CHAPTER ONE

The Physiology and Molecular Biology of Sponge Tissues

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Contents

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Abstract

Sponges have become the focus of studies on molecular evolution and the evolution of animal body plans due to their ancient branching point in the metazoan lineage. Whereas our former understanding of sponge function was largely based on a morphological perspective, the recent availability of the first full genome of a sponge (*Amphimedon queenslandica*), and of the transcriptomes of other sponges, provides a new way of understanding sponges by their molecular components. This wealth of genetic information not only confirms some long-held ideas about sponge form and function but also poses new puzzles. For example, the *Amphimedon* sponge genome tells us that sponges possess a repertoire of genes involved in control of cell proliferation and in regulation of development. *In vitro* expression studies with genes involved in stem cell maintenance confirm that archaeocytes are the main stem cell population and are able to differentiate into many cell types in the sponge including pinacocytes and choanocytes. Therefore, the diverse roles of archaeocytes imply differential gene expression within a single cell ontogenetically, and gene expression is likely also different in different species; but what triggers cells to enter one pathway and not another and how each archaeocyte cell type can be identified based on this gene knowledge are new challenges. Whereas molecular data provide a powerful new tool for interpreting sponge form and function, because sponges are suspension feeders, their body plan and physiology are very much dependent on their physical environment, and in particular on flow. Therefore, in order to integrate new knowledge of molecular data into a better understanding the sponge body plan, it is important to use an organismal approach. In this chapter, we give an account of sponge body organization as it relates to the physiology of the sponge in light of new molecular data. We focus, in particular, on the structure of sponge tissues and review descriptive as well as experimental work on choanocyte morphology

and function. Special attention is given to pinacocyte epithelia, cell junctions, and the molecules present in sponge epithelia. Studies describing the role of the pinacoderm in sensing, coordination, and secretion are reviewed. A wealth of recent work describes gene presence and expression patterns in sponge tissues during development, and we review this in the context of the previous descriptions of sponge morphology and physiology. A final section addresses recent findings of genes involved in the immune response. This review is far from exhaustive but intends rather to revisit for non-specialists key aspects of sponge morphology and physiology in light of new molecular data as a means to better understand and interpret sponge form and function today.

Key Words: Functional morphology; sponge physiology; signalling molecules; cell biology; developmental regulatory molecules

1. Introduction

Sponges are unusual animals which, due to their ancient heritage, can shed light on fundamental questions such as the origin of multicellularity, the evolution of tissues, signaling pathways, body polarity, and coordination systems. Sponge body plans are so different from those of other animals that it is difficult to compare even basic features, yet their molecular framework—which was revealed with the first full sponge genome from *Amphimedon queenslandica* (Srivastava *et al.*, 2010), as well as from transcriptomic and other gene data from other sponges (Nichols *et al.*, 2006; Harcet *et al.*, 2010)—shows that they have a very similar complement of genes and gene pathways to those in other animals. Although at the time of this review we have only one full sponge genome on which to base our comparison, it appears that some of the molecules that may underlie important morphological innovations in animals might be missing in sponges. Examples are the absence of sodium channels from the *A. queenslandica* genome and yet their presence in choanoflagellates, the closest unicellular ancestor to animals (Liebeskind *et al.*, 2011), and the presence of most components of the post-synaptic density in the sponge genome, including the ligand binding sites, but the absence of the ligands (Sakaraya *et al.*, 2007; Alie and Manuel, 2010). This, together with the suggestion that ctenophores might be basal to sponges (Dunn *et al.*, 2008), implies that sponges may not be 'witnesses to the pre-history' of animal systems as was suggested earlier by Pavans de Ceccatty (1974a) but may instead have lost complex animal characters, even neurons, however, unusual that might seem. Increasingly, it becomes clear that we need to revisit the morphology and physiology of sponges in order to better understand the relationship between gene and protein, and between cell and tissue function.

Sponges are suspension feeders, with a body plan designed to process as much water as possible, or needed, for feeding and respiration. Thus, the evolution of the sponge body plan can be seen as being guided by the fluid environment: sponges in low food environments find ways to process more water (use passive flow, e.g. Maldonado and Young, 1998; Leys et al., 2011) or eat different food as in the case of carnivorous sponges (e.g. Vacelet and Boury-Esnault, 1995); sponges in high food environments, or which have formed symbioses with microbes, may need to process less water (Weisz *et al.*, 2008).

