than he estimated would cause damage to the ostia and a fraction of what might damage the osculum if it is closed. Therefore, constricting canals can stop water flow through the sponge, even though the flagella pumps do not stop.

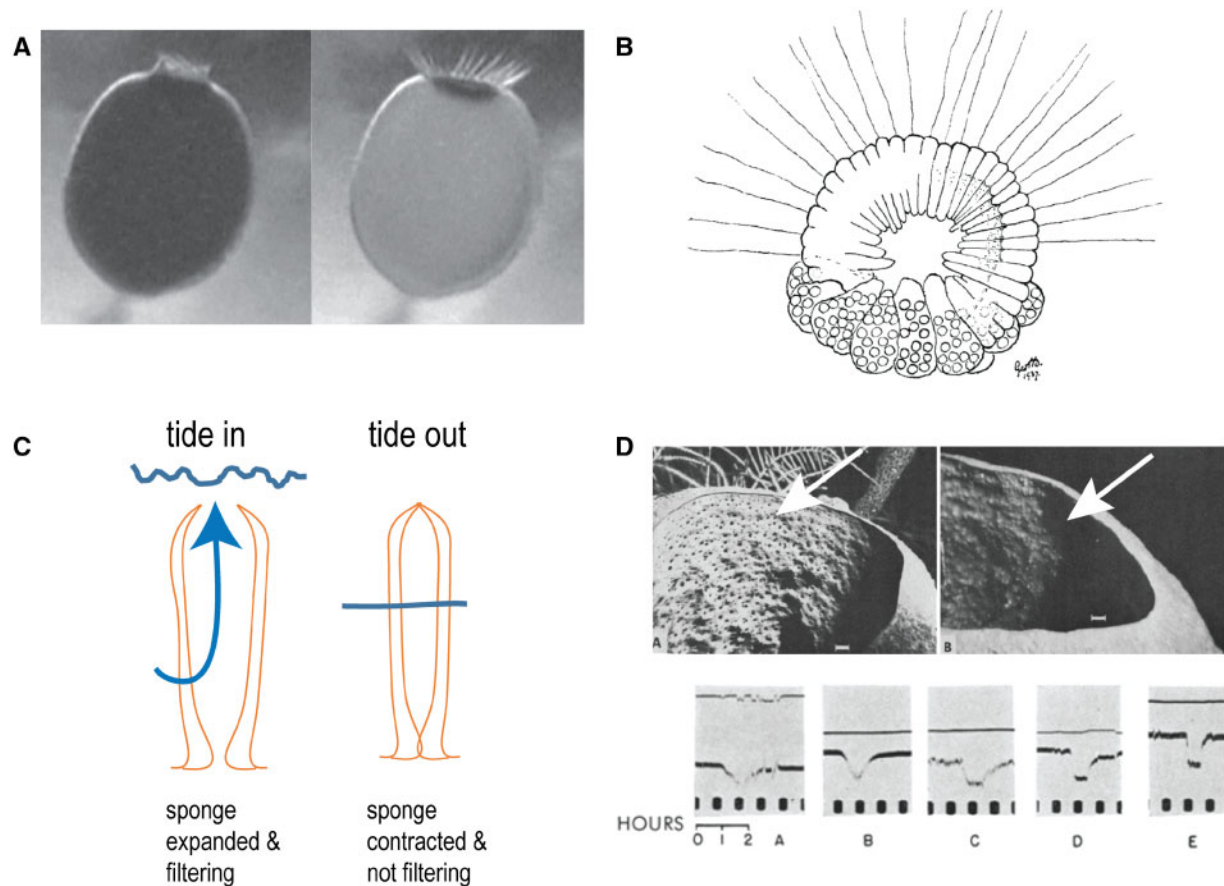


Fig. 1 Sponge behaviors: **A**) Straightening and bending of the ciliary tuft in response to changes in light intensity in the *Amphimedon queenslandica* larva (from Leys et al. 2002). **B**) Amphiblastula larva drawing by Bidder (1937). **C**) Parker's (1910) observation that the osculum contracts in response to contact with air as the tide goes out. **D**) Upper panel, Reiswig's (1971) photographs of *Verongula* spp. showing open (left) and closed (right) excurrent canals into the atrial cavity (arrows); lower panel, flow sensor recordings from *Verongula* showing reduction in excurrent flow that correlated with constriction of the excurrent canals.

Reiswig (1971) documented a range of behaviors *in situ* using both flow sensors and photography and concluded that reductions in flow were caused by contraction of sphincters in canals. Some contractions lasted 1 h each day, and while some species contracted episodically, one species had diurnal patterns and others didn't contract at all. He found that all the sponges' excurrent flow rate changed seasonally, reducing during the fall and especially during storms (Reiswig 1971). Recent work has found the same results for other species. *Cliona orientalis* has diurnal rhythms of excurrent flow and contractions, and reduces excurrent flow and contracts in response to sediment disturbance (Strehlow et al. 2017).

Based on Parker's and Reiswig's observations, and in agreement with Bidder's (1937) conclusions, it seems likely that judiciously located sphincters are able to reduce the flow through the whole canal system. Observations of sensory cells in the osculum and elsewhere in the canal system (Elliott and Leys 2007; Nickel 2010; Hammel and Nickel 2014;

Ludeman et al. 2014) strongly suggest that sponges sense water movement around themselves, probably at the tip of the osculum, and through themselves, at various points in the pump system. Given the sensitivity to water movement, it is likely sponges also respond to pressure changes which compress water.

New observations

In 2015 Ocean Networks Canada placed an undersea observatory at a rocky outcrop 40 m deep near Bamfield, British Columbia, Canada. A fist-sized orange demosponge, *Suberites concinnus*, happened to colonize the spot under a camera and instrument array so we could watch its behavior over several years at the same time monitoring ambient current, light, temperature, oxygen, and pressure. The sponge (nicknamed "Belinda") has a range of behaviors, with twitches, ripples, and cringes (Fig. 2; Leys and Hamonic 2019). Many movements of the sponge are

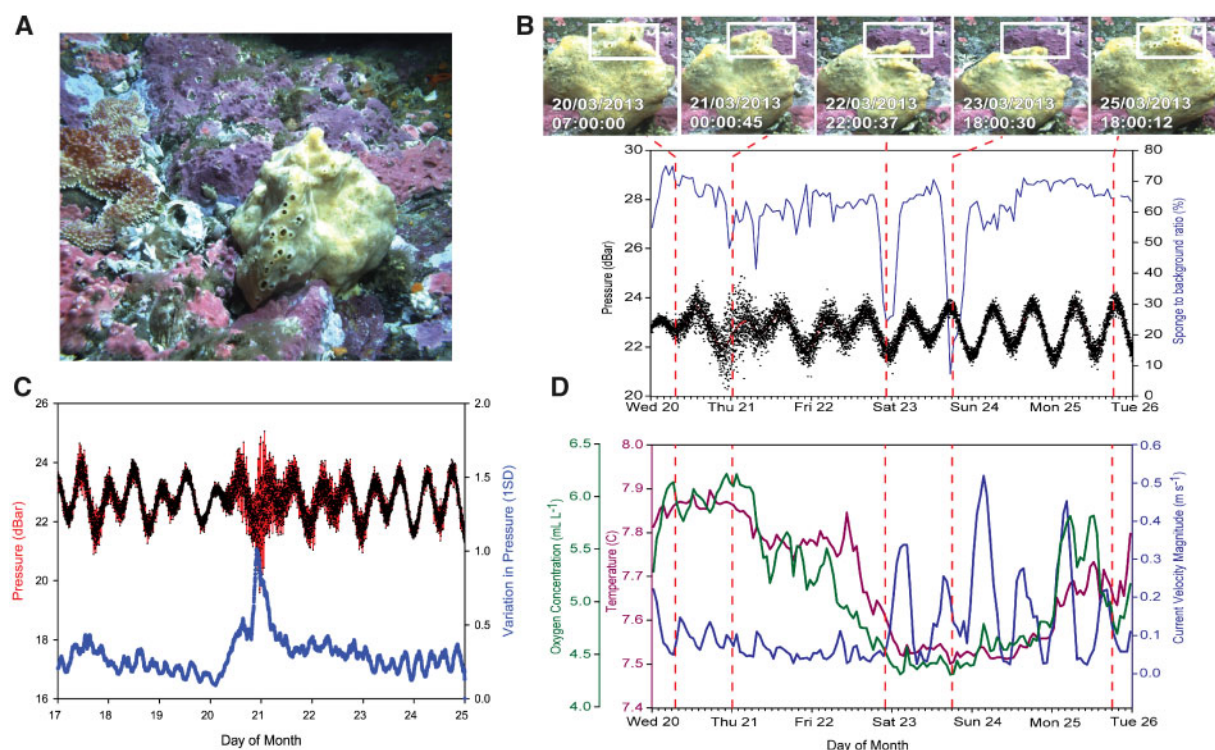


Fig. 2 Response of the demosponge *Suberites concinnus* to a storm. **A**) The demosponge *S. concinnus* and a neighboring sea star in the camera's field of view at the Folger Node of the Ocean Networks Canada "Folger Platform." **B**) A plot of the change in sponge size and change in pressure over time. White square on example images indicates the region of interest that was monitored for changes in sponge size. **C**) Plot of pressure (red, upper line) and pressure anomalies (lower line, 1 SD of pressure) over time. The region shown in the box reflects the time period shown in B and D. The wave-form of the upper line reflects tidal changes. **D**) A plot of temperature, oxygen, and current velocity at the Folger platform over time.

difficult to connect to a stimulus, but several large cringes were clearly associated with a storm whose arrival could be detected by changes in pressure.

For one storm event in 2013 we plotted change in area of the sponge together with pressure, oxygen, temperature, and current, and found that a massive pressure anomaly preceded several contractions of the sponge (Fig. 2C,D). The anomaly gave rise to a change in oxygen/temperature over the next 2 days, and after 3 days there were two pulses of increased current, from 0 to 30 cm s⁻¹ (Fig. 2D). Importantly two large cringes by the sponge preceded the pulses of current. The sponge reduced its whole size before being hit by the first wave of current. It is possible that the sum of changes in water properties caused a threshold in sensitivity to be reached, but it is also possible that the sponge responded to the pressure wave and reduced its size before the current increased. Another possibility is that fish shelter behind the sponge, disturbing the sponge mechanically. More storm responses have been recorded and must still be analyzed.

Another sponge watched by cameras is at Station M, a long-term study site 4000 m deep that is monitored by the Monterey Bay Aquarium Research

Institute (Fig. 3A–D). There, a field of view includes a white blob that seems to be a sponge on a stalk—no specimen has been collected to date so it is referred to by the MBARI Deep-Sea Guide as "Hexactinellida sp. 2" and described as being a ball-shaped sponge with multiple stalks of non-twisted spicule columns that project into the sediments (MBARI 2019). This sponge may be a rossellid hexactinellid because its contractions are extremely slow. Simply plotting change in size over time shows that the sponge contracts and expands several times over 5 months (Fig. 3A). Contractions can take several days—just the start of a contraction is 4 h (Fig. 3B)—and the sponge remains contracted for over a week. It is mystifying what use that long a contraction is—presumably there is no feeding while contracted. Some objects (perhaps pyrosomes, hagfish, or large detrital aggregates that reach the seafloor as marine snow) appear to hit the base of the sponge, but these interactions are not obviously correlated with times the sponge contracts (Fig. 3C).

In summary sponges carry out a range of behaviors and respond to a range of stimuli in various ways—some responses are local and others are global; global behavior appears coordinated but

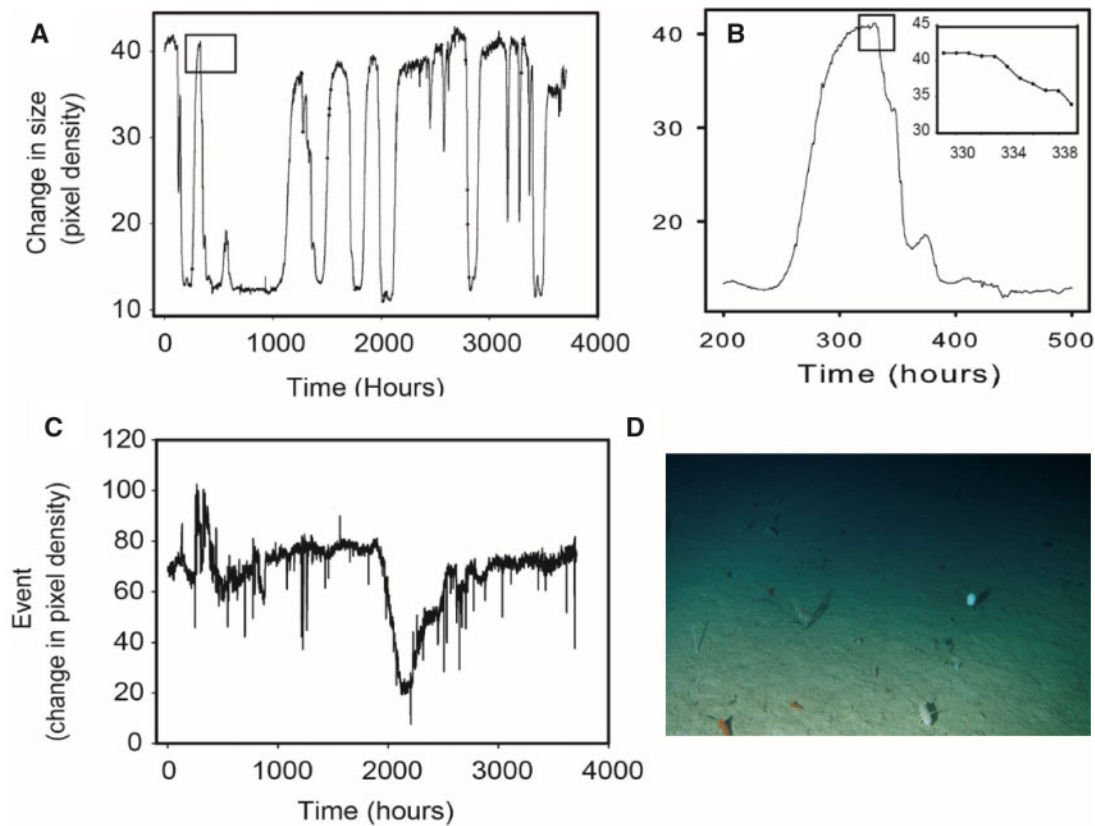


Fig. 3 Behavior of “*Hexactinellida* sp. 2,” a deep-sea sponge imaged by MBARI at 4000 m at Station M off the coast of California. **A**) A plot of pixel density as a proxy for change in size of the sponge over time. Images were captured hourly for nearly 5 months. The boxed region is shown in (B). **B**) Expansion of the sponge takes nearly 75 h and full contraction, at least 25 h. The inset provides an expanded view of the boxed region in (B) showing that a small reduction in area of the sponge takes 8 h, indicating that contraction of the whole sponge is extremely slow. **C**) Analysis using pixel density changes to plot a single “event” which was an object intersecting with the sponge, at roughly 2000 h. Objects linger around the sponge for long periods. **D**) The camera’s view with *Hexactinellida* sp. 2 as the white object on the right. Image copyright MBARI 2012.

how that would happen is not yet understood. Some stimuli are chemical in nature, many are mechanical. The contractions are all slow, at least an order of magnitude slower than electrical signaling in glass sponges (see Leys [2015] for a comparison of signaling rates), and more than three orders of magnitude slower than electrical signaling in cnidarians. The MBARI sponge is curious because it is particularly slow. If that sponge is indeed a hexactinellid glass sponge, then it suggests hexactinellids are able to have two types of response: fast responses to irritants that immediately arrest the flagella using electrical signals (Leys and Mackie 1997; Leys et al. 1999, reviewed in Leys 2015), as well as extremely slow contractions—a remarkably slow contraction might be due to the very unusual syncytial tissue. Contractions have so far not been seen in any glass sponge, so identifying the sponge in the MBARI video is a priority.

Cellular sponges have a number of tissues that might be contractile. One is the epithelium

(pinacocytes), another is a smooth muscle-like cell in the mesohyl (myocytes). Both have been together termed “actinocytes” (Boury-Esnault and Rützler 1997; Nickel 2010), however because lots of sponge cells have actin, the term actinocyte does not seem a distinct enough character. Given the large amount of collagen in many sponges it is hard to believe that cells in the mesohyl do not have a role especially since we know that collagen can change stiffness in *Chondrosia reniformis* (Wilkie et al. 2006). But regardless of the cell type involved, the signal must be chemical, between cells or, as in the case of *C. reniformis*, between cells and mesohyl.

Chemical signaling

Neurotransmitters, their enzymes and synaptic structural proteins, significantly pre-date the origin of neural tissues (Ryan and Grant 2009; Alie and Manuel 2010; Burkhardt et al. 2014). Compounds we associate with electrochemical signaling are found in bacteria, fungi, protists, and sponges, all creatures

without conventional nervous systems (e.g., Plugge et al. 2000; Ren et al. 2001; Liebeskind et al. 2011). The question is when did the functional biochemical signaling pathways arise? Possibly we should be looking for very different biochemical signaling systems in the different non-bilaterians. Animals probably experimented with different combinations of molecules and enzymes before settling on what we consider conventional chemical signaling in vertebrates. But what is conventional? Most knowledge about how neurotransmitters interact comes from studies on rat and mouse, but studies on invertebrates show that those same neurotransmitters may respond to different inhibitors (Tierney 2018). Work on the annelid *Platynereis dumerilii* shows that a broad range of neuropeptides are involved (Conzelmann et al. 2013; Bauknecht and Jékely 2015). Clearly neural chemistry in invertebrates is highly varied, and studies on non-bilaterians with neurons show an even greater range of peptidergic and small molecule chemical signals (Putnam et al. 2007; Anctil 2009; Moroz et al. 2014; Moroz 2015; Bosch et al. 2017).

Conventional neurotransmitters in vertebrate nervous systems include monoamines (serotonin, histamines, and catecholamines [noradrenalin/adrenalin]), acetylcholine (ACh), and amino acid signaling molecules (e.g., glutamate/GABA) (Krnjević 1974). A range of data are used to evaluate their presence and absence in animal tissues. To understand the mechanisms of behavior, first approaches are often application of neurotransmitter substances onto a tissue and recording of response, either by behavior (movement) or electrical impulses, and also to use pharmacology by adding known inhibitors of the receptors. In early days enzyme assays were used to test for substrates produced, more modern approaches involve antibody labeling, but often using non-specific antibodies (i.e., using antibodies raised in mammals on cnidarians or sponges). A third approach is to search transcriptomes and genomes for the genes as they are known from bilaterians.

The challenge in interpreting the collective data is that almost all views are from the bilaterian perspective because what we know of the enzyme interactions, antibody specificity, and gene sequences comes from bilaterians. In short, we are looking for classical bilaterian type molecules and pathways in non-bilaterians.

We searched genomes and transcriptomes for a wide range of signaling molecules and their synthesis pathways (Fig. 4). What we found is good evidence for everything *except* conventional serotonergic and adrenergic signaling, and each of

the four non-bilaterian groups seems to have specialized in use of a subset of signaling molecules. These data agree with work by others (Moroz et al. 2014; Bosch et al. 2017; Francis et al. 2017; Senatore et al. 2017).

Neurotransmitter small molecules in sponges and other non-bilaterians

Several reviews summarize the behavioral and histological evidence for neurotransmitter molecules in sponges (Renard et al. 2009; Nickel 2010; Leys 2015; Leys and Farrar 2016). From these, we know that sponges show histochemical staining for acetylcholinesterase, monoamine oxidase, serotonin, epinephrine, and that some staining patterns are blocked by inhibitors (e.g., Lentz 1966). Also many substances including serotonin, epinephrine, ACh, nitric oxide donors, and amino acids such as glutamate and GABA elicit contractions of ostia, oscula, or the whole body (Ellwanger et al. 2004; Ellwanger and Nickel 2006; Elliott and Leys 2010). Antibody work is limited because generally cross-reactivity between vertebrate-derived antibodies and sponge tissues is extremely poor. One report showed the whole of a larva labeled for serotonin (Weyrer et al. 1999) and another suggested rabbit anti-GABA and GAD label a range of cells from epithelia to choanocytes and mesohyl cells in the demosponge *Chondrilla nucula* (Ramoino et al. 2007).

However genes and transcriptomes tell a different story, and our genome and transcriptome searches (Fig. 4) simply confirm those findings of others (Srivastava et al. 2010; Ryan et al. 2013; Riesgo et al. 2014; Moroz 2015; Francis et al. 2017). Not only are the genes coding for serotonin and dopamine receptors missing from transcriptomes and genomes currently available for a range of sponges from all classes, but the key enzymes involved in synthesizing these molecules are also absent (Fig. 4). Although a number of enzymes used in the serotonergic and adrenergic biosynthesis pathways are present, key molecules are absent from sponge genomes and transcriptomes. These include serotonin (5HT)-receptors and the enzyme tryptophan hydroxylase (Tph), which would convert tryptophan to serotonin. Also missing are conventional adrenergic receptors and strong orthologs of the enzymes phenylethanolamine N-methyltransferase (PNMT) and tyrosine hydroxylase (Th), which convert norepinephrine to epinephrine, and tyrosine to dopamine, respectively (Fig. 4).

More surprisingly perhaps, the same is found for other non-bilaterians (cnidarians, ctenophores, and placozoans) (see Supplementary Data Files from

	Chordata		Cnidaria	Placozoa	Porifera		Ctenophora		Choanoflagellata		Filasterea
Molecule	Hsa	Nve	Tad	Emu	Twi	Aqu	Mlei	Pba	Mbr	Sro	Cow
Monoamine neurotransmitter molecules											
5HT receptor	●	○	○	○	○	○	○	○	○	○	○
TpH	●	●	○	○	○	○	○	○	○	○	○
AADC	●	●	●	●	●	●	●	○	○	○	●
TH	●	○	○	○	○	○	○	○	○	○	○
QDPR	●	●	●	●	●	●	●		●		●
VMAT (slc18a2)	●	○	○	●	●	●	●		●		●
PaH	●	●	●	●	●	●	●	●	●	●	●
DbH	●	●	●	●	●	●	○	○	○	○	○
PNMT	●	○	○	○	○	○	○	○	○	○	○
Dopamine receptor	●	○	○	○	●	○	○	○	○	○	○
Amino acids											
glutaminase	●	●	●	○	●	●	●	●	●	●	●
GLUD1	●	●	●	○	●	●	●	●	●	●	●
mGluR	●	●	●	●	●	●	●	●	○	○	○
GAD	●	●	●	●	●	●	●	●		●	●
EAAT	●	●	●	●	●	●	●	●	○	○	○
VGlut1	●	●	●	●	●	●	●	●	●	●	●
GABA	●	●	●	●	●	●	●	○	○	○	○
ABAT	●	●	●	○	●	●	○	●	○	○	●
TAT	●	●	●	●	●	●	●	●		●	●
Nitric Oxide											
NOS	●	●	●	●	●	●	●	○	○	○	○
sGC	●	●	●	●	●	●	●	●	●	●	
Prkg1	●	●	●	●	●	●	●	○	●	●	●
Acetylcholine											
Acetylcholinesterase	●	●	●	●	●	●	●	○	●	●	●

Fig. 4 Presence of bilaterian (human, mouse, or rat) genes involved in the biosynthetic pathways which form signaling molecules, searched from genomes and transcriptomes of human (*Homo sapiens*, Hsa), Cnidaria (*Nematostella vectensis*, Nve), Placozoa (*Trichoplax adhaerens*, Tad), Porifera (*Ephydatia muelleri* Emu, *Tethya wilhelma* Twi, *Amphimedon queenslandica*, Aqu), Ctenophora (*Mnemiopsis leidyi* Mlei, *Pleurobrachia bachei* Pba), Choanoflagellata (*Monosiga brevicollis* Mbr, *Salpingoeca rosetta* Sro), and Filasterea (*Capsaspora owczarzaki* Cow). Genes are categorized by the particular signaling molecules they are involved in producing (highlighted in beige). Filled indicates presence, empty indicates absence, blank indicates inconclusive due to poor availability of genome or transcriptome data. Presence indicated by e-value cut-off e^{-10} . Legend to abbreviations is in [Supplementary File S3](#).

Ryan et al. 2013; Moroz et al. 2014). Each of these groups lacks strong orthologs for serotonin and dopamine receptors, and also lacks PNMT and Th (Fig. 4). Anctil (2009) found that although many transcripts from the anemone *Nematostella vectensis* show similarity to histamines none clearly group with serotonin or dopamine/adrenergic receptors; in phylogenetic analysis, these genes grouped most strongly with GPCR rhodopsin family proteins. One review concluded “while there are tantalizing hints of transmitter pathways [in cnidarians], in almost all cases the evidence is ambiguous” (Anderson 2004). Despite data showing anti-serotonin labeling in tissues of different anthozoans (Westfall et al. 2000;

Kass-Simon and Pierobon 2007), unequivocal evidence for conventional serotonin and epinephrine signaling is missing (Bosch et al. 2017). Similarly, Moroz et al. (2014) concluded that the ctenophore *Pleurobrachia* genome and other ctenophore species’ transcriptomes “lack all but one (glutamate/GABA) of the enzymes to synthesize any of the conventional low molecular weight neurotransmitters.” It is fairly safe to conclude therefore, that sponges also do not use classical serotonin, epinephrine, or histamine chemical signaling.

Sponges have acetylcholinesterase, suggesting they are able to break down Ach (Fig. 4), which suggests Ach might be used in signaling. However

acetylcholinesterase is also present in ctenophores, cnidarians, choanoflagellates, as well as a range of plants, and its non-neuronal role in humans indicates this is an ancient signaling system that is used for cellular responses in growth, regeneration, and tissue homeostasis (Wessler et al. 2001).

Where the non-bilaterian groups differ is in the type of glutamate signaling and use of peptides. Sponges have a number of metabotropic glutamate (mGluR) and GABA receptors (Perovic et al. 1999; Srivastava et al. 2010; Krishnan et al. 2014; Guzman and Conaco 2016; Francis et al. 2017), as well as a full complement of the enzymes necessary for their biosynthesis. These are G-protein coupled receptors that mediate slow responses. In *Geodia cydonium* Perovic et al. (1999) found a hybrid GABA-mGluR receptor that is structurally similar to vertebrate mGluR4 and mGluR5 receptors. A hybrid type might be more common across other sponge groups because functional work shows different responses to glutamate and GABA. In some sponges both glutamate and GABA can cause contractions (e.g., *Tethya wilhelma*, Ellwanger et al. 2007), while in others GABA works antagonistically to glutamate (e.g., *Ephydatia muelleri*, Elliott and Leys 2010), as in the vertebrate nervous system. The competitive inhibitors AP3 and KYN also block glutamate-triggered contractions (Elliott and Leys 2010) and given the similarity of contractions in sponges it seems likely that glutamatergic signaling is common in many sponge species. Faster acting ionotropic glutamate receptors (iGluRs) have been found in a couple of sponge transcriptomes (Riesgo et al. 2014), but it is unknown if they are functional. In contrast while ctenophores do have mGluRs they also have a surprisingly large number of iGluRs which show distinct expression patterns (Moroz et al. 2014; Moroz 2015). Ctenophores also have neurons and millisecond responses for predatory behavior (Moroz et al. 2014).

Peptides are so far not known from sponges (Srivastava et al. 2010). In ctenophores prohormone precursors for peptides were found in *Pleurobrachia bachei*, although no work has tested their functionality (Moroz et al. 2014). In contrast peptides seem to be the dominant chemical signaling molecule in both cnidarians and placozoans (Srivastava et al. 2008; Anctil 2009; Senatore et al. 2017; Varoqueaux et al. 2018).

Chemical synapses: synaptic proteins and vesicle exocytosis

Another often sought set of proteins involved in chemical signaling are those involved in the formation and function of synapses. Neurons package chemicals

into vesicles for localized release and many of the molecules required for vesicle exocytosis are present in sponges (Riesgo et al. 2014; Francis et al. 2017). Indeed, many synaptic proteins are pre-metazoan (Supplementary File S1) (Ryan and Grant 2009; Burkhardt et al. 2014; Yang et al. 2015). For instance, fungi possess some postsynaptic density proteins, while choanoflagellates possess presynaptic proteins that localize to the apical pole (Alie and Manuel 2010; Burkhardt 2015). However, this does not necessarily suggest that synapse-like secretory mechanisms arose long before the bilaterian nervous system. Synaptic complexes are mechanistically complex—many proteins must be co-expressed in space and time. Sponges possess a near-complete catalog of postsynaptic density genes (Riesgo et al. 2014). In the sponge *A. queenslandica*, seven genes otherwise known to be involved in the postsynaptic density are co-expressed in larval globular cells (Sakaraya et al. 2007; Ueda et al. 2016). However, although some modules are co-expressed, the majority of these genes are not co-expressed at various stages of the life cycle (Conaco et al. 2012) or within the same cell type (Seb  -Pedr  s et al. 2018). We should not expect the secretory apparatuses of sponges to resemble bilaterian-like synapses because even in non-bilaterians that do possess nerves the organization of synaptic components can be distinct, as exemplified by the ctenophore presynaptic triad (Hernandez-Nicaise 1973).

A non-bilaterian view of chemical signaling molecules

Our biggest challenge is thinking outside of the bilaterian box. When we see a lot of the same enzymes and receptor molecules we expect to find, it is easy to assume the signaling pathway is there. And it might be, but functioning slightly differently. Several reviews have concluded that there are probably cnidarian-specific molecules that interact with the serotonin-like (melatonin GPCR) receptors (Anderson 2004; Anctil 2009; Moroz 2015; Bosch et al. 2017). Functional studies in cnidarians suggest that some enzymes might substitute for one another. In mammals tyrosinase is found in epidermal tissues and Th in nervous tissues, and they have different targets (Esposito et al. 2012; Lai et al. 2018). The sea anemone *Metridium senile* lacks Th. However, Carlberg et al. (1984) extracted a tyrosinase from *Metridium* tentacles and found that the *Metridium* tyrosinase had broad enough activity to hydroxylate L-DOPA (dopamine). However, there are conflicting views on whether PNMT, needed to make dopamine,

occurs in cnidarians (Marlow et al. 2009; Moroz et al. 2014; Krishnan and Schiöth 2015; Moroz 2015; Francis et al. 2017). In *P. bachei*, L-DOPA-like molecules may play a role in the adhesive properties of colloblasts (Townsend and Sweeney 2019). It is therefore not certain that cnidarians use conventional serotonergic or adrenergic signaling, and fairly well agreed that other non-bilaterians do not.

What neurotransmitter-type molecules actually do in animals without a nervous system is a good question. In placozoans peptides are thought to be used to signal to other individuals that there is food (Senatore et al. 2017; Varoqueaux et al. 2018). In the calcareous sponge *Leucandra aspera* GABA is suggested to regulate feeding, and experiments using dissociated cells showed enhanced uptake of dextrans (used as a food analog) in the presence of GABA (Ramoino et al. 2011). Antibodies to mammalian GABA_B receptors labeled choanocytes, the cells that generate flow through the sponge, and which typically phagocytose food (Ramoino et al. 2011). Nitric oxide is a short-lived gaseous signaling molecule that functions in bilaterians in relaxation of smooth muscle during peristalsis. Nitric oxide synthase is present in sponges and other non-bilaterians, and while nitric oxide was found to modulate peristalsis in the sponge *T. wilhelma* as it does in the cnidarian *Renilla koellikeri* (Anctil et al. 2005; Ellwanger and Nickel 2006), it is also suggested to be an important signal triggering the metamorphosis of sponge larvae (Ueda et al. 2016). One hypothesis is that *A. queenslandica* larval flask cells are both required for metamorphosis and may also be neurosecretory (Nakanishi et al. 2015). Secretory cells occur in many invertebrates and in some they may be simply involved in mucus secretion, but in others like *Platynereis* and cnidarians they release peptides to induce metamorphosis (Erwin and Szmant 2010; Conzelmann et al. 2013).

Another non-bilaterian perspective is that the metabolites that sponge GPCRs may be receptive to could be produced by symbionts. For example, tryptamine alkaloids (serotonin metabolites) and dopamine have been extracted from demosponges (Salmoun et al. 2002; Liu et al. 2004), but both are suggested to be produced by microbial symbionts, not the sponge. Dopamine was isolated from *Neopetrosia exigua* along with several other novel chemicals, and was suggested to be produced by a *Synechococcus*-like cyanobacterium; 5-hydroxytryptamine-derived alkaloids were extracted from two *Hyrtios* spp. demosponges, and were also considered produced by symbionts (Liu et al. 2004). No research has examined whether these molecules have a role in sponge tissue growth, repair, feeding, or other aspects of

homeostasis. However, there are hints that microbial metabolites may be involved in controlling canal formation. In *Suberites domuncula* CD36/LIMPII receptors are expressed on pinacocytes during canal formation, and their expression is suppressed by the bacterial metabolite 2-methylthio-1,4-naphthoquinone (MTN) (Müller et al. 2004). If some chemicals are synthesized by microbial symbionts it is conceivable the sponge recognizes and uses them, possibly to the microbes' advantage, to dilate canals to flush tissues with more oxygen, or to take in more dissolved carbon at different tide cycles, or to direct chambers where to join canals during the ongoing process of adjusting canal morphology during homeostasis. In *Hydra* the nervous system is tightly coupled to microbial symbionts and to previously considered non-neural roles that are involved in the tight relationship between symbiont and host (Klimovich and Bosch 2018). Clearly it is important to think broadly about how chemical signaling might work in an animal that does not use neurons.

Summary and conclusions

Sponges show extensive behavior, but sponge behavior is slow: contractions propagate locally at $2\text{--}300\ \mu\text{ s}^{-1}$ —the slowest is in regions of canals around the sponge, the fastest is the contraction of the osculum recorded by McNair (1923). These rates of propagation are at least an order of magnitude slower than in nervous systems (reviewed in Leys 2015). Effectors are sphincters in canals, canal epithelia, and we cannot rule out contractile cells in the collagenous mesohyl (Elliott and Leys 2007). Stimuli in the natural environment are low tides (water level dropping) and storms (including waves but also stirred up sediments and possibly pressure waves). Diurnal rhythms also exist, and so light is sensed (Nickel 2004; Jindrich et al. 2017).

It is tempting to think there is an overall coordination of behavior, but years of research indicate that the spread of stimuli is local (Parker 1910; Bidder 1937; Mackie 1979; Elliott and Leys 2007; Nickel 2010, reviewed in Leys and Farrar 2016). Massive effects on one osculum have no effect on a neighboring osculum. Global responses are most likely cumulative responses to the same stimulus.

Sponges have no neurons and lack conventional neurotransmitters (serotonin, epinephrine, histamine), but so do other non-bilaterians despite having neurons (e.g., Moroz et al. 2014). In sponges there are slow acting G-protein coupled receptor pathways using glutamate (mGluRs/GABA) and there is evidence they use other commonly used

small signaling molecules like nitric oxide (Ellwanger et al. 2007; Elliott and Leys 2010; Ueda et al. 2016). It seems that each non-bilaterian phylum has a specialized chemical signaling complement: sponges, mGluRs/GABA (Elliott and Leys 2010; Francis et al. 2017), ctenophores, iGluRs (Moroz et al. 2014), placozoans, peptides (Schuchert 1993; Senatore et al. 2017; Varoqueaux et al. 2018), cnidarians, neuropeptides and histamines (Bosch et al. 2017; Satterlie 2019). In bilaterians small amine neurotransmitters become established, in addition to a rich range of neuropeptide and ion channel signaling systems (Liebeskind et al. 2011, 2015; Paps and Holland 2018) (Supplementary File S2).

The adult sponge responsive system is most similar to that used for smooth muscle in bilaterians. Biosynthesis pathways used for local signaling may have been re-tooled for faster signaling in other animals. In sponges the most obvious use of chemical signaling is to control water flow through the animal in response to a range of short and long-term environmental stimuli. However, we might also expect cells to use chemical signaling in guiding cell migration in growth and regeneration, and also in responding to temperature, daylight, etc. There are not enough pieces of the biosynthesis pathways to hint at loss of a more complex system. Although some responses and the chemical signals involved could be very similar to those used to control smooth muscle in other invertebrates, the overall picture of chemical signaling in sponges seems to reflect a sponge-specific system. What is needed for future work on chemical signaling in non-bilaterians is physiological approaches to test specific hypotheses. In the post-genomic world we now need to return to the types of experimentation that gave such a wealth of data in bilaterians before genomes were available. Using genomic data to guide the choice of molecules and inhibitors promises massive leaps in understanding signaling in non-bilaterians.

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Supplementary data

Supplementary data are available at *ICB* online.

References

- Alie A, Manuel M. 2010. The backbone of the post-synaptic density originated in a unicellular ancestor of choanoflagellates and metazoans. *BMC Evol Biol* 10:34.
- Ancil M. 2009. Chemical transmission in the sea anemone *Nematostella vectensis*: a genomic perspective. *Comp Biochem Physiol D Genomics Proteomics* 4:268–89.
- Ancil M, Poulain I, Pelletier C. 2005. Nitric oxide modulates peristaltic muscle activity associated with fluid circulation in the sea pansy *Renilla koellikeri*. *J Exp Biol* 208:2005–17.
- Anderson PAV. 2004. Cnidarian neurobiology: what does the future hold? *Hydrobiologia* 530:107–16.
- Bauknecht P, Jékely G. 2015. Large-scale combinatorial deorphanization of *Platynereis* neuropeptide GPCRs. *Cell Rep* 12:684–93.
- Bergquist PR, Sinclair ME. 1968. The morphology and behaviour of larvae of some intertidal sponges. *N Z J Mar Freshw Res* 2:426–37.
- Bidder GP. 1937. The perfection of sponges. *Proc Linn Soc Lond* 149:119–46.
- Bosch TCG, Klimovich A, Domazet-Lošo T, Gründer S, Holstein TW, Jékely G, Miller DJ, Murillo-Rincon AP, Rentzsch F, Richards GS, et al. 2017. Back to the basics: cnidarians start to fire. *Trends Neurosci* 40:92–105.
- Boury-Esnault N, Rützler K. 1997. *Thesaurus of sponge morphology*. Washington (DC): Smithsonian Institution.
- Burkhardt P. 2015. The origin and evolution of synaptic proteins—choanoflagellates lead the way. *J Exp Biol* 218:506–14.
- Burkhardt P, Grønborg M, McDonald K, Sulur T, Wang Q, King N. 2014. Evolutionary insights into premetazoan functions of the neuronal protein Homer. *Mol Biol Evol* 31:2342–55.
- Carlberg M, Jergil B, Lindbladh C, Rosengren E. 1984. Enzymatic 5-hydroxylation of L-DOPA by a tyrosinase isolated from the sea anemone *Metridium senile*. *Gen Pharmacol Vasc Syst* 15:301–7.
- Collin R, Mobley AS, Busutil Lopez L, Leys SP, Cristina Diaz M, Thacker RW. 2010. Phototactic responses of larvae from the marine sponges *Neopetrosia proxima* and *Xestospongia bocatorensis* (Haplosclerida: Petrosiidae). *Invertebr Biol* 129:121–8.
- Conaco C, Bassett DS, Zhou H, Arcila ML, Degnan SM, Degnan BM, Kosik KS. 2012. Functionalization of a protosynaptic gene expression network. *Proc Natl Acad Sci U S A* 109:10612–8.
- Conzelmann M, Williams EA, Krug K, Franz-Wachtel M, Macek B, Jékely G. 2013. The neuropeptide complement of the marine annelid *Platynereis dumerilii*. *BMC Genomics* 14:906.

- Dunn CW, Hejnal A, Matus DQ, Pang K, Browne WE, Smith SA, Seaver E, Rouse GW, Obst M, Edgecombe GD, et al. 2008. Broad phylogenomic sampling improves resolution of the animal tree of life. *Nature* 452:745–9.
- Elliott G, Leys SP. 2007. Coordinated behaviour in a freshwater sponge: orchestrated contractions effectively expel water from the aquiferous system. *J Exp Biol* 210:3736–48.
- Elliott GRD, Leys SP. 2010. Evidence for glutamate, GABA and NO in coordinating behaviour in the sponge, *Ephydatia muelleri* (Demospongiae, Spongillidae). *J Exp Biol* 213:2310–21.
- Elliott GRD, MacDonald TA, Leys SP. 2004. Sponge larval phototaxis: a comparative study. *Boll Mus Ist Biol Univ Genova* 68:291–300.
- Ellwanger K, Brümmer F, Nickel M. 2004. Glutamate, GABA and serotonin induce contractions in the sponge *Tethya wilhelma* (Porifera: Demospongiae). In: *Jahrestagung der Deutschen Zoologischen Gesellschaft Abstractband*. Rostock: Zoologisches Institut der Universität Rostock p. 157.
- Ellwanger K, Eich A, Nickel M. 2007. GABA and glutamate specifically induce contractions in the sponge *Tethya wilhelma*. *J Comp Physiol A* 193:1–11.
- Ellwanger K, Nickel M. 2006. Neuroactive substances specifically modulate rhythmic body contractions in the nerveless metazoan *Tethya wilhelma* (Demospongiae, Porifera). *Front Zool* 3:7.
- Erwin P, Szmant A. 2010. Settlement induction of *Acropora palmata* planulae by a GLW-amide neuropeptide. *Coral Reefs* 29:929–39.
- Esposito R, D'Aniello S, Squarzone P, Pezzotti MR, Ristatore F, Spagnuolo A. 2012. New insights into the evolution of metazoan tyrosinase gene family. *PLoS ONE* 7:e35731.
- Fernandez-Valverde SL, Calcino AD, Degnan BM. 2015. Deep developmental transcriptome sequencing uncovers numerous new genes and enhances gene annotation in the sponge *Amphimedon queenslandica*. *BMC Genomics* 16:387.
- Feuda R, Dohrmann M, Pett W, Philippe H, Rota-Stabelli O, Lartillot N, Wörheide G, Pisani D. 2017. Improved modeling of compositional heterogeneity supports sponges as sister to all other animals. *Curr Biol* 27:3864–3870.
- Fortunato S, Adamski M, Bergum B, Guder C, Jordal S, Leininger S, Zwafink C, Rapp HT, Adamska M. 2012. Genome-wide analysis of the sox family in the calcareous sponge *Sycon ciliatum*: multiple genes with unique expression patterns. *EvoDevo* 3:14.
- Fortunato SAV, Leininger S, Adamska M. 2014. Evolution of the Pax-Six-Eya-Dach network: the calcisponge case study. *EvoDevo* 5:23.
- Francis W, Eitel M, Vargas S, Adamski M, Haddock S, Krebs S, Blum H, Wörheide G. 2017. The genome of the contractile demosponge *Tethya wilhelma* and the evolution of metazoan neural signalling pathways. *bioRxiv*120998.
- Guzman C, Conaco C. 2016. Comparative transcriptome analysis reveals insights into the streamlined genomes of haplosclerid demosponges. *Sci Rep* 6:18774.
- Haddock SHD. 2007. Comparative feeding behavior of planktonic ctenophores. *Integr Comp Biol* 47:847–853.
- Hammel JU, Nickel M. 2014. A new flow-regulating cell type in the demosponge *Tethya wilhelma*—functional cellular anatomy of a leuconoid canal system. *PLoS ONE* 9:e113153.
- Hernandez-Nicaise M-L. 1973. The nervous system of ctenophores III. Ultrastructure of synapses. *J Neurocytol* 2:249–263.
- Jackson D, Leys S, Hinman V, Woods R, Lavin M, Degnan B. 2002. Environmental regulation of development: induction of marine invertebrate metamorphosis. *Int J Dev Biol* 46:679–86.
- Jindrich K, Roper KE, Lemon S, Degnan BM, Reitzel AM, Degnan SM. 2017. Origin of the animal circadian clock: diurnal and light-entrained gene expression in the sponge *Amphimedon queenslandica*. *Front Marine Sci* 4.
- Kass-Simon G, Pierobon P. 2007. Cnidarian chemical neurotransmission, an updated overview. *Comp Biochem Physiol A* 146:9–25.
- Klimovich AV, Bosch TCG. 2018. Rethinking the role of the nervous system: lessons from the *Hydra* holobiont. *BioEssays* 40:1800060.
- Krishnan A, Dnyansagar R, Almén MS, Williams MJ, Fredriksson R, Manoj N, Schiöth HB. 2014. The GPCR repertoire in the demosponge *Amphimedon queenslandica*: insights into the GPCR system at the early divergence of animals. *BMC Evol Biol* 14:270.
- Krishnan A, Schiöth HB. 2015. The role of G protein-coupled receptors in the early evolution of neurotransmission and the nervous system. *J Exp Biol* 218:562–571.
- Krnjević K. 1974. Chemical nature of synaptic transmission in vertebrates. *Physiol Rev* 54:418–540.
- Kumala L, Riisgård HU, Canfield DE. 2017. Osculum dynamics and filtration activity in small single-ostium explants of the demosponge *Halichondria panicea*. *Mar Ecol Prog Ser* 572:117–28.
- Lai X, Wichers HJ, Soler-Lopez M, Dijkstra BW. 2018. Structure and function of human tyrosinase and tyrosinase-related proteins. *Chemistry Eur J* 24:47–55.
- Leininger S, Adamski M, Bergum B, Guder C, Liu J, Laplante M, Bråte J, Hoffmann F, Fortunato S, Jordal S, et al. 2014. Developmental gene expression provides clues to relationships between sponge and eumetazoan body plans. *Nat Commun* 5:3905.
- Lentz TL. 1966. Histochemical localization of neurohumors in a sponge. *J Exp Zool* 162:171–9.
- Leys SP, Cronin T, Degnan B, Marshall J. 2002. Spectral sensitivity in a sponge larva. *J Comp Physiol A* 188:199–202.
- Leys SP, Degnan B. 2001. Cytological basis of photoreceptive behaviour in a sponge larva. *Biol Bull* 201:323–38.
- Leys SP, Ereskovsky A. 2006. Embryogenesis and larval differentiation in sponges. *Can J Zool* 84:262–87.
- Leys SP, Farrar N. 2016. Chapter 3: Porifera. In: Schmidt-Rhaesa A, Harzsch S, Purschke G, editors. *Structure and Evolution of Invertebrate Nervous Systems*. Oxford: Oxford University Press.
- Leys SP, Hamonic L. 2019. Supplementary Files for: Sponge behaviour and the chemical basis of responses: a post-genomic view. *Integr Comp Biol*. Available online at Education and Research Archive, University of Alberta. <https://doi.org/10.7939/r3-3f9m-m216>.
- Leys SP, Mackie G. 1997. Electrical recording from a glass sponge. *Nature* 387:29–30.

- Leys SP, Mackie G, Meech R. 1999. Impulse conduction in a sponge. *J Exp Biol* 202:1139–50.
- Leys SP. 2015. Elements of a ‘nervous system’ in sponges. *J Exp Biol* 218:581–91.
- Leys SP, Kahn AS. 2018. Oxygen and the energetic requirements of the first multicellular animals. *Integr Comp Biol* 58:666–76.
- Liebeskind BJ, Hillis DM, Zakon HH. 2011. Evolution of sodium channels predates the origin of nervous systems in animals. *Proc Natl Acad Sci U S A* 108:9154–9.
- Liebeskind BJ, Hillis DM, Zakon HH. 2015. Convergence of ion channel genome content in early animal evolution. *Proc Natl Acad Sci U S A* 112:E846.
- Liu H, Mishima Y, Fujiwara T, Nagai H, Kitazawa A, Mine Y, Kobayashi H, Yao X, Yamada J, Oda T, et al. 2004. Isolation of Araguspongine M, a new stereoisomer of an Araguspongine/Xestospongine alkaloid, and dopamine from the marine sponge *Neopetrosia exigua* collected in Palau. *Mar Drugs* 2:154–63.
- Ludeman DA, Farrar N, Riesgo A, Paps J, Leys SP. 2014. Evolutionary origins of sensation in metazoans: functional evidence for a new sensory organ in sponges. *BMC Evol Biol* 14:3.
- Ludeman DA, Reidenbach MA, Leys SP. 2017. The energetic cost of filtration by demosponges and their behavioural response to ambient currents. *J Exp Biol* 220:995–1007.
- Mackie GO. 1979. Is there a conduction system in sponges? *Colloques Int CNRS* 291:145–51.
- Mah JL, Leys SP. 2017. Think like a sponge: the genetic signal of sensory cells in sponges. *Dev Biol* 431:93–100.
- Maldonado M. 2006. The ecology of the sponge larva. *Can J Zool* 84:175–94.
- Maldonado M, Bergquist PR. 2002. Phylum Porifera. In: Young CM, Sewell MA, Rice ME, editors. *Atlas of marine invertebrate larvae*. San Diego (CA): Academic Press. p. 21–50.
- Maldonado M, Durfort M, McCarthy DA, Young CM. 2003. The cellular basis of photobehavior in the tufted parenchymella larva of demosponges. *Mar Biol* 143:427–41.
- Marlow H, Srivastava M, Matus D, Rokhsar D, Martindale M. 2009. Anatomy and development of the nervous system of *Nematostella vectensis*, an anthozoan cnidarian. *Dev Neurobiol* 69:235–254.
- Martindale MQ, Henry JQ. 1999. Intracellular fate mapping in a basal metazoan, the ctenophore *Mnemiopsis leidyi*, reveals the origins of mesoderm and the existence of indeterminate cell lineages. *Dev Biol* 214:243–57.
- McNair GT. 1923. Motor reactions of the fresh-water sponge *Ephydatia fluviatilis*. *Biol Bull* 44:153–66.
- Monterey Bay Aquarium Research Institute (MBARI) 2019. Hexactinellida sp. 2. The Deep-Sea Guide (DSG) at <http://dsg.mbari.org/dsg/view/concept/Hexactinellida%20sp.%202>. Consulted on 2019-01-03.
- Moroz LL. 2015. Convergent evolution of neural systems in ctenophores. *J Exp Biol* 218:598–611.
- Moroz LL, Kocot KM, Citarella MR, Dosung S, Norekian TP, Povolotskaya IS, Grigorenko AP, Dailey C, Berezikov E, Buckley KM, et al. 2014. The ctenophore genome and the evolutionary origins of neural systems. *Nature* 510:109–14.
- Müller WEG, Thakur NL, Ushijima H, Thakur AN, Krasko A, Pennec GL, Indap MM, Perović-Ottstadt S, Schröder HC, Lang G, et al. 2004. Matrix-mediated canal formation in primmorphs from the sponge *Suberites domuncula* involves the expression of a CD36 receptor–ligand system. *J Cell Sci* 117:2579–90.
- Nakanishi N, Stoupin D, Degnan SM, Degnan BM. 2015. Sensory flask cells in sponge larvae regulate metamorphosis via calcium signaling. *Integr Comp Biol* 55:1018–27.
- Nickel M. 2004. Kinetics and rhythm of body contractions in the sponge *Tethya wilhelma* (Porifera: Demospongiae). *J Exp Biol* 207:4515–24.
- Nickel M. 2010. Evolutionary emergence of synaptic nervous systems: what can we learn from the non-synaptic, nerveless Porifera? *Invertebr Biol* 129:1–16.
- Nickel M, Scheer C, Hammel JU, Herzen J, Beckmann F. 2011. The contractile sponge epithelium sensu lato—body contraction of the demosponge *Tethya wilhelma* is mediated by the pinacoderm. *J Exp Biol* 214:1692–8.
- Paps J, Holland PWH. 2018. Reconstruction of the ancestral metazoan genome reveals an increase in genomic novelty. *Nat Commun* 9:1730.
- Parker GH. 1910. The reactions of sponges with a consideration of the origin of the nervous system. *J Exp Zool* 8:1–805.
- Perovic S, Krasko A, Prokic I, Muller IM, Muller WEG. 1999. Origin of neuronal-like receptors in Metazoa: cloning of a metabotropic glutamate/GABA-like receptor from the marine sponge *Geodia cydonium*. *Cell Tissue Res* 296:395–404.
- Pett W, Adamski M, Adamska M, Francis WR, Eitel M, Pisani D, Wörheide G. 2019. The role of homology and orthology in the phylogenomic analysis of metazoan gene content. *Mol Biol Evol* 36:643–9.
- Philippe H, Derelle R, Lopez P, Pick K, Borchellini C, Boury-Esnault N, Vacelet J, Renard E, Houliston E, Queinnee E, et al. 2009. Phylogenomics revives traditional views on deep animal relationships. *Curr Biol* 19:706–12.
- Plugge B, Gazzarrini S, Nelson M, Cerana R, Van Etten JL, Derst C, DiFrancesco D, Moroni A, Thiel G. 2000. A potassium channel protein encoded by chlorella virus PBCV-1. *Science* 287:1641–4.
- Presnell JS, Vandepas LE, Warren KJ, Swalla B, Amemiya C, Browne WE. 2016. The presence of a functionally tripartite through-gut in Ctenophora has implications for metazoan character trait evolution. *Curr Biol* 26:2814–20.
- Putnam NH, Srivastava M, Hellsten U, Dirks B, Chapman J, Salamov A, Terry A, Shapiro H, Lindquist E, Kapitonov VV, et al. 2007. Sea anemone genome reveals ancestral eumetazoan gene repertoire and genomic organization. *Science* 317:86–94.
- Ramoino P, Gallus L, Paluzzi S, Raiteri L, Diaspro A, Fato M, Bonanno G, Tagliaferro G, Ferretti C, Manconi R. 2007. The GABAergic-like system in the marine demosponge *Chondrilla nucula*. *Microsc Res Tech* 70:944–51.
- Ramoino P, Ledda FD, Ferrando S, Gallus L, Bianchini P, Diaspro A, Fato M, Tagliaferro G, Manconi R. 2011. Metabotropic γ -aminobutyric acid (GABAB) receptors modulate feeding behavior in the calcisponge *Leucandra aspera*. *J Exp Zool A Ecol Genet Physiol* 315A:132–40.
- Reiswig HM. 1971. *In situ* pumping activities of tropical Demospongiae. *Mar Biol* 9:38–50.

- Reiswig HM. 1979. A new sponge with rapid contraction systems. In: Annual Meeting of the Canadian Society of Zoology. Quebec: Laval University. p. 83a.
- Ren D, Navarro B, Xu H, Yue L, Shi Q, Clapham DE. 2001. A prokaryotic voltage-gated sodium channel. *Science* 294:2372–5.
- Renard E, Leys SP, Wörheide G, Borchellini C. 2018. Understanding animal evolution: the added value of sponge transcriptomics and genomics. *BioEssays* 40:1700237.
- Renard E, Vacelet J, Gazave E, Lapébie P, Borchellini C, Ereskovsky AV. 2009. Origin of the neuro-sensory system: new and expected insights from sponges. *Integr Zool* 4:294–308.
- Riesgo A, Farrar N, Windsor PJ, Giribet G, Leys SP. 2014. The analysis of eight transcriptomes from all poriferan classes reveals surprising genetic complexity in sponges. *Mol Biol Evol* 31:1102–20.
- Rivera AS, Ozturk N, Fahey B, Plachetzki DC, Degnan BM, Sancar A, Oakley TH. 2012. Blue-light-receptive cryptochrome is expressed in a sponge eye lacking neurons and opsin. *J Exp Biol* 215:1278–86.
- Russell JJ, Theriot JA, Sood P, Marshall WF, Landweber LF, Fritz-Laylin L, Polka JK, Oliferenko S, Gerbich T, Gladfelter A, et al. 2017. Non-model model organisms. *BMC Biol* 15:55.
- Ryan JF, Pang K, Schnitzler CE, Nguyen A-D, Moreland RT, Simmons DK, Koch BJ, Francis WR, Havlak P, Smith SA, et al. 2013. The genome of the ctenophore *Mnemiopsis leidyi* and its implications for cell type evolution. *Science* 342:1242592.
- Ryan TJ, Grant SGN. 2009. The origin and evolution of synapses. *Nat Rev Neurosci* 10:701–12.
- Sakaraya O, Armstrong KA, Adamska M, Adamski M, Wang I, Tidor B, Degnan BM, Oakley TH, Kosik KS. 2007. A post-synaptic scaffold at the origin of the animal kingdom. *PLoS ONE* 2:e506.
- Salmoun M, Devijver C, Daloze D, Braekman J-C, van Soest RWM. 2002. 5-Hydroxytryptamine-derived alkaloids from two marine sponges of the genus *Hyrtios*. *J Nat Prod* 65:1173–6.
- Satterlie R. 2017. Cnidarian neurobiology. In: Byrne JH, editor. *The Oxford handbook of invertebrate neurobiology*. Oxford: Oxford University Press (DOI:10.1093/oxfordhb/9780190456757.013.7).
- Schnitzler C, Pang K, Powers M, Reitzel A, Ryan J, Simmons D, Tada T, Park M, Gupta J, Brooks S, et al. 2012. Genomic organization, evolution, and expression of photoprotein and opsin genes in *Mnemiopsis leidyi*: a new view of ctenophore photocytes. *BMC Biol* 10:107.
- Schuchert P. 1993. *Trichoplax adhaerens* (Phylum Placozoa) has cells that react with antibodies against the neuropeptide RFamide. *Acta Zool* 74:115–7.
- Sebé-Pedrós A, Chomsky E, Pang K, Lara-Astiaso D, Gaiti F, Mukamel Z, Amit I, Hejnal A, Degnan BM, Tanay A. 2018. Early metazoan cell type diversity and the evolution of multicellular gene regulation. *Nat Ecol Evol* 2:1176–88.
- Senatore A, Reese TS, Smith CL. 2017. Neuropeptidergic integration of behavior in *Trichoplax adhaerens*, an animal without synapses. *J Exp Biol* 220:3381–90.
- Srivastava M, Begovic E, Chapman J, Putnam NH, Hellsten U, Kawashima T, Kuo A, Mitros T, Salamov A, Carpenter ML, et al. 2008. The *Trichoplax* genome and the nature of placozoans. *Nature* 454:955–60.
- Srivastava M, Simakov O, Chapman J, Fahey B, Gauthier M, Mitros T, Richards GS, Conaco C, Dacre M, Hellsten U, et al. 2010. The *Amphimedon queenslandica* genome and the evolution of animal complexity. *Nature* 466:720–7.
- Strehlow BW, Pineda M-C, Duckworth A, Kendrick GA, Renton M, Abdul Wahab MA, Webster NS, Clode PL. 2017. Sediment tolerance mechanisms identified in sponges using advanced imaging techniques. *PeerJ* 5:e3904.
- Tamm SL. 2014. Cilia and the life of ctenophores. *Invertebr Biol* 133:1–46.
- Tierney AJ. 2018. Invertebrate serotonin receptors: a molecular perspective on classification and pharmacology. *J Exp Biol* 221:jeb184838.
- Tiselius P, Möller LF. 2017. Community cascades in a marine pelagic food web controlled by the non-visual apex predator *Mnemiopsis leidyi*. *J Plankton Res* 39:271–9.
- Townsend JP, Sweeney AM. 2019. Catecholic compounds in ctenophore colloblast and nerve net proteins suggest a structural role for DOPA-like molecules in an early-diverging animal lineage. *Biol Bull* 236:55–65.
- Ueda N, Richards G, Degnan B, Kranz A, Adamska M, Croll R, Degnan S. 2016. An ancient role for nitric oxide in regulating the animal pelagobenthic life cycle: evidence from a marine sponge. *Sci Rep* 6:37546.
- Varoqueaux F, Williams EA, Grandemange S, Truscetto L, Kamm K, Schierwater B, Jékely G, Fasshauer D. 2018. High cell diversity and complex peptidergic signaling underlie placozoan behavior. *Curr Biol* 28:3495–3501.e3492.
- Wahab M, de Nys R, Webster N, Whalan S. 2014. Larval behaviours and their contribution to the distribution of the intertidal coral reef sponge *Carteriospongia foliascens*. *PLoS ONE* 9:e98181.
- Warburton FE. 1966. The behavior of sponge larvae. *Ecology* 47:672–4.
- Wessler I, Kilbinger H, Bittinger F, Kirkpatrick CJ. 2001. The biological role of non-neuronal acetylcholine in plants and humans. *Jpn J Pharmacol* 85:2–10.
- Westfall JA, Elliott SR, MohanKumar PS, Carlin RW. 2005. Immunocytochemical evidence for biogenic amines and immunogold labeling of serotonergic synapses in tentacles of *Aiptasia pallida* (Cnidaria, Anthozoa). *Invertebr Biol* 119:370–8.
- Weyer S, Rützel K, Rieger R. 1999. Serotonin in Porifera? Evidence from developing *Tedania ignis*, the Caribbean fire sponge (Demospongiae). *Mem Qld Mus* 44:659–65.
- Wilkie IC, Parma L, Bonasoro F, Bavestrello G, Cerrano C, Carnevali MDC. 2006. Mechanical adaptability of a sponge extracellular matrix: evidence for cellular control of mesohyl stiffness in *Chondrosia reniformis* Nardo. *J Exp Biol* 209:4436–43.
- Yang X, Pei J, Kaeser-Woo YJ, Bacaj T, Grishin NV, Südhof TC. 2015. Evolutionary conservation of complexins: from choanoflagellates to mice. *EMBO Rep* 16:1308