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REVIEW / SYNTHÈSE

Physiology of coordination in sponges[1]

S.P. Leys and R.W. Meech

Abstract: All multicellular organisms need a means of communicating between cells and between regions of the body. The evolution of a nervous system, by the Cnidaria, provided a fast means of communication and enabled the colonization of rapidly changing environments. Sponges, the descendants of the first multicellular animals, lack nerves but nevertheless have a number of different systems that allow coordinated behaviour, albeit rather slow coordinated behaviour. It is from elements within these systems that the origins of the nervous and endocrine systems, the grand organizing principles of higher animals, seem likely to have appeared. Electrical activity has not been found in cellular sponges, yet local contractions are elicited in response to a variety of stimuli and, in some cases, contractions propagate across the body to control the hydrodynamics of the feeding current. The mechanism of propagation is thought to involve hormones or a combination of other signaling molecules and direct mechanical action of one cell on the next, leading to increased intracellular calcium. In other instances cellular sponges respond to stress, such as heat shock, by elevating intracellular calcium by way of second messengers such as cyclic ADP-ribose. Electrical communication, well known in plants and protists, was first demonstrated in a sponge in 1997. Hexactinellids (glass sponges), which arrest their feeding current within 20 s of mechanical or electrical stimulation, do so via an electrical impulse that propagates through syncytial tissues. These unusual syncytial tissues are cytoplasmically coupled from outside to inside and top to bottom so that there are no membrane boundaries to impede the electrical currents. Pharmacological tests suggest that Ca^{2+}, rather than Na^{+}, drives the action potential. The conduction velocity is slow (0.27 $cm \cdot s^{-1}$) and is highly temperature sensitive (Q_{10} ~3). At present, glass sponges are the only poriferans known to have propagated electrical signals. In addition, reports of directional swimming in sponge larvae, of the rapid and coordinated changes in the tensile strength of the extracellular matrix in *Chondrosia* Nardo, 1847, and of the rapid closure of ostia of some cellular sponges in response to mechanical stimuli further illustrate the variety of coordinating mechanisms that evolved in the Porifera in the absence of a nervous system.

Résumé : Tous les organismes multicellulaires ont besoin d'un moyen de communication entre les cellules et entre les différentes régions du corps. L'évolution d'un système nerveux chez les cnidaires a fourni un moyen rapide de communication et a permis la colonisation d'environnements qui changent vite. Les éponges, les descendants des premiers animaux multicellulaires, n'ont pas de nerfs, mais elles possèdent plusieurs systèmes différents qui assurent un comportement coordonné, bien que ce soit un comportement coordonné plutôt lent. C'est vraisemblablement à partir d'éléments de ces systèmes que se sont développés les systèmes nerveux et endocriniens, les grands principes d'organisation chez les animaux supérieurs. On ne trouve pas d'activité électrique chez les éponges cellulaires; il se produit néanmoins des contractions locales en réaction à une variété de stimulus et, dans certains cas, les contractions peuvent se propager à travers le corps afin de contrôler l'hydrodynamique du courant alimentaire. On croit que le mécanisme de propagation implique des hormones ou une combinaison d'autres molécules de signalisation, ainsi que l'action mécanique des cellules sur leurs voisines, ce qui augmente le calcium intracellulaire. Dans d'autres cas, les éponges cellulaires réagissent au stress, par exemple à un choc thermique, en accroissant le calcium intracellulaire au moyen de seconds messagers, tels que l'ADP-ribose cyclique. En 1997, on a pu démontrer pour la première fois l'existence de communication électrique, bien connue chez les plantes et les protistes, chez une éponge. Les hexactinelles (éponges de verre), qui interrompent leur courant alimentaire en moins de 20 s après une stimulation mécanique ou électrique, le font au moyen d'une impulsion électrique qui se propage à travers les tissus syncytiaux. Ces curieux tissus syncytiaux sont reliés par leur cytoplasme de l'extérieur à l'intérieur et du haut en bas de manière à ce qu'il n'y

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[1]This review is one of a series dealing with aspects of the biology of the phylum Porifera. This series is one of several virtual symposia on the biology of neglected groups that will be published in the Journal from time to time.
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ait pas de frontière membranaire pour bloquer les courants électriques. Des tests pharmacologiques indiquent que le Ca^{2+}, plutôt que le Na^{+}, est le moteur du potentiel d'action. La vitesse de conduction est lente (0,27 $cm \cdot s^{-1}$) et elle est fortement affectée par la température (Q_{10} ~3). À l'heure actuelle, la transmission des courants électriques n'est signalée que chez les éponges de verre. De plus, on a observé de la nage orientée chez des larves d'éponges, des changements rapides et coordonnés de la force de tension de la matrice extracellulaire chez *Chondrosia* Nardo, 1847 et la fermeture rapide des ostiums chez certaines éponges cellulaires en réaction aux stimulus mécaniques; ces exemples illustrent davantage la variété de mécanismes de coordination qui se sont développés chez les porifères en l'absence d'un système nerveux.

[Traduit par la Rédaction]

Introduction

Before we discuss coordination in the Porifera, it might be useful to make a few general statements about its role in the evolution of the Metazoa. Whatever the actual origins of the Metazoa, we suppose that multicellularity provides a competitive edge that stems from increased size and the appearance of cell specialization. Even in its most basic form, where the efficiency gain comes from a simple association of different specialized cell types, any advantage will be lost unless cell interactions ensure that there are appropriate numbers of each cell class. Hence, even the most basic cooperation between cells requires some kind of "coordination".

A sponge is an animal whose primary function is to filter water for food to provide energy for growth and reproduction.[3] This apparent simplicity belies the complexity of the processes that support it. These processes include not only the choanocyte pump but also the particular form of sponge epithelia that line the water channels (pinacocytes), the incurrent pores (ostia), and the excurrent canals (oscula), as well as a myriad of cells that move within the central collagenous mesohyl or mesenchyme. Molecular analysis suggests that this structural complexity exists within a body plan set up by adhesion molecules, morphogens, and a suite of regulatory genes (Müller and Müller 2003; Larroux et al. 2006).

Given the growing understanding of the cellular complexity of sponges, we have had to limit this review to the coordination of the behaviour of sponges in response to specific environmental stimuli. A consideration of the complex cell-matrix interactions that take place during the growth and development of the sponge will not be covered here. We also do not examine a host of other complex areas of sponge physiology such as nutrient uptake, translocation of metabolites, and immune responses. For glass sponges, some of these topics are covered in a separate review (Leys et al. 2006).[4]

Though the terms "sponge" and "behaviour" are not frequently associated, reports of sponges contracting or otherwise controlling the flow of water through their bodies date as far back as Aristotle's *History of Animals* (see Mackie 1979). Here we review four specific coordination/conduction systems in sponges. We first discuss the slow contractile events that propagate through cellular sponges and cause changes in feeding current. Second, we review propagation of electrical signals through syncytial sponges ("glass sponges") that cause arrests in water flow. A third signaling system involves second messengers that function in heat signaling pathways. The fourth category includes examples of behaviour that have been documented in relatively few sponges, but which may be widespread within the phylum. Among these are extracellular matrix mediated responses to mechanical stress, directed swimming in sponge larvae mediated by ciliary photoreceptors, and rapid closure of tissue around ostial pore fields in response to mechanical stimuli.

In higher animals, whole societies are coordinated by environmental factors such as day length and by chemical signals such as pheromones. At the tissue level, coordination is accomplished by the grand organizing systems of hormones and electrical impulses. In the cytoplasm, molecular interactions are coordinated by the simple expedient of ensuring that the cell architecture aligns appropriate enzymes alongside mobile molecular messengers. At each level of organization, many of the signal receptors, message generators, message paths or conduits, and effectors have been identified. In sponges, the nature of the effector is in some cases still unclear, and it is possible that more than one effector exists. Identification of the receptor, signal, and signal pathway may also be uncertain. Nevertheless, sponge mechanisms have long intrigued those interested in the evolution of coordinating and conducting systems in the Metazoa. In part, there is an expectation that elements of more complex coordinating systems will play a simplified role in these, the first of the Metazoa.

In defining the phylum, Grant (1936) concluded that the Porifera lack "perceptible nervous or muscular filaments or organs of sense"; they are animals that simply generate water flow through the body to feed. This is easy to say but rather more difficult to prove, and it took many years of careful histological and behavioural studies before it could be finally established that sponges really do not possess a nervous system (Jones 1962; Pavans de Ceccatty 1974). Sponge signaling and coordination systems are complex nevertheless. The observed local slow behaviours can be explained in the bulk of sponges (demosponges and calcareous sponges) by local release of hormones, chemicals, or by the local mechanical interaction of one cell on the next (Mackie 1979; Lawn 1982), processes similar to signaling in non-excitable tissues in many metazoans. Furthermore, electrical

[3] Except in some carnivorous species that lack choanocyte chambers and feed by capturing small crustaceans in hook-like spicules in their outer epithelium (Vacelet and Boury-Esnault 1995).
[4] S.P. Leys, G.O. Mackie, and H.M. Reiswig. 2006. The biology of glass sponges. In preparation.

impulses do control behaviour in the syncytial glass sponges. Here we survey data derived from the many technological advances — fluorescent probes, digital time-lapse microscopy, and electrophysiology and molecular approaches — that have lead us to our present view of coordination systems in sponges.

Contractions in cellular sponges

Cellular sponges respond to mechanical and photic stimuli in situ and, in addition, to electrical and chemical stimuli in laboratory experiments. Such stimuli reduce the feeding current and, if maintained, may abolish it completely for an extended period. Responses include closure of the oscula in both marine and freshwater sponges (e.g., McNair 1923; Prosser et al. 1962; Emson 1966; Pavans de Ceccatty 1969), and contractile waves that travel up the osculum or across the entire sponge (De Vos and Van de Vyver 1981), sometimes compressing it to a fraction of its previous size (Pavans de Ceccatty 1960; Gaino et al. 1991; Nickel 2004). Although changes in feeding current have been observed during the passage of these contractile waves, all evidence suggests that it is the constriction of the aquiferous system — ostia, canals, or oscula (Parker 1910) — or the compression of the flagellated chambers (De Vos and Van de Vyver 1981) which is responsible for the decreased water flow, rather than the arrest of flagellar beating. While some responses appear to be local, observations from multiple preparations of both marine and freshwater sponges suggest that many of the events are propagated, although neither the true nature of the event nor the means by which it propagates are completely understood.

Rates of propagation

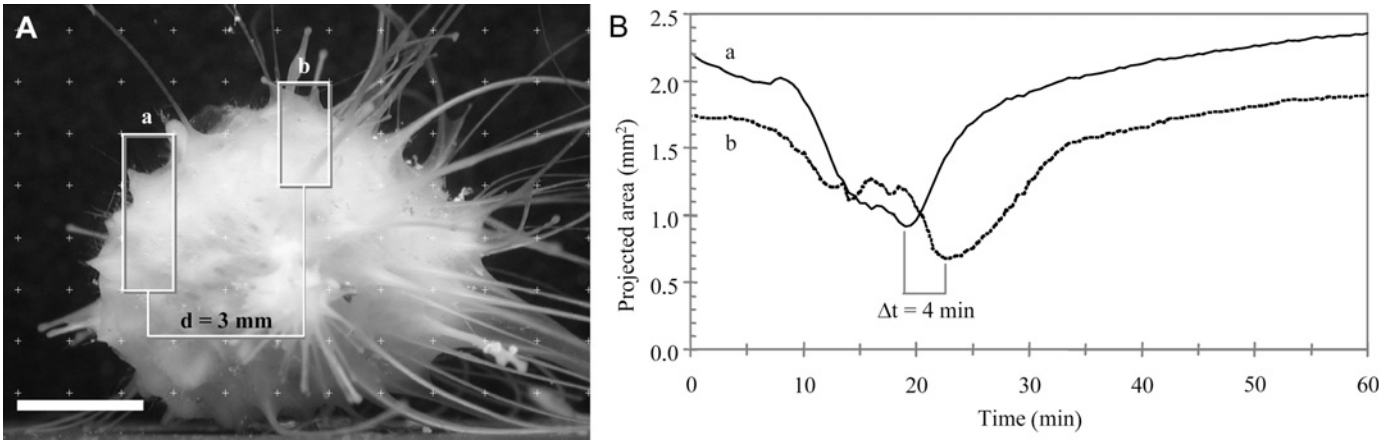
The rate of propagation of the contractile wave can be informative about the type of signal involved. However, reported propagation rates vary greatly depending on the type of preparation (whole opaque animal or transparent microscope preparation) and on the cell layer being observed. For example, in the freshwater sponge *Ephydatia fluviatilis* (L., 1758), McNair (1923) observed waves of contraction traveling down the osculum at 35 $\mu m \cdot s^{-1}$ and up the osculum at 17 $\mu m \cdot s^{-1}$. According to Jones (1962), this may represent conduction down the endopinacoderm (inside the osculum) and up the exopinacoderm (outside of the osculum). These rates are somewhat faster than the 8 $\mu m \cdot s^{-1}$ recorded to cross the entire aquiferous system during the so-called "spontaneous contractions" in *E. fluviatilis* reported by De Vos and Van de Vyver (1981). Recent observations by G.R.D. Elliott and S.P. Leys (in preparation) show that waves of contraction which travel through the canals of the choanosome in 10–15 min move more slowly (4 $\mu m \cdot s^{-1}$) than waves that run up the osculum in 11–60 s (30–100 $\mu m \cdot s^{-1}$), and some contractions appear to be spasms or convulsive movements that occur simultaneously over the entire sponge. The rates of propagation in different regions of the sponge appear to be related to the overall function of forcing water out of the aquiferous system in a single coordinated action (see "Coordination of contractions" on p. 292).

Freshwater sponges are thin-walled animals, i.e., their epithelia line a cellular mesohyl that may be less than 5 µm thick. Thus, the wave of contraction is thought to travel through the pinacocyte layer (epithelia) rather than through cells in the mesohyl (De Vos and Van de Vyver 1981). In contrast, many marine sponges have a thick mesohyl that contains a variety of cell types within a dense collagenous matrix. In these sponges, the contractile cells (referred to as myocytes or actinocytes) are generally considered to lie within the mesohyl in specialized sphincters around the osculum and exhalent canals, and waves of contraction are presumed to travel from cell to cell within the mesohyl. In each case, the signal may also travel short distances through the mesohyl itself — perhaps mediated by some chemical signal released extracellularly. An observation supporting this idea is that cells crawling through the mesohyl stop suddenly when a wave of contraction passes over them even though they do not make contact with the pinacoderm or any other cell type (De Vos and Van de Vyver 1981).

Local contractions of the osculum occur in many marine sponges, but propagation of the contractions over extended distances have been reported in only a few instances, perhaps because of the difficulty of measuring such contractions in large opaque animals. Only two examples stand out. In the first, Pavans de Ceccatty (1969) used time-lapse cinematography to study waves of contraction moving across the oscular crown and body wall of the genera *Spongia* (*Euspongia*) L., 1759 and *Hippospongia* Schulze, 1879. The contractile waves caused a change in the apparent density of the surface epithelia, allowing him to chart its movement across the sponge at 8–30 $\mu m \cdot s^{-1}$. These two sponge genera, however, are both large, and detailed study of their contractile behaviour is difficult. Sponges in the genus *Tethya* Lamarck, 1814 on the other hand are smaller, generally 2–10 cm, spherical animals that are easily maintained in aquaria. Many species have a global contractile response that has caught the attention of several researchers. In early studies, Pavans de Ceccatty (1960) showed that a sharp mechanical stimulus to the osculum of *Tethya lyncurium* (L., 1767) (currently known as *Tethya aurantium* (Pallas, 1766); a marine sponge) caused the entire sponge to contract within minutes to one-third its original size. Multiple stimuli applied to different places on its surface caused an even faster response and the osculum closed within 30 s. In a different approach, Reiswig (1971) used thermistor flow probes and photography to monitor feeding current and changes in morphology of three genera in situ. He demonstrated that different genera have different pumping rhythms. *Tethya crypta* de Laubenfels, 1949 had such a contractile osculum that flow probes could not record changes in pumping over more than a couple of hours. Furthermore, the contractions occurred with a predictable diurnal rhythm that was upset by changes in weather and water conditions (Reiswig 1971).

Tethya wilhelma Sará, Sará, Nickel, and Brümmer, 2001 studied in aquaria by Nickel (2004), also has rhythmic contractions that compress the entire animal to nearly a quarter of the original volume each time (Fig. 1). There are two types of contraction: (1) one that travels a short distance ("sub-contraction") at 5–25 $\mu m \cdot s^{-1}$ and (2) one that causes the whole animal to shrink to one-third of its original size. The latter involves a 20 min contraction phase and a much longer (45 min) expansion phase. The contraction cycle is shorter during the day than at night, which suggests that some sort of pacemaker activity may be involved. Nickel

Fig. 1. (A) Regions used to measure the spreading of local contractile waves over the surface of *Tethya wilhelma*. Field *a* represents an area of 3 mm^2; field *b* represents 2 mm^2. Both regions are at the periphery of the sponge and are used to show changes in area based on the contrast difference between sponge (whitish) and background (black); distance *d* between *a* and *b* is 3 mm. Scale bar = 2.5 mm. (B) Changes in the projected areas of fields *a* and *b* during a contraction event. The maximum contraction spreads as a wave over the sponge surface, taking 4 min to traverse the 3 mm distance, at a rate of 12.5 $\mu m \cdot s^{-1}$. (From Nickel 2004, reproduced with permission of M. Nickel and of J. Exp. Biol., vol. 207, p. 4520, © 2004 The Company of Biologists Ltd.)



(2004) suggests that the contraction is caused by actin-rich cells (actinocytes) which line canals in the outer cortex of the sponge, equivalent to the pinacoderm of freshwater sponges, rather than cells of the mesohyl, as is presumed to be the case in other marine sponges. Presumably, expansion follows the natural dilation of the canals with water upon relaxation of the "actinocytes".

A really interesting observation from *T. wilhelma* is that local responses can be initiated by the proximity or "attack" of animals such as amphipods (Nickel 2004). Such events occur on the background of the endogenous, rhythmic activity; the frequency of which can be quite constant for periods of hours or even days. Another intriguing finding was that clones formed by budding can fuse and, when they do, each clone retains its contraction rhythm until individual skeletal structures are lost and a new unit is formed, which then has a single rhythm. Both Pavans de Ceccatty (1960) and Reiswig (1976) have suggested that these kinds of global contractions may be involved in clearing debris from the body and ejecting gametes.

It should be noted that global contractions are not restricted to demosponges; calcareous sponges are also capable of substantial changes in body size. Photographs of sponges taken over 24 h showed that *Clathrina clathrus* (Schmidt, 1864) contracted different parts of its body at different rates (Gaino et al. 1991). Although exact rates were not obtained, the authors noted that areas several centimetres in diameter contracted in less than 1 h. Notably, no equivalent contractions have ever been reported in hexactinellid (glass) sponges, and it has been supposed that their tissue lacks the required contractile apparatus (Mackie and Singla 1983).

Evidence for the contractile apparatus

Rhythmic contractions like those in the genus *Tethya* imply the presence of a versatile cytoskeleton of actin and myosin. In fact, actin was first identified in cells of a variety of demosponges using thin-section transmission electron microscopy (Bagby 1965; Vacelet 1966; Pavans de Ceccatty et al. 1970), and later in the basal epithelial cells of freshwater sponges using immunocytochemistry and heavy meromyosin (Pavans de Ceccatty 1981).

The "almost-muscles" (Mackie 1990), or myocytes, of the marine sponges *Microciona prolifera* (Ellis and Solander, 1786) and *Tedania ignis* (Duchassaing and Michelotti, 1864) are fusiform cells, 50 µm long and 2–3 µm wide. In *T. ignis*, they are said to be similar to smooth muscle cells because they stain red with Mallory's trichrome stain (Bagby 1965). Thin sections of myocytes from both *T. ignis* and *M. prolifera* show bundles of filaments 150–250 Å (1 Å = 0.1 nm) in diameter; in *M. prolifera*, there are thinner filaments 50–70 Å in diameter, which in transverse sections appear to form a ring around the thick filaments (Bagby 1965).

What we know of the cytoskeleton of epithelial cells in freshwater sponges comes mostly from studies on organelle transport. In this work, the upper region of the sponge was removed to create a single layer of flat or plate-like basal epithelial cells in which organelles could be seen moving along the cytoskeletal proteins. Immunohistochemistry and rhodamine–phalloidin labeling have revealed a cytoskeletal organization like that present in fibroblasts (Pavans de Ceccatty 1981; Weissenfels 1990; Wachtmann and Stockem 1992*a*, 1992*b*). Bundles of actin filaments line the periphery of stationary cells and form long tracts across elongate motile cells. A network of microtubules radiates out from the central nucleus, spatially organizing the Golgi apparatus and other organelles within the cell.

Recent work with new probes for actin (Bodipy Phalloidin, Molecular Probes) on *Ephydatia muelleri* (Lieberkühn, 1856) show that dense tracts of actin occur in endopinacocytes which form the underside of the apical pinacoderm (the upper surface of the sponge). Not only do two or three such tracts traverse individual cells, but they link up with identical tracts in neighboring cells at strong focal adhesion plaques to form a nearly continuous pathway of polymerized microfilaments (Elliott 2004). In contrast, actin in basal endopinacocytes (cells forming the basal endopinacoderm, a tissue that anchors the animal to the substrate) is mostly around the periphery of the cell; in only a few such

cells is the actin organized into tracts across the middle of the cell as it is in the apical pinacoderm. Although some tracts of actin are found in the endopinacocytes that line canals, the distribution of actin here is more diffuse. This distribution of actin throughout the animal is quite revealing, because time-lapse video microscopy shows that the apical pinacoderm (a tent-like tissue that covers the animal and joins with the osculum) moves up and down like a single diaphragm during contractions; presumably the dense tracts are involved in this movement — a study of myosin distribution is now needed (see "Coordination of contractions" section below).

Evidence for junctions

For propagation of contractile waves to occur across distances of many millimetres, it is presumed that cells must be tightly linked to one another, yet many authors consider sponge epithelia to be poorly held together or leaky (Mackie 1984, 1990; Tyler 2003), and the early ultrastructural studies of the genera *Verongia* Bowerbank, 1845, *Tedania* Gray, 1867, and *Microciona* Bowerbank, 1862 (Bagby 1965; Vacelet 1966) suggested that myocytes appear to rarely contact one another. In fact, Bagby (1965) proposed that because nearly 50% of the volume of the sponge osculum in *Tedania* and *Microciona* is extracellular space, each myocyte could act as an independent contractile unit, presumably working against the collagenous extracellular matrix.

Pavans de Ceccatty et al. (1970) tested this hypothesis by carrying out a detailed ultrastructural study of cells in the oscula of four demosponges that are known to contract (*Hippospongia communis* (Lamarck, 1814), *Spongia* (*Euspongia*) *officinalis* (L., 1759), *Haliclona rosea* (Bowerbank, 1866), and *Verongia cavernicola* Vacelet, 1959 (currently known as *Aplysina cavernicola* (Vacelet, 1959)). They found that sufficient contacts between myocytes do occur to allow effective reduction of ocular diameter (Pavans de Ceccatty et al. 1970). Though five classes of cells were described, the majority of junctions were between pinacocytes and between contractile cells of the mesohyl. Most junctions involved the simple juxtaposition of plasma membranes with no special features in apposing cells. However, quite a variety of junctions were found in cells in the oscula of *H. communis*, including desmosome-like junctions, septate junctions, and junctions involving the exchange of material through vesicles. Notably, gap junctions have not been observed in any sponges (Green and Bergquist 1979; Garrone et al. 1980; Lethias et al. 1983), although there is one study that suggests differently. Loewenstein (1967) reported that electrical coupling could develop over a period of about 20 min when isolated cells of *Haliclona* (*Haliclona*) *oculata* (Pallas, 1766) were placed in contact in vitro. However, it is not clear whether such coupling could take place in vivo. Experiments on molluscan neurons show that coupling between isolated neurites can develop over a similar time period, but that gap junctions do not form between neurites from the same neuron unless they are first separated from the cell body (Guthrie et al. 1994).

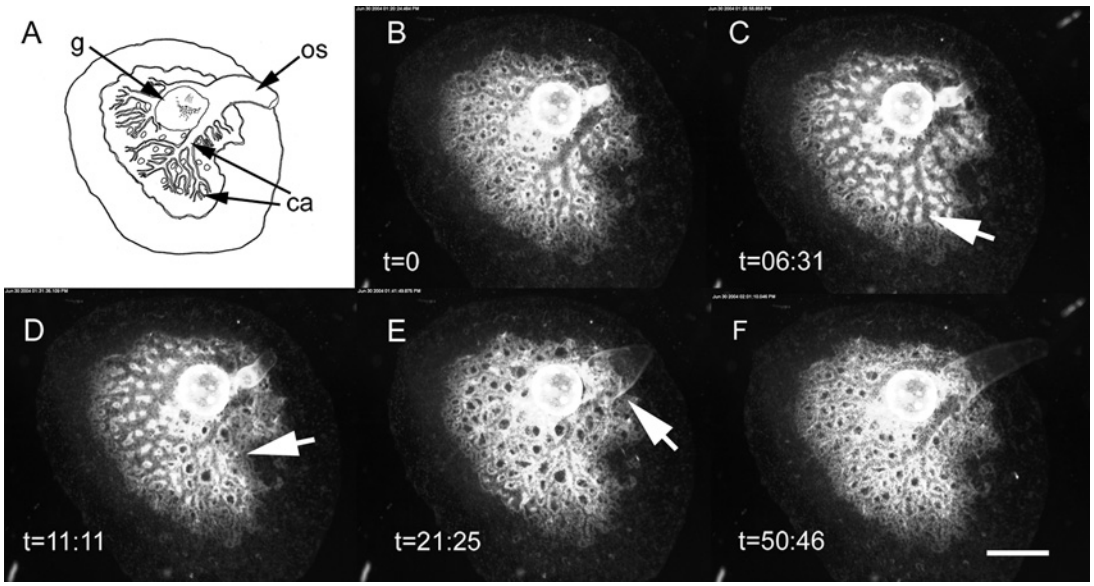
Coordination of contractions

Despite views that freshwater sponges were too rigid and full of spicules to observe global contractions (Pavans de Ceccatty 1960), in fact the juvenile freshwater sponge turns out to be a very practical model for studying both contractions and arrest of flow because of its transparency and small size (1 mm in diameter). Detailed observations by De Vos and Van de Vyver (1981) suggested that the contraction events might be coordinated across the entire sponge, but how exactly this happened or what effect it might serve was unknown. To understand how this might occur, Elliott and Leys (2003) used time-lapse video microscopy to re-examine contraction events in *E. muelleri*. They identified six stages involved in expelling waste from the sponge, which illustrate quite clearly that contractions are coordinated throughout the entire animal (Fig. 2). Addition of inedible dye (Sumi black calligraphy ink) or shaking the dish triggers a series of contractile events starting with the immediate contraction of the osculum. Over the next 10 min, the excurrent canals dilate, many expanding to twice their original diameter. The inflation process propagates as a wave starting at the large canals at the base of the osculum and moving gradually out to the small peripheral canals. At the moment of maximum inflation, fields of ostia in the apical pinacoderm close, and immediately afterwards a wave of contraction starts at the periphery of the sponge and propagates back along the canals to the base of the osculum. While individual ostia can close in 40 s, fields of 10 or more ostia typically close within 1–3 min (Fig. 3). It takes about 15 min (depending on the size of the sponge) for the contractile wave to propagate across the sponge to the base of the osculum, but in doing so all water is forced into the osculum, which swells to several times its normal diameter. When the contraction reaches the base of the osculum, it rapidly (in 30–60 s) propagates up the osculum expelling the pool of water and waste dye.

In time-lapse video images, the sponge surface (apical pinacoderm) can be seen to contract as a single sheet several times. Presumably, this involves the actin network that links endopinacocytes. Thus, throughout the entire event, from dye stimulus to final contraction of the osculum (a 30–40 min period), multiple contraction and signaling events take place. Fields of ostia close and open in synchrony; the wave of contraction initiates simultaneously from the periphery of the sponge and propagates along the canals in a single peristaltic wave to the base of the osculum; finally, the osculum opens when the contractile wave reaches its base and water is expelled as the wave moves from the base to the tip of the osculum. Perhaps the most intriguing signal, however, is that which controls the initial closure of the osculum. What triggers its closure when the apparent stimulus is inedible dye that builds up and clogs the flagellated chambers or rapid movement of the dish? Though some aspects of this behaviour may be explained by multiple dispersed stimuli occurring over a wide area, the first signal — the initial closure of the osculum — is less easy to explain in this way.

The stereotypical and highly orchestrated sequence of events is a proven method of clearing clumps of waste from the aquiferous system. This is not to say, however, that the "condensation" contractions of Weissenfels (1990) do not occur. The sponge appears to carry out minor "endogenous" contractions, much as the genus *Tethya* has "subcontractions" on a regular basis, and it is likely that these function to assist the choanocytes in flushing water from

Fig. 2. Stages in a contraction event in the sponge *Ephydatia muelleri*. (A) Diagrammatic representation of the sponge attached to a cover slip illustrating the location of the osculum (os) and branching canals (ca). (The juveniles hatch from overwintering gemmules; g.) (B) One minute after being shaken, the osculum contracts and closes while the canals begin to expand as water is drawn in through the ostia. (C) Maximum expansion of canals (arrow). (D) A wave of contraction begins at the periphery of the sponge (arrow) and moves across through the whole sponge. (E) The contractile wave has traveled across the whole sponge, compressing water into the osculum, which appears inflated (arrow). (F) At the end of the contraction, water is vented out of the osculum and the canals relax. Time (*t*) indicates minutes and seconds. Scale bar = 0.5 mm. (G.R.D. Elliott and S.P. Leys, unpublished data.)



specific regions of the canals. It is interesting to note the similarities between the use of contractions to move fluid through the canals of the sponge and the peristaltic contractions used by anthozoan cnidarians such as the sea pansy *Renilla koellikeri* Pfeffer, 1886 to move fluid through its gastrovascular cavity (Anctil 1989).

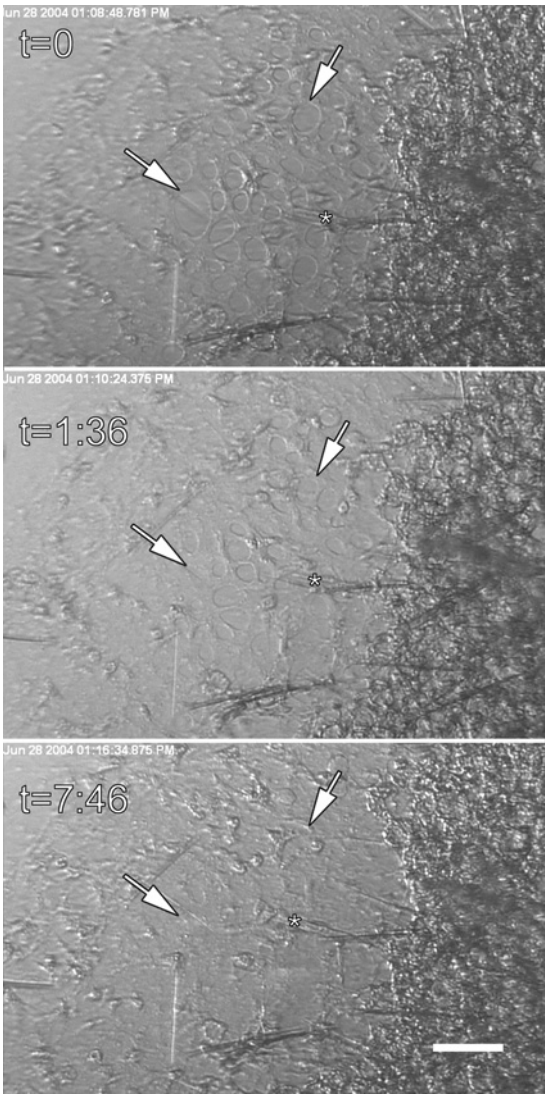
Pharmacology of signal transduction

Some of the earliest approaches to exploring the components of signal transduction during contractions in sponges involved the application of different stimuli — electrical, mechanical, and chemical — with the expectation that chemicals known from higher animals may play a similar role in these primitive metazoans. The difficulty in assessing and comparing these studies arises from the lack of uniform stimuli, the range of different effectors observed, and the large number of different species used. For example, electrical stimuli were studied with success in *E. fluviatilis* (McNair 1923), but unsuccessfully in the genera *Spongia* (*Euspongia*) (Pavans de Ceccatty 1971) and *Microciona* and in a suite of other species (Prosser 1967). Then, where mechanical stimuli were used uniformly across several studies, some researchers noted changes in oscular diameter, whereas others studied changes in ostial diameter. This section summarizes the effect on conducted waves of contraction of a great variety of pharmacological agents used in these studies. There is little recent data on the effects of hormones or other chemicals on sponge contractions, but knowledge of the presence of glutamate receptors in some demosponges (Perovic et al. 1999) and preliminary evidence that glutamate initiates contractions in the genus *Tethya* (Ellwanger et al. 2004) suggest that these experiments would be worthwhile pursuing.

In studies of the genus *Stylotella* Lendenfeld, 1888, Parker (1910) found that both ether and chloroform caused a rapid closure of the ostia and a slower closure of the osculum; strychnine evoked slow closure of the ostia; and both cocaine and atropine caused the ostia to open or remain open. Emson (1966) applied over a dozen chemicals to *Cliona celata* Grant, 1826 and found that four — eserine, histamine, tryptamine, and adrenaline — arrested the flagella in the choanocyte chambers. Pavans de Ceccatty (1971) found that while neither adrenaline nor acetylcholine alone affected the diameter of the osculum of the genus *Euspongia*, when used in succession they acted as synergistic excitors and caused constriction of the osculum. A detailed histochemical study of whole mounts of the calcareous sponge *Sycon* Risso, 1826 (= *Scypha* Gray, 1821) by Lentz (1966) showed acetylchonlinesterase, monoamine oxidase, adrenaline, noradrenaline, and 5-hydroxytryptamine to be localized in the cytoplasm of cells with either a multipolar or a spindle-shaped (bipolar) morphology. Multipolar cells were found in the mesohyl, while spindle-shaped cells formed a ring around the osculum.

The exchange of ions has been attempted with a variety of marine and freshwater demosponges again with varied results. From his experiments largely on the genus *Microciona*, Prosser (1962) concluded that contraction of the osculum required a univalent cation (usually Na^+, which could be replaced with K^+ or Li^+ without effect, but not by choline) and a divalent cation (usually Ca^{2+}, which could be replaced with Mg^{2+} or Sr^{2+}). On the other hand, Pavans de

Fig. 3. Closure of ostia (arrows) in the freshwater sponge *E. muelleri* prior to the start of a contractile wave. Time (*t*) is indicated in minutes and seconds. Scale bar = 20 μm. (G.R.D. Elliott and S.P. Leys, unpublished data.)



Ceccatty (1971) observed spontaneous contractions in the genus *Euspongia* and found that Mg^{2+} was not equivalent to Ca^{2+}, neither was K^+ equivalent to Na^+. The requirement for extracellular calcium in cell crawling has also recently been demonstrated (Lorenz et al. 1996).

Possible mechanisms of coordination

The single common factor from all pharmacological experiments is the requirement for extracellular calcium, or at least a divalent cation, for contraction. The observation that cells in the mesohyl cease crawling when a contractile wave passes (De Vos and Van de Vyver 1981) suggests that an extracellular signaling molecule may be released by contracting cells as proposed by Pavans de Ceccatty (1974). It may be that the local signal opens calcium channels and causes calcium influx. This not only produces contraction but also the further release of the extracellular signal, thereby propagating the contraction. Presumably, this agent also has an inhibitory effect on cell crawling. The exact nature of the signal remains unknown, but from our growing knowledge of calcium signaling in plants, protists, and non-excitable tissues of many metazoans, likely candidates are glutamate (Anctil and Carette 1994; Nedergaard 1994; Devlin and Schlosser 1999), ATP (Osipchuk and Cahalan 1992), nitric oxide (NO) (Colasanti et al. 1997; Moroz 2001; Melarange and Elphick 2003; Anctil et al. 2005), cyclic AMP (Anctil 1989), cyclic GMP (Anctil et al. 1991), or calcium itself (Hofer 2005). Evidence for stress-induced NO synthase activity in two demosponges (Giovine et al. 2001) suggests that NO may be involved. Given the role of NO in muscle relaxation in starfish and cephalopods (Schipp and Gebauer 1999; Melarange and Elphick 2003), it is possible that two signaling systems work in synergy during the expansion and contraction states of the sponge just as NO and amino acids have been shown to modulate peristaltic muscle contractions for fluid circulation in the sea pansy *R. koellikeri* (Anctil et al. 2005). However, the rapid (<60 s) diaphragm-like pulses of the apical pinacoderm and the quick contractions that run up the osculum (a structure 1.5 mm long) point to even faster modes of signaling. Although aqueous (gap) junctions are not known in sponges, an extensive search should be carried out for transient connections that could be enhanced under certain conditions as glial-cell gap junctions are in the presence of glutamate or high K^+ (Enkvist and McCarthy 1994).

Control of flagellar beating

Contraction of oscula, ostia, and canals would clearly restrict the flow of water through a sponge, but is it also possible that cellular sponges can control (i.e., arrest) their flagellar beating? If not, as Mackie (1979) notes in his review, this would make these sponges very different from other filter-feeding invertebrates. Some workers have reported that sponges pump almost continuously (Parker 1910; Jorgensen 1966), whereas others have observed variations in the strength of pumping (Bowerbank 1856, 1857) or periodic arrests in the demosponges *Verongia gigantea* Hyatt, 1875 and *T. crypta* (Reiswig 1971). The difficulty in differentiating between contraction of canals or oscula and arrests of flagella arises from the inability to observe the flagella because of the opacity of most sponges.

However, the freshwater sponge genera *Ephydatia* and *Spongilla* Lamarck, 1816 are so transparent that the flagella can be observed beating by light microscopy while waves of contraction move through the sponge body. Both Wintermann (1951) and Kilian (1952) report that the feeding current arrests temporarily during a contractile wave, but suggest that arrest is due to contraction of the incurrent pores or ostia. By examining the contraction in *E. fluviatilis* using time-lapse film and simultaneous fixation of specimens for electron microscopy, De Vos and Van de Vyver (1981) found that the choanocyte chambers can become so contorted as the contractile wave passes over them that they do temporarily arrest beating.

Arrests of flagella in other filter-feeding invertebrates are coordinated by the rapid propagation of action potentials that bring about an influx of calcium, similar to the mechanism of ciliary reversal during the avoidance response in *Paramecium* O.F. Müller, 1773 (Naitoh, 1982). For arrest

of pumping in demosponges to occur by the cessation of flagella caused by propagated electrical impulses like those that cause flagella arrest in glass sponges (see below), their cells would have to be coupled together with low-resistance pathways, and this does not appear to be the case as seen above (Garrone et al. 1980; Green and Bergquist 1982; Lethias et al. 1983). It is more likely that variations in pumping rates in these sponges represent either a long-term periodic change in the endogenous activity of the whole organism, perhaps co-ordinated by ectohormones, or responses of the whole organism to changes in its environment such as a change in ambient temperature (Annandale 1907).

In summary, there is no evidence that cellular sponges can control their flagellar beating … but presumably they do, although not over the short term. Short-term changes in flow can be explained by constriction of canals or pores, but longer term changes in flow are more likely to result from a combination of contraction of the ostia, sphincters, and oscula, possibly combined with flagellar arrest. The only observation of changed flagellar activity is in *E. fluviatilis*, and there the arrest of beating was caused by, or accompanied, contortion of the chambers.

Electrical signaling in glass sponges

Conduction pathway and effectors

Glass sponges (class Hexactinellida) differ from other sponges in that their major tissue consists of a giant multinucleated syncytium — the trabecular reticulum. This reticular tissue traverses the entire animal, forming the outer (dermal) and inner (atrial) surfaces (called the dermal and atrial membranes, respectively), and lines all incurrent and excurrent canals, as well as the flagellated chambers (Reiswig 1979*a*; Mackie and Singla 1983; Leys 1995, 1999). Cells are also present, but these are joined to the trabecular reticulum by cytoplasmic bridges that may be plugged with a unique proteinaceous junction, the perforate plugged junction (Mackie 1981; Mackie and Singla 1983). In this manner, glass sponges manage to segregate cytoplasmically distinct regions, while maintaining communication pathways throughout the animal by way of the trabecular tissue and cytoplasmic bridges.

Water flows into the sponge through 10–15 μm diameter pores in the dermal membrane, into large incurrent canals that branch and narrow as they approach the prosopyles of the flagellated chambers. The prosopyles consist of two layers of perforated reticular tissue that form the walls of the flagellated chambers and that permit the passage of water. The branched choanocytes that are embedded in tissue between the prosopyles in the first trabecular layer project with their collars and beating flagella through the perforations in the second layer. Water leaves the chambers through large openings (apopyles) and vents through a series of canals that lead to the central atrium and osculum.

Indirect evidence of signal propagation

Flow recordings from the oscula of glass sponges in both the field and in laboratory aquaria have shown that arrests in feeding current occur spontaneously and in response to either mechanical or electrical stimuli (Lawn et al. 1981; Mackie et al. 1983). Sediment in the incurrent water provides a natural stimulus (see below). Close observation of the dermal membrane during arrests confirm that the pores do not contract (Mackie et al. 1983), and although it is not possible to observe other openings such as the prosopyles, the speed of the arrest (less than 20 s) suggests that cessation of flagellar beating is the most likely mechanism. Such arrests are well known in other ciliary and flagellar filter feeders (Aiello 1974; Mackie et al. 1974).

The use of thermistor flow probes established that arrests are all-or-none and propagate through the sponge at 0.27 $cm \cdot s^{-1}$ (Lawn et al. 1981). The signal can spread in all directions — horizontally and vertically — and pass from a sponge to its bud, causing arrest in the latter even though the atrial chambers are completely separate (Mackie et al. 1983). It seemed likely that the trabecular tissue acts as a single unit for conducting electrical signals through the sponge (Lawn et al. 1981), but early attempts to confirm this with electrical recordings were thwarted by the special nature of the trabecular strands (see next section).

Electrophysiology of glass sponges

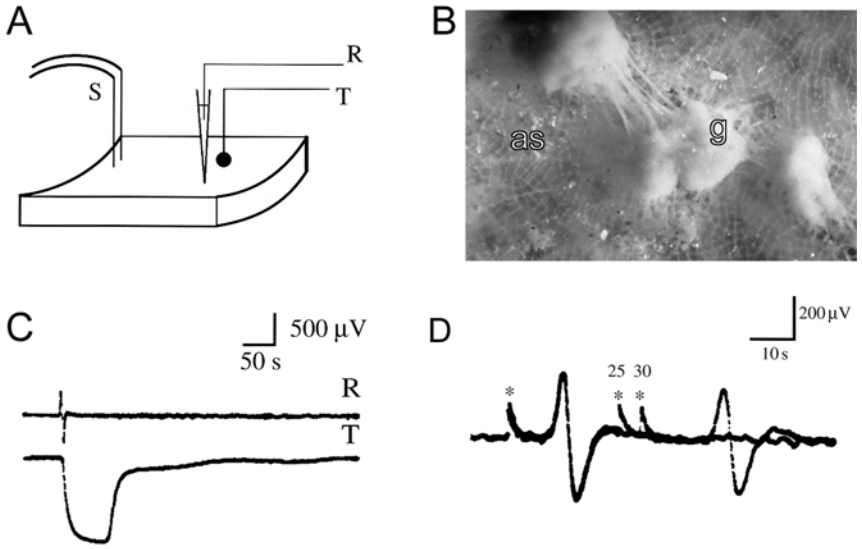
In his 1982 review, Lawn wrote "The sponges are the only major animal group in which electrical activity has yet to be demonstrated."

Until the early 1980s, the major barrier to using electrophysiological techniques to study sponges was the insubstantial nature of sponge tissue. Glass sponges such as *Rhabdocalyptus dawsoni* (Lambe, 1892) consist of thin sheets, perhaps no more than 1 μm thick, draped on a scaffold of spicules "like a three-dimensional cobweb" (Mackie and Singla 1983). The tissue was too fragile to use external recording techniques such as suction electrodes and too thin to use intracellular micropipettes. Even piezoelectric devices designed for incremental movement jolted the micropipette right through the cell rather than lodging it in the cell cytoplasm.

As with many other small and delicate cells, the invention of the patch pipette (Hamill et al. 1981) appeared to provide a relatively easy approach. All that was needed was to lower the pipette on to the cell surface, suck the sponge membrane on to the glass tip, obtain a gigaseal, and watch the data appear on the computer monitor. Unfortunately, it proved remarkably difficult to obtain a "tight" seal between the sponge cell surface and the patch pipette, something that has been attributed to the glycocalyx on the cell surface of cellular sponges (Carpaneto et al. 2003). It took a further 16 years before electrical activity was finally demonstrated in sponges (Leys and Mackie 1997) and a further 4 years before the first patch-pipette data were reported (Zocchi et al. 2001).

The first great breakthrough used the well-known ability of dispersed sponge cells to "clump" together in aggregates. Leys and Mackie (1997) grafted an aggregate of tissue (spherical multinucleate pieces of the sponge) back onto the surface of a slab of body wall from the original sponge (Fig. 4). They found that within 24 h cytoplasm could be seen streaming down the pale yellow strands of tissue which had formed between the aggregate and the sponge surface. There was enough tissue in the aggregate for them to be able to suck a solid plug of material into an 80 μm diameter polyethylene "suction" pipette. Note that aggregates would only

Fig. 4. (A, B) Electrical recording from the glass sponge *Rhabdocalyptus dawsoni*. A stimulus (S) is applied to the atrial surface (as) of a slab of body wall while a suction electrode (R) records from a graft (g) of aggregated tissue; a thermistor probe (T) is used to measure changes in water flow through the nearby body wall. (C) An action potential (R) is recorded immediately prior to arrests in the feeding current (T). (D) Superimposed traces show the effect of the refractory period. With two shocks 30 s apart, the second shock elicits an impulse with a reduced conduction velocity and amplitude, but with shocks 25 s apart the sponge is absolutely refractory and no impulse is generated. The shock artefacts are indicated with an asterisk. (A, C, D from Leys et al. 1999, reproduced with permission of J. Exp. Biol., vol. 202, pp. 1140 (B) and 1142 (A, C, D), © 1999 The Company of Biologists Ltd.)



fuse with body wall from the same specimen (autografts). Allografts placed on body wall taken from another specimen were rejected and "scar" tissue formed around the base of the aggregate.

Upon stimulation at a remote site, a long-lasting biphasic action potential appeared at the external recording electrode. At 11 °C, the action potential lasted about 5 s and propagated at a speed of 0.17–0.3 $cm \cdot s^{-1}$ (Fig. 4C). A flow meter placed at the recording site revealed that the appearance of the action potential at the suction electrode coincided with a cessation of feeding current across the nearby body wall. The absolute refractory period was 29 s and subsequent shocks within 30–120 s of the first produced a response with a lower amplitude and reduced conduction velocity (Fig. 4D).

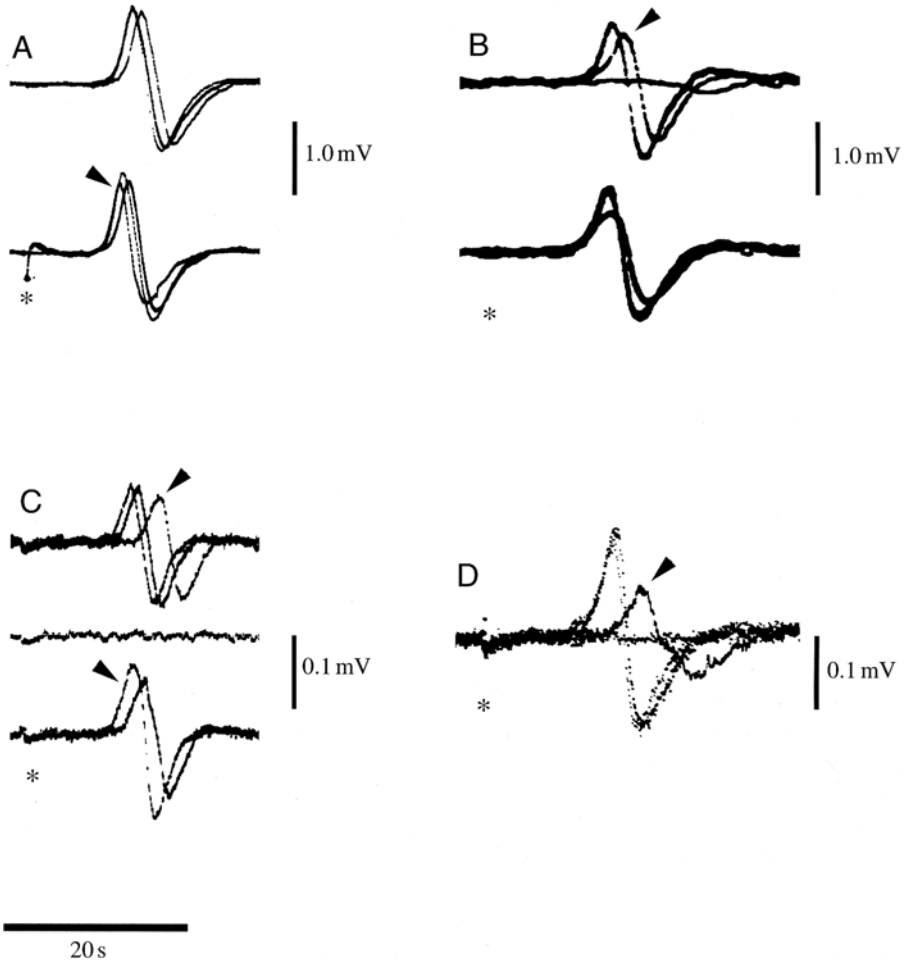
The ability to record an electrical impulse demonstrated that the conduction system was separate from the effector system, as electrical impulses continued to propagate unchanged even when flow had completely stopped. Without this knowledge, it might have been supposed that the arrests in one chamber could spread to the next by some hydromechanical process much as cilia become entrained into metachronal waves in other animals (Gueron and Levit-Gurevich 1999). The electrical recordings show that there really is a separate impulse pathway. Leys and Mackie (1997) have suggested that the electrical activity is conducted through the trabelular syncytium; there are no membrane barriers within these syncytia or between the flagellated chambers that are responsible for generating the feeding current.

Identification of the ionic basis of the sponge action potential was based on the following observations made on the externally recorded impulse recorded in different artificial seawaters (see Leys et al. 1999) (Fig. 5): (*a*) substituting choline chloride for 75% of the sodium chloride in the seawater bathing the preparation, produced only a small reduction in amplitude; (*b*) the amplitude was reversibly reduced by adding 5 $mmol \cdot L^{-1}$ Co^{2+} to the seawater and reversibly abolished by 10 $mmol \cdot L^{-1}$ Co^{2+} or 10 $mmol \cdot L^{-1}$ Mn^{2+}, which are well known as calcium channel blockers; (*c*) adding the organic calcium channel inhibitor nimodipine (12 $\mu mol \cdot L^{-1}$) reversibly blocked the impulse; and (*d*) the potassium channel inhibitor tetraethylammonium chloride (TEA chloride) also produced a reversible block at low levels (1 $mmol \cdot L^{-1}$).

From this data, we conclude that the sponge impulse is a calcium-dependent action potential lasting at least 5 s. It is not clear whether the repolarizing phase follows potassium channel activation as it does in other propagated action potentials because, although TEA blocks the propagated impulse, it does not appear to prolong the action potential. It is possible that the blocking effect comes about because TEA blocks resting potassium channels, depolarizes the sponge syncytia, and inactivates the impulse-generating calcium channels. It seems more likely that termination of the action potential depends upon inactivation of the inward calcium current, as this would account for the refractory period observed during dual stimulus experiments.

It seems likely that the prolonged calcium influx during the action potential provides the signal that brings about flagellar arrest. There is good evidence that changes in the pattern of ciliary beating (arrests or reversals) are triggered by increases in intracellular Ca^{2+} in ciliate and flagellate protozoans (Naitoh and Eckert 1974), as well as in lamellibranch molluscs (Satir 1975), ascidians (Mackie et al. 1974), and ctenophores (Moss and Tamm 1987). Leys et al. (1999) suggest that the arrest of feeding current is a protective response to sediment in the water and guards against the entry of unsuitable materials into the filtration system. The presence of the refractory period ensures that the action potential

Fig. 5. Pharmacology of the action potential in *R. dawsoni*. (A) The delay in time to peak in 50% external Na^+ (top) fully recovered on return to normal seawater; with 25% external Na^+ (bottom), the amplitude of the externally recorded impulse was reduced irreversibly. Sodium chloride was replaced with choline chloride. (B) Co^{2+} (10 mmol·L^{-1}; top) reduced the amplitude of the action potential at 5 min (arrowhead) and blocked it completely after 8.6 min. At 5 mmol·L^{-1} (bottom), Co^{2+} produced a partial block. Control traces before and after Co^{2+} addition fully superimpose in each case. (C) Nimodipine (12 μmol·L^{-1}), a calcium channel antagonist, progressively reduces action potential amplitude (top) until it is completely blocked at 13.3 min (middle). Conduction velocity is also reduced. Recovery (bottom) follows a similar pattern. (D) Tetraethylammonium ions (TEA^+, 1 mmol·L^{-1}) block the action potential after 10 min, with partial recovery after 5 min (arrowhead) and full recovery after 6–7 min of washing in normal sea water. (From Leys et al. 1999, reproduced with permission of J. Exp. Biol., vol. 202, p. 1144, © 1999 The Company of Biologists Ltd.)



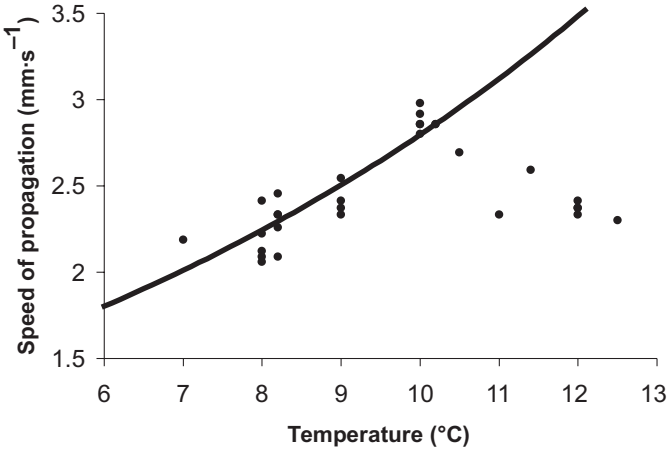
does not go back on itself and generates inappropriate arrests. When shocks are less than 30 s apart, the second shock usually fails to evoke a propagated impulse regardless of shock strength (Fig. 4D). This is the absolute refractory period. With a 30 s shock interval, the response enters the relative refractory period and the action potential is of lower amplitude and of reduced conduction velocity. Overall, this relative refractoriness lasts about 150 s and the conduction velocity follows an exponential trajectory (time constant 22s) during the course of the recovery.

The different conduction velocities during the relative refractory period mean that the pumping activity in the region of the stimulus is likely to be different to that of the flagellated chambers farther away. For example, two stimuli 30 s apart will give a prolonged arrest of pumping in the region close to the stimulus. However, as the impulses propagate farther away from the stimulus, the time delay between them will increase. Not until the stimuli are 150 s apart (i.e., when the relative refractoriness is over) will the impulses maintain a constant separation as they propagate throughout the tissue.

Effect of temperature

In preliminary experiments (S.P. Leys, G.O. Mackie, and R.W. Meech, unpublished data), we have used flow meter recordings to indirectly monitor the conduction of the sponge action potential at different ambient temperatures in the range 6–12.5 °C. The arrest response is unusually sensitive to temperature (see Fig. 6). Below 7 °C, water flow was consistently arrested, but it restarted once the temperature had returned to 10 °C. In other measurements on the same sponge, arrests were reversibly abolished at 12 °C as though impulse conduction was blocked at higher temperatures. Other sponges may continue to arrest at temperatures up to 19 °C (G.J. Tompkins, personal communication).

Fig. 6. Effect of ambient temperature on the speed of propagation in the glass sponge *R. dawsoni*. The conduction velocity was calculated from the delay between the stimulus and the onset of the water arrest 14 mm away. The line through the points shows the slope of the relationship expected for a Q_{10} value of 3. (S.P. Leys, G.O. Mackie, and R.W. Meech, unpublished data.)



In summary, (*a*) feeding arrests in *R. dawsoni* appear to have a limited temperature range, i.e., pumping is abolished below 7 °C and is continuous at 12.5 °C; (*b*) the apparent speed of propagation of the electrical signal is not linearly related to temperature but reaches a peak value at about 10 °C, i.e., there is a temperature optimum; and (*c*) between 7 and 10 °C, the Q_{10} is about 3.

What mechanisms set the temperature optimum in the sponge? We have considered the following four possibilities:

1. Temperature effects on ion channel properties: in the giant neurones of the water snail *Lymnaea* Lamarck, 1801, both calcium activation and the magnitude of the calcium current are strongly dependent on temperature (Byerly et al. 1984). The Q_{10} for the activation rate constant is 4.9 and the Q_{10} for the maximum current is 2.3.
2. Increased intracellular Ca^{2+}: in the demosponge *Axinella polypoides*, an increase in temperature from 14 to 20 °C for as little as 10 min causes a marked increase in the cytoplasmic Ca^{2+} concentration (Zocchi et al. 2001). Intracellular Ca^{2+} ($[Ca^{2+}]_i$) is well known to contribute to the inactivation of L-type calcium channels (see Eckert and Chad 1984), and it is possible that the maintained rise in $[Ca^{2+}]_i$ in *R. dawsoni* brings about a steady reduction in the number of available calcium channels and reduces the speed of conduction. A test of the hypothesis would be to use low levels of abscisic acid or cyclic ADP-ribose (cADPR) to raise $[Ca^{2+}]_i$, or 8-bromo-cADPR as an antagonist (see the "Temperature signaling cascade" on p. 299, as well as Zocchi et al. 2001).
3. Branched structure: the high temperature sensitivity of *R. dawsoni* may arise from the highly branched structure of the trabecular reticulum through which the impulse propagates. Spike propagation through a three-dimensional network with multiple branch points might well be highly temperature sensitive. Westerfield et al. (1978) have studied the temperature sensitivity of conduction failure at axonal branch points in the stellar nerves of *Loligo pealeii* Lesueur, 1821. Action potentials fail to propagate above a critical ratio of post-branch to pre-branch diameters. The value of this ratio is markedly reduced at higher temperatures, an effect that appears related to the width of the action potential rather than to other factors such as threshold, which seems relatively insensitive to temperature (Guttman 1966). Consequently, at higher temperatures, blocks will occur at progressively larger numbers of junction points and the slower conduction velocity at the sponge surface may reflect the increasingly circuitous route taken by the propagating impulse.
4. Temperature sensitivity of ciliary arrest is a property of the choanoderm. We know that pumping is continuously arrested below 6 °C, but it is also possible that the choanocytes pump continuously above 12 °C for reasons unconnected with impulse propagation. For example, if pumping arrests follow increases in $[Ca^{2+}]_i$, a rise in temperature might stimulate the Ca^{2+} pumps in the cell membrane and keep $[Ca^{2+}]_i$ at a low level.

Although points 2–4 above might account for the apparent conduction block at 12 °C, they do not explain why conduction of ciliary arrest appears to have such a high Q_{10} value. The effect of temperature on impulse conduction has been best studied using squid giant motor axons. Most recently, a comparison of propagated action potentials from tropical and temperate squid species has shown that conduction velocity is approximately linearly related to ambient temperature over the range 0–35 °C (Rosenthal and Bezanilla 2002). The Q_{10} values for propagation varied from 1.2 to 2.4, depending on species and temperature range. Hodgkin and Katz (1949) have reported similar findings for a single species of *Loligo* Lamarck, 1798. For conduction in myelinated nerve fibres, Paintal (1965) reports Q_{10} values of 1.8.

A theoretical analysis of the squid axon by Huxley (1959) suggests that the single channel ionic conductances upon which the action potential depends are not very temperature sensitive, but the kinetic processes of activation and inactivation have Q_{10} values of about 3. Huxley (1959) found that beyond a certain point the rate of rise of the action potential does not increase with temperature and he suggested that the membrane time constant was limiting. The rate of repolarization, on the other hand, continued to increase so that the amplitude and duration of the action potential became progressively reduced (see Hodgkin and Katz 1949). At about 33 °C, the repolarization process occurred too early for sufficient current to be generated for propagation (see also Jack et al. 1983). An analytical model (Hunter et al. 1975) confirms that at temperatures near the physiological range the conduction velocity is influenced largely by changes in the rate of rise of the action potential, whereas at higher temperatures the effect of the recovery processes becomes significant. At no stage does an increase in ambient temperature decrease the conduction velocity, which is something that appears to occur in glass sponges (Fig. 6).

Significance of the findings

The propagation of feeding arrests in *R. dawsoni* is greatly affected by small changes in ambient temperature, perhaps because of the sensitivity of the calcium channels upon which the action potential depends (Byerly et al.

1984). Enzymic processes such as phosphorylation markedly affect the open time of L-type calcium channels, and changes in temperature can be expected to produce marked changes in calcium-dependent processes as a result. Sodium-dependent action potentials, such as those in the squid axon (Rosenthal and Bezanilla 2002), are much less temperature sensitive and it may be for this reason that they have been favoured for impulse propagation in most metazoan axons.

The optimum temperature for conduction of feeding arrests corresponds well with the temperature of the water at the collection site (see Pickard 1963). But for specimens of *R. dawsoni* exposed to, for example, arctic waters, optimum conduction might be expected to occur at significantly colder temperatures. It seems possible that the temperature signaling cascade reported by Zocchi et al. (2001) may be involved in this kind of adaptation (see the "Temperature signaling cascade" section below).

Response to natural stimuli — sediment

Arrests of water flow have also been recorded in the cloud sponge, *Aphrocallistes vastus* Schulze, 1887 (Hexactinellida, Hexactinosida, Aphrocallistidae) — one of three species of glass sponge that form massive reefs at 160–240 m depths on the continental shelf of western Canada (Leys and Tompkins 2005). As in *R. dawsoni*, arrests occur in response to mechanical stimuli and sediment, but features of the arrest differ between the two sponges and recovery after arrests appears to be much more robust in *A. vastus* than in *R. dawsoni*. Single doses of a sediment solution (approximately 100 $mg \cdot L^{-1}$), which mimic a disturbance made by fish, cause all-or-none arrests of 1–2 min in duration in both sponges (Leys and Tompkins 2005). The arrests can be of much longer duration in *R. dawsoni*, but the arrests are very brief in *A. vastus*. Furthermore, whereas continued addition of filtered sediment (<25 μm particles) caused several 2–5 min arrest–recovery responses in *R. dawsoni*, in *A. vastus* this treatment caused the pump rate to decline in a stepped manner over a period of 3 min or longer. The gradual decline in pumping could be due to a series of arrest–recovery responses or to the gradual clogging of canals with sediment. When sediment was no longer added, the full pumping rate was only achieved after a series of arrest–recovery responses, as though the animal was flushing itself out. Thus, *A. vastus* is capable of resuming pumping after each arrest regardless of the number of stimuli it receives; *R. dawsoni*, on the other hand, appears to be far more sensitive to sediment stimuli. Field observations using a remote-operated vehicle (ROV) confirm that the sponges have different pumping behaviours in their natural environment (S.P. Leys, personal observation). Fluorescein dye squirted onto sponges on fjord walls at 160 m depth immediately came flowing out of the osculum of *A. vastus* but not of most *R. dawsoni*. Many *R. dawsoni* did not take up the dye even 30 min after the arrival of the ROV.

Temperature signaling cascade

The role of Ca^{2+} as an intracellular messenger in muscle contraction, synaptic transmission, and ion channel activation is well known in the Metazoa, while in Protozoa such as *Paramecium* a rise in intracellular calcium-ion concentration ($[Ca^{2+}]_i$) reverses ciliary beating. Stomatal movement in the guard cells of plants is also connected with $[Ca^{2+}]_i$ oscillations and transients (Allen et al. 2000, 2001). In each of these examples, delivery of the message can be modulated by a large number of different enzymes and is markedly affected by ambient temperature.

The "Ca^{2+} balance" in the cell cytoplasm is not dependent simply on inflows and outflows across the plasma membrane but also on the storage and release of Ca^{2+} at intracellular sites. Nucleotides such as cyclic ADP-ribose (cADPR) promote Ca^{2+} release in a wide range of cells from protists to higher mammals (Lee 1997). cADPR is synthesized from nicotine adenine dinucleotide (NAD) by ADP-ribosyl cyclase (E.C. 3.2.2.5). Zocchi et al. (2001) have examined ADP-ribosyl cyclase activities in a number of different sponge species from both the Demospongiae and the Calcispongiae (Calcarea) (see Table 1). Some ADP-ribosyl cyclases are bifunctional and go on to degrade cADPR to ADP-ribose, but in sponges this hydrolase activity was found to be low with a cyclase/hydrolase ratio of 100 or more. Zocchi et al. (2001) show that in most cases cGDP-ribosyl cyclase (E.C. 3.2.2.5) activity is also low.

Although cADPR (but not cGDPR) acts intracellularly to release Ca^{2+} from intracellular stores, cyclase activity can also be demonstrated at the outer surface of intact cells. In fact, the activity of intact *A. polypoides* tissue is about 500 times higher than that of any mammalian cells (Zocchi et al. 2001). Transport of cADPR into cells has been demonstrated in vertebrate cell lines (Zocchi et al. 2001). Once in the cell cytoplasm, cADPR binds to ryanodine receptors (RyR) on the surface of the endoplasmic reticulum and modulates the release of Ca^{2+} through calcium-permeable RyR channels. In permeabilized *A. polypoides* cells, 10 $\mu mol \cdot L^{-1}$ cADPR rapidly releases Ca^{2+} from ryanodine-sensitive stores (Zocchi et al. 2001).

Zocchi et al. (2001) have observed a maintained increase in ADP-ribosyl cyclase activity in *A. polypoides* after increasing the ambient temperature from 14 to 26 °C for as little as 2 min. There was also an increase in abscisic acid (ABA). Furthermore, low levels of ABA (5–50 $nmol \cdot L^{-1}$) stimulated a 4-fold increase in cyclase activity and an associated increase in intracellular cADPR (Zocchi et al. 2001). Either 50 $nmol \cdot L^{-1}$ ABA or increased temperature produced a marked increase in $[Ca^{2+}]_i$, which was inhibited by prior exposure to the membrane-permeant cADPR antagonist 8-Br-cADPR (20 $\mu mol \cdot L^{-1}$). In summary, heat stress induces an increase in ABA, which activates ADP-ribosyl cyclase and causes a rise in cADPR.

The ABA activation appears to require protein kinase A (PKA; E.C. 2.7.1.37) activity because addition of the kinase inhibitor K252a (1 $\mu mol \cdot L^{-1}$) blocked both the ABA and the temperature-induced increase in cyclase activity. The cell-permeant PKA activator 8-Br-cAMP had the opposite effect, producing an 8-fold increase in cyclase activity in intact cells. In plants, ABA is a phytohormone and it mediates responses to stresses such as cold, drought, and salinity via a cADPR-initiated increase in Ca^{2+}; Ca^{2+}-dependent phosphorylation and dephosphorylation follows (Wu et al. 1997).

The immediate response of *A. polypoides* to heat stress or ABA is an increase in amino acid incorporation, oxygen

Table 1. Cyclase and hydrolase activities (nmol·min^{-1}) of marine sponges (supporting information to Zocchi et al. 2001).

Sponges	ADP-ribosyl cyclase (nmol cADPR·min^{-1}·mg^{-1})	GDP-ribosyl cyclase (nmol cGDPR·min^{-1}·mg^{-1})	cADPR hydrolase (nmol ADPR·min^{-1}·mg^{-1})	*n*
Calcispongiae (Calcarea)				
Clathrina cerebrum (Haeckel, 1872)	2.18	0.11	0.003	4
Demospongiae				
Aplysina aerophoba Nardo, 1843	31.0	1.20	0.12	6
Axinella damicornis (Esper, 1794)	1.51	0.40	0.002	6
Axinella polypoides Schmidt, 1862	1400	23.8	2.52	10
Axinella verrucosa (Esper, 1794)	1.26	0.32	0.002	6
Cliona nigricans (Schmidt, 1862)	32.2	2.24	0.16	6
Petrosia ficiformis (Poiret, 1789)	1.23	0.06	0.003	6

Note: Mechanically dissociated sponge cells were lysed with 0.01% Triton-X100 and incubated at 14 °C. Mean values, calculated from the indicated number of experiments, are shown. The SD was ≤20% of the mean in all cases.

consumption, and filtration rate, but this is a short-lived effect and is followed by long-term depression (Zocchi et al. 2003). Interrupting the temperature signaling cascade with the cell-permeant cADPR antagonist 8-Br-cADPR or with the $[Ca^{2+}]_i$ chelator EGTA-AM prevented both effects. Zocchi et al. (2003) also observed temperature-induced and ABA-induced stimulation of respiration and filtration in experiments on *Chondrosia reniformis* Nardo, 1847 (Demospongiae, Hadromerida) (Zocchi et al. 2003).

Intact *A. polypoides* cells produced a slow increase in $[Ca^{2+}]_i$ in response to extracellular cADPR, and addition of purified cyclase increased sponge respiration by 40%, as well as stimulating filtration (Zocchi et al. 2003). ADP-ribosyl cyclase activity in the seawater surrounding control sponges increased 20-fold after 30 min at 24 °C and there was an associated increase of the cADPR concentration in the seawater from 0.2 ± 0.06 to 4.02 ± 0.6 nmol·L^{-1}. Thus, cADPR may act as an ectohormone that coordinates the metabolic activity of the whole branching structure. It may be that the heat signaling pathway is only present in some cell types and a coordinated response in the whole animal depends on the external dissemination of cADPR.

Significance of the heat signaling pathway

Axinella polypoides is confined to the Mediterranean Sea, where it is considered an endangered or threatened species (Annex II of the *Protocol Concerning Specially Protected Areas and Biological Diversity in the Mediterranean Sea* adopted in the Barcelona Convention in 1996; revised in the Bern Convention, 1998). It lives at a depth of between 30 and 40 m, where the ambient temperature will be close to that at the surface and is likely to range from about 14 to 26 °C during the course of the year. This 12 °C temperature range will markedly affect the metabolism of the animal unless it undergoes some form of cyclical acclimation.

The increase in oxygen consumption induced by heat stress can be inhibited by 8-Br-cADPR (Zocchi et al. 2003) and is unlikely to arise from a simple increase in rates of reaction. Nevertheless, it seems counterproductive because any stimulation of metabolism will only serve to increase body temperature still further. A long-term inhibition of metabolism seems a more protective reaction, although it is likely to be equally lethal if the increase in ambient temperature is maintained for an extended period.

Under natural conditions, the slow changes in $[cADPR]_i$ and $[Ca^{2+}]_i$ during seasonal cycles of ambient temperature might be involved in acclimation. The acclimation might decrease enzyme concentrations at higher temperatures to compensate for increased activity or it might modify the cytoplasmic environment and thereby modulate enzymic activity. In addition, alternative sets of enzymes with different temperature optima might be expressed and utilized (Somero 2004). In each case, the change in ambient temperature must send some signal to the cell nucleus and alter gene expression. Calcium handling by the cell is likely to be so affected by changes in ambient temperature that changes in $[Ca^{2+}]_i$ might be a suitable signal; the work of Zocchi et al. (2001) tends to confirm this. They suggest that temperature signaling in the sponge depends on a specific heat and mechano-gated cation channel in the cell membrane (see below).

In plants, a brief period of low temperature triggers an increase in $[Ca^{2+}]_i$ and induces gene expression (Henriksson and Trewavas 2003). Increases in $[Ca^{2+}]_i$ trigger transcription factors in a number of different plants including maize (Sheen 1997) and *Arabidopsis* Heynh. (Braam 1992). In the mammalian cortex, calcium binding by calmodulin triggers several nuclear transcription factors, including the cAMP response element binding protein (Dolmetsch et al. 2001). Zocchi et al. (2001) have already drawn attention to the links between the "cell learning" during acclimation and the learning exhibited by neurons in the genus *Aplysia* L., 1758 involving activation of the cAMP response elements (or CREs) that initiate gene transcription.

Effect of mechanical stress

Zocchi et al. (2001) report that repeated centrifugation also produced cyclase activation in *A. polypoides*. They attribute this to the effect of mechanical stress on mechanosensitive channels in the cell membrane. By analogy with the mechano-activated channel TREK-1, they suggest that low levels of arachidonic acid (see Patel et al. 1998) mimics the effects of mechanical stress. In confirmation of their prediction, there was a 4-fold increase in cyclase activity and a 6-fold increase in the concentration of ABA in the presence of 50 μmol·L^{-1} arachidonic acid (AA). TREK-1 is also heat sensitive (Maingret et al. 2000), so TREK-1-like channels could respond to both heat and mechano-stress.

Ion channels

Patch-clamp experiments have revealed the presence of several different classes of ion channels in sponge cell membranes (Zocchi et al. 2001; Carpaneto et al. 2003). This is an impressive achievement, as the technical difficulties involved in making any form of electrophysiological recording from sponge material are considerable (see Mackie et al. 1983). To make single channel recordings, it is first necessary to make a high-resistance "gigaseal" between the recording pipette and the cell surface (Hamill et al. 1981). In many cells under cultured conditions, the surface is sufficiently "clean" that this is relatively easy. However, investigators have had to resort to treatment with proteolytic enzymes in some cells to clear the surface of cell "debris". Carpaneto et al. (2003) suggest that the difficulty with forming gigaseals on sponge cells is associated with the presence on the cell surface of a complex glycocalyx-containing acid mucopolysaccharides, glycoproteins, and proteoglycans. Long-chain fatty acids and sterol residues in the cell membrane may also increase the fluidity of the cell membrane and further increase the difficulty in forming gigaseals.

To obtain the gigaseals required for patch-clamp recordings from *A. polypoides*, Carpaneto et al. (2003) found that it was essential to include trivalent cations in the seawater bathing the cells. Once the seal had been formed, the trivalent ions (0.01–1 mmol·L^{-1} La^{3+} or Gd^{3+}) could be washed away. The effect of the trivalent ions is unknown, but Carpaneto et al. (2003) suggest that they may stiffen the cell membrane. Any toxic effect they may have is difficult to assess, but it is notable that the sponge cells all appear to have positive resting potentials rather than the negative value expected under the whole cell recording conditions used by Zocchi et al. (2001).

Single channel recordings from cell-attached patches reveal the presence of a mechano-activated channel with a single channel conductance of 13 pS and a reversal potential near –40 mV (Zocchi et al. 2001), which is close to the potassium equilibrium potential. In mammalian sensory neurons, the heat-sensing receptor is a mechano-gated K^+ channel with two-pore domains, which may be activated by low levels of arachidonic acid (TREK-1) (Maingret et al. 2000). When whole cell clamped sponge cells were exposed to arachidonic acid (100 µmol·L^{-1}), the increased conductance obtained had a positive reversal potential (Zocchi et al. 2001) that was somewhat different to the expected potassium equilibrium potential (–68 mV). If the arachidonic acid does activate the stretch-activated K^+ channels, it must also activate other channels as well.

Zocchi et al. (2001) also used whole cell clamped sponge cells to examine the effect of heat stress on the cell membrane. They observed that an outwardly rectifying conductance induced by a temperature jump from 14 to 26 °C could be blocked by 100 µmol·L^{-1} Gd^{3+}. The effect was at least partially reversible. Whether the inhibition is fully reversible is not known because, as explained above, the conductance can only be measured after the cells have been exposed to trivalent ions. The reversal potential of the heat-activated conductance was even more positive than the resting potential.

In summary, it appears that in addition to the large (~70 pS) K^+-selective channel identified by Carpaneto et al. (2003), sponge cell membranes have a small stretch-activated channel (13 pS) with a reversal potential close to the K^+ equilibrium potential. There is also a heat-activated conductance with a positive reversal potential and a conductance activated by arachidonic acid. Differences in reversal potential mean that it is not clear whether or not the AA activates the heat-sensitive conductance. Zocchi et al. (2001) suggest that the heat-sensitive cation conductance which they have identified acts as a sensor for heat stress and sets off the entire temperature signaling cascade by stimulating the production of APA. The mechanism by which this happens has not been established.

Other coordination systems

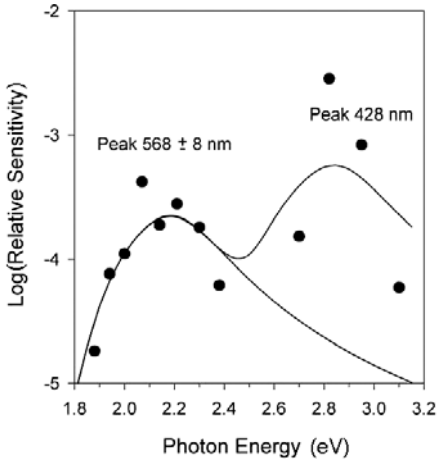
Coordinated ciliary beat in larval photoreceptors

With few exceptions, most sponge larvae are ciliated propagules 50–5000 µm in length (Maldonado and Bergquist 2002). In parenchymellae larvae, which are common to many demosponges, the bulk of the outer surface is formed by a ciliated columnar epithelium, but the anterior and posterior poles of the larva may be bare (Maldonado and Bergquist 2002). In many parenchymellae, especially long cilia arise from cells that form a ring around the posterior pole. The larvae typically swim unidirectionally — although some can reverse and others spend equal amounts of time swimming in both directions — rotating in either a right-handed fashion or a left-handed fashion (Leys and Degnan 2001). Many sponge larvae respond to photic stimuli; the majority swim away, but some swim towards the light, while others change phototaxis during their larval life, which may range from 12 h to several weeks (Wapstra and van Soest 1987). The force for forward movement is generated by the small cilia whose beat is thought to be entrained into metachronal waves by viscous coupling (Aiello 1974; Sleigh 1974; Gueron and Levit-Gurevich 1999). Directionality is thought to be conferred by irregular beating of the long posterior cilia (Leys and Degnan 2001; Maldonado et al. 2003).

The parenchymella larva of *Reniera*, a tropical reef flat sponge, is negatively phototactic for the first 6–12 h of larval life, a behaviour that may steer the larva towards the shaded regions on the reef for settlement (Leys and Degnan 2001). A sudden increase in light intensity causes the long posterior cilia to rigidly straighten; the reverse occurs when light intensity suddenly decreases. Furthermore, as the larva rotates, cilia on two halves of the posterior ring are exposed to different light levels. Preparations in which the posterior half of the larva was placed posterior side up in a dish of seawater with light shone from one side showed that as the cilia reach the lit side they straighten and as they continue around to the shaded side they bend over the posterior pole (Leys and Degnan 2001). It is suggested that the individual responses of the posterior cilia to light allows a coordinated response of the whole larva. Maldonado et al. (2003) confirm that this behaviour is found in other haploscerid and dictyoceratid sponge larvae. However, they also show that distal protrusions of the posterior ciliated cells — regions that contain the pigment granules which shade the cilium — are connected to one another by cytoplasmic bridges, which could be involved in coordinating movement of the cilia.

Although neither the exact location of the photoreceptor nor its molecular basis is known, the spectral sensitivity of

Fig. 7. The action spectrum of the ciliary response to light in the larva of the genus *Reniera*, a marine demosponge. The first peak at 568 ± 8 nm indicates that the photoreceptor has the characteristics of rhodopsin. (K. Foster and S.P. Leys, unpublished data.)



the ciliary photoresponse has been characterized (Leys et al. 2002). The action spectrum for the genus *Reniera*, which shows a peak relative sensitivity at 450 nm and a smaller peak at 600 nm, has been interpreted as reflecting the absorbance spectra of flavins or carotenoids, rather than that of a rhodopsin (Leys et al. 2002). However, the spectrum is not unlike that of the unicellular alga *Euglena* Ehrenberg, 1838 (Wolken 1971), which has been shown to be a rhodopsin after reanalysis using photon energy rather than wavelength of light (Gualtieri et al. 1992); photon energy gives a better description of the light energy affecting the photoreceptor than wavelength of light. Similar reanalysis of the data from the sponge *Reniera* sp. also reveals two distinct rhodopsin peaks at 428 and 568 nm (K. Foster and S. Leys, unpublished data) (Fig. 7).

Closure of ostial pore fields in *Phorbas amaranthus*

An unusually rapid contraction system was reported by Reiswig (1979*b*) in the cellular sponge *Phorbas amaranthus* Duchassaing and Michelotti, 1864 (Poecilosclerida, Hymedesmiidae). *Phorbas amaranthus* belongs to a group of sponges in which incurrent pores (ostia) are restricted to circular fields or "pore fields". Such fields are 1–4 mm in diameter and are surrounded by a corona containing elevated spicules and a contractile sphincter. The closure of ostia and of sphincters lining incurrent and excurrent canals is not unusual among cellular sponges as has been discussed above. However, the response by *P. amaranthus* is unusual because of the speed at which the pore field can close and the rapid coordination among pore fields via "inter-field" tissues. In situ and laboratory experiments showed that contraction of the field and closure of the corona occurred within 0.25–0.5 s of stimulation; relaxation was slower at 10–3000 s. The rate of contraction of the sphincter and corona (0.2–16 $cm \cdot s^{-1}$ estimated for 1–4 mm diameter fields) is faster than any known event in cellular sponges. That the response is global, and thus propagated, is also suggested from field guides for divers that say that *P. amaranthus* responds to touch by slowly closing its pores (Humann 1992).

Mechanical properties of the extracellular matrix in *C. reniformis*

Although we have specifically excluded from this review a consideration of the complex cell-matrix interactions that take place during the growth and development of sponges, we make an exception in the case of the extracellular matrix of *C. reniformis*. This is based on speculations that changes in the tensile strength of collagen in *C. reniformis* is a coordinated — albeit slow — behavioural response of the whole animal to its environment (Bonasoro et al. 2001). *Chondrosia reniformis* is a common sponge in shallow waters world wide, but species in the Mediterranean are unusual in that they generate attenuated outgrowths that can reach downward or horizontally 10 cm or more away from the parental body (Bonasoro et al. 2001). Various interpretations of this phenomenon have been offered, from asexual reproduction by budding (Bavestrello et al. 1998), passive responses to environmental change (Sàra and Vacelet 1973), or localized locomotion, possibly preceding asexual reproduction (Bond and Harris 1988).

A detailed ultrastructural study of the sponge tissue, its ectosome (cortex) and endosome (choanosome), revealed only localized cytological features that could be associated with plasticization of the sponge (Bonasoro et al. 2001). No contractile cells or myocytes were found; instead spherulous cells — cells with spherulous inclusions — were implicated as the principal cell type occurring where changes in sponge texture were noticed.

To test whether the changes in stiffness is under direct physiological control, Wilkie et al. (2004) observed the effect of different ions on the flexural stiffness of slabs of ectosome and choanosome. They found that the collagenous mesohyl stiffens marginally in solutions containing 10 times normal calcium levels (100 $mmol \cdot L^{-1}$) and stiffens substantially in 0.38 $mol \cdot L^{-1}$ $CaCl_2$, which is isotonic with seawater. The effect of Ca^{2+}-free seawater was to soften the tissue irreversibly, while the addition of a calcium chelator increased stiffness drastically. The calcium channel blockers Co^{2+} and Mn^{2+} produced a similar stiffness increase. Finally, use of a detergent (or freezing) to lyse the cells dramatically stiffened the mesohyl, as did soaking slabs of tissue in an extract of lysed cells. Wilkie et al. (2004) conclude that mesohyl cells contain a stiffening factor that, when released, changes the passive mechanical properties of the extracellular matrix.

The results suggest that the stiffening and easing phenomena are reminiscent of mutable collagenous tissue (MCT) in echinoderms, which can change mechanical properties in a time scale that ranges from less than 1 s to a few minutes (Wilkie 1996, 2002; Wilkie et al. 2004). The mesohyl of *C. reniformis* has many similarities to the MCT of echinoderms — sensitivity to changes in calcium concentration and treatments that cause cell lysis, organization of fibrillar collagen, and the probable ability to change interfibrillar connections (Wilkie 1996).

Summary

In this review we have concentrated on the coordinated response of sponges to specific environmental stimuli, the

main difficulty being that the nature of the receptor, signal, signal pathway, and effector are unclear in many cases.

Perhaps the best example of behavioural coordination in sponges, because so many of the elements are understood, is the directed swimming in larvae mediated by ciliary photoreceptors. It is true that the exact location of the photoreceptor remains unknown, but the photopigment has been partially characterized and the role of the long posterior cilia in determining direction of swimming seems well established. All that remains is to determine the link between the two.

In the glass sponges, the conducting pathway is relatively well understood. The effectors are the pumping choanocytes and the link can at least be hypothesized as being due to an increase in internal calcium. The difficulty here is to identify the natural stimulus and the sensory receptor. Perhaps the most important environmental variable in an animal designed for filtering water for food and reproduction is the presence of particulate matter that may damage its delicate pumping apparatus. Thus, the stimulus is identifiable but what is the receptor? We can only speculate that there must be stretch-activated ion channels in the incurrent canals.

The glass sponges are unusual in depending on an electrical signal for the coordinated response to danger and we might wonder why. Perhaps the system of calcium action potentials that the sponges inherited from the Protozoa is not suited to environments where there is a wide range of temperatures. So, although this electrical signaling system might be satisfactory for the deep ocean or for rapid responses to local mechanical stimuli, any species in shallower waters might need to evolve a whole new signaling system to cope with diurnal changes in ambient temperature.

Alternatives include the use of already-present chemical pathways to provide a mechanism for slow adaptation, and the use of slowly propagated contractions that are probably dependent on the local release of hormones and "transmitter" chemicals or on the local mechanical interaction of one cell on the next.

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