Since the sponge is essentially a piping system, whose pressure differences are essential to generate the proper flow rates over the filter, at the cellular level modifications to a canal filtration system require constant readjustments, and therefore, cells and tissues should have the flexibility to adjust and modify the canal system in response to changes in flow to maintain the correct pressure differential. This dependence on flow penetrating all regions of the animal means that regional specialization is less evident than in other animals. In carnivorous sponges, where the tissues are freed up from that dependence, regionalization is often overt with distinct spatial separation of food capture regions and reproductive regions, separation of male and female gametes; where they are still present, choanocyte (feeding) chambers are also segregated to a small region of the sponge body. But all sponges have some regionalization of tissues, and in many groups, skeletal types are regionalized, while in others, reproductive structures are separated from choanosomal feeding tissues. Despite the commonality of the aquiferous system, there are many ways of building it, and the millennia during which sponges have been doing this have generated enough variations on the theme that interpretation of regions as tissues that carry out a common function requires a good understanding of the cell type, origin, and function. Today, molecular expression data can help fill in the picture.

Simpson's 'The cell biology of sponges' (1984) is an invaluable comprehensive resource on the structure and function of sponges. It covers a period of research that used ultrastructure to study the fine details of sponge cell function, associations, and lineage, and highlights several areas of uncertain knowledge. Some of these areas we now know more about by the use of new techniques, in particular scanning electron microscopy and immunocytochemistry, but also X-ray microtomography, new physiological approaches, and new molecular data. In order to understand what genes and their products do in sponges, we need to have a good idea of sponge cell biology and physiology. The aim of this review is to re-evaluate aspects of sponge structure and function upon which molecular and physiological data have shed new light. We are selective in our approach and do not intend to try to match Simpson's scope, but rather touch on topics in which new advances have been made with respect to our understanding of sponge tissues, tissue function, differentiation, and patterning. We first provide an overview of sponge body

organization and then discuss new interpretations of the cell biology and physiology of sponges, highlighting, where relevant, new knowledge yielded by the study of gene expression.

2. General Organization of Sponges

2.1. Gross morphology

Sponges can be massive and spherical, thin and encrusting, tall and tubular, and many variations on these forms. In many texts, one will see sponges described as having ascon, sycon, and leucon grades of structure, terms which refer to the organization of choanocyte feeding chambers in the body wall, ascon, a simple tube lined by choanocytes; sycon, a tube with fingers lined by choanocytes; and leucon, with canals leading to sperhical or ovoid chambers lined with choanocytes (Ruppert *et al.*, 2004). However, since only Calcarea have all three grades (Fig. 1.1A), and there are many variations of leuconoid form in demosponges, hexactinellids, and homoscleromorph sponges (Fig. 1.1B–D), it is not a very useful way of thinking about the body structure. To understand how sponges actually function, we need to get away from this view, and yet at the same time work toward understanding what the implications are of having the variation of grades of structure in the Calcarea.

Body form has been most useful for taxonomists, but for the sponge, it is clearly a matter of feeding and excretion and, therefore, water flow. In the case of hexactinellids, massive incurrent canals penetrate deep into the choanosome which can give a syconoid appearance (Fig. 1.1B), and in the case of homoscleromorph sponges, incurrent canals appear as depressions in the outer surface and penetrate deep into the wall of the sponge, also giving a syconoid-like, 'sylleibid' appearance in section (Fig. 1.1C). In leuconoid forms, where the body wall is filled with many choanocyte chambers (the choanosome), across the body wall some incurrent canals are short, while the respective excurrent canals are long, and vice-versa for chambers deeper into the body wall (Fig. 1.1D): that is to say choanocyte chambers lie in parallel, never in series. The grades of structure were originally considered grades of evolutionary complexity (reviewed in Manuel *et al.*, 2002) presumably since the leuconoid sponges would have less issue with filtering the same water. That is, in asconoid Calcarea, flow is unidirectional down the length of a tube lined by potential feeding cells, so it appears that the same flow would be used by all cells; in syconoid Calcarea, the water current goes into separate, albeit large, chambers, and therefore, fewer cells would compete for the same water flow; in leuconoid sponges, the flow would be divided up, each portion to be filtered by only very few cells. However, if it were more valuable to be able to capture a larger item, we might view this in reverse. In this case, larger

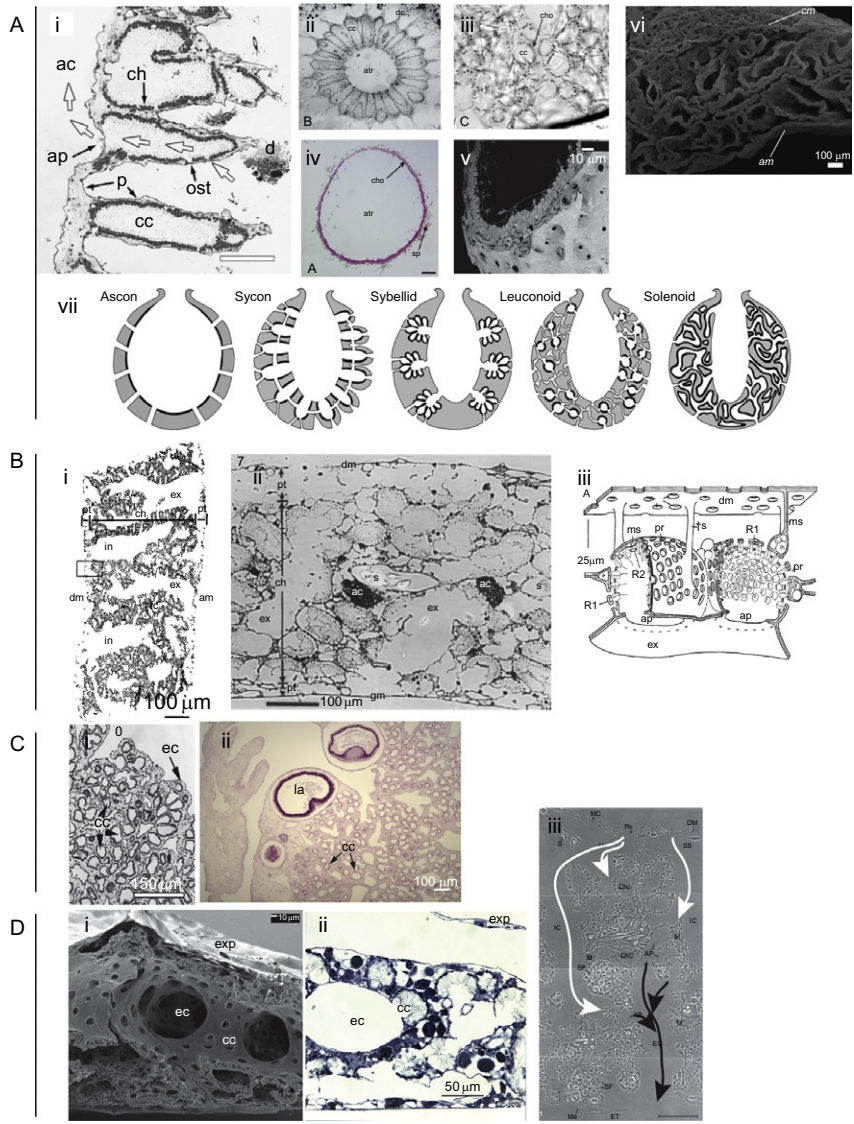

Figure 1.1 Body structure of sponges. A. Calcarea (i) *S. coactum* longitudinal section, (ii) *S. raphanus* cross section, (iii) *Leuconia johnstoni* transverse section, (iv) *Leucosolenia variabilis* cross section, (v) *Leucosolenia eleanor* cross-section (SEM), (vi) *Leucascus roseus* transverse section (SEM), (vii) diagrams illustrating the different body wall structures found in Calcarea. (i, Eerkes-Medrano and Leys, 2006; ii-iv, from Manuel, 2009; v, Leys, unpublished; vi, vii, from Cavalcanti and Klautau, 2011). (B) Hexactinellida. (i) Cross section of the body wall of *Rhabdocalyptus dawsoni* from a composition of images of wax sections, (ii) Cross-section and (iii) diagram of the chamber structure in *Farrea occa* (i, Leys, 1999; ii, iii, Reiswig and Mehl,

chambers would allow several cells to cooperatively capture a protist in syconoid sponges. In asconoid sponges, having a large single tube might even encourage the entry of unwary intruders which could be disabled using toxins, allowing the sponge to feed on something much larger than is normally able to enter via the incurrent flow.

2.2. Body wall overview

Sponges have quite diverse body constructions. We focus here on the haplosclerid-type morphology, many aspects of which can be generalized to other groups. The body wall consists of an outer layer (cortex), a canal system that brings water into chambers where the pumping and feeding cells are (the choanosome), and lacunae (epithelial-lined spaces) or canals that bring water to converge on the excurrent vent or osculum (the atrial tissue) (Fig. 1.2).

The outermost tissue has been called the dermal membrane because early researchers thought the epithelium in all sponges was syncytial (Wilson, 1907). Electron microscopy has confirmed that only hexactinellid sponges have syncytial tissues; however, in some species, boundaries of surface cells are hard to see even by scanning electron microscopy, so it is understandable that they were difficult to see by light microscopy (Fig. 1.2C). The dermal membrane (as used by earlier workers) consists of a thin single layer of cells, pinacocytes (see Section 3.1) that lies on a collagenous middle layer, the mesohyl. It is worth noting that some authors used the term dermal membrane to refer to the entire outer region of the sponge—whether a single exopinacoderm, or an exopinacoderm and endopinacoderm with fine mesohyl inbetween—and others used it to refer to all pinacocytes lining the surface and canals of the sponge. Recent workers no longer use this term and refer instead only to the 'exopinacoderm' (Boury-Esnault and Rützler, 1997).

The nature of the sponge surface layers varies greatly. In some sponges, the exopinacoderm covers a cortex—a distinct rind that consists of particular cell types, spicule types, and occasionally foreign material embedded into the tissue (e.g. Teragawa, 1986). In other sponges, the skin is formed by a thin single layer of exopinacocytes covering a massive collagenous mesohyl, while in others, the skin forms a 3-μm-thin layer over an equally meagre mesohyl, which is sandwiched by another epithelium, the endopinacoderm (or in syconoid and asconoid sponges, the choanoderm). Under the exopinacoderm lies the first of

1991). (C) Homoscleromorpha. Cross-sections of (i) *Oscarella kamchatkensis*, Gazave *et al.*, 2010; (ii) *Corticium candelabrum* (cc, choanocyte chamber, la, larva) (courtesy of A. Riesgo). (D) Demospongiae. (i) Scanning EM and (ii) cross section of *Ephydatia muelleri* (ec, excurrent canal; cc, choanocyte chamber; exp, exopinacoderm) (iii). Cross-section of *Haliclona elegans* showing the different paths water might take through the sponges. (i and ii, Elliott and Leys, unpublished; iii, Langenbruch and Scalera-Liaci, 1986.

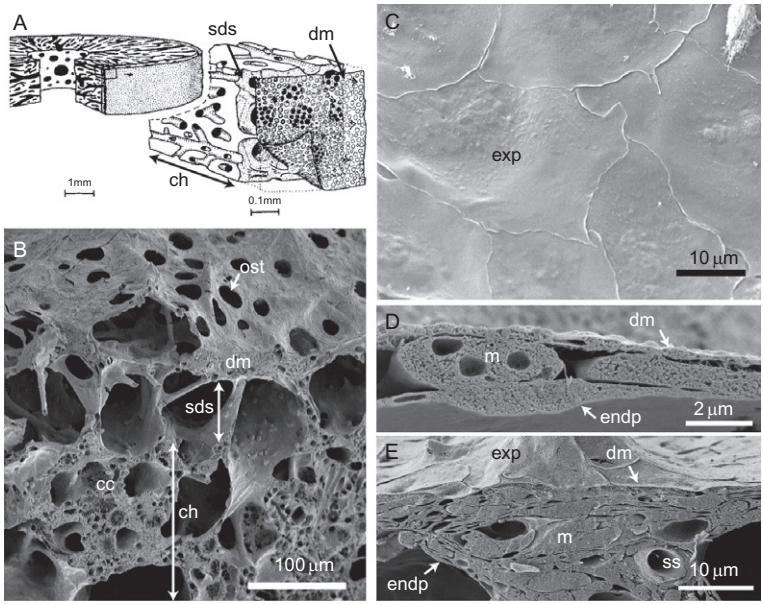

Figure 1.2 Regionalization in sponges. (A) Diagram of a cross-section and cutaway showing the aquiferous system of *Haliclona permollis*. (B) Fractured specimen of *Neopetrosia problematica* showing the dermal tissues with surface dermal membrane (dm) and ostia (ost), subdermal space (sds) and choanosome (ch) with choanocyte chambers (cc). (C–E) Aspects of the dermal membrane. (C) Exopinacoderm (dermal membrane proper) of *Ephydatia muelleri*, SEM. (D) Fracture through the dermal membrane of *E. muelleri*, SEM. (E) Fracture through the dermal membrane and mesohyl of *Haliclona mollis*. (Dm, dermal membrane; sds, subdermal space; ch, choanosome; cc, choanocyte chamber, exp, exopinacoderm; end, endopinacoderm; m, mesohyl cell; ss, spicule space) (A from Reiswig, 1975; B–E, Leys and Adams, unpublished.)

a set of epithelial-lined spaces into which the water first enters. These may be lacunae (small epithelial-lined spaces) or a single large cavity (called the subdermal cavity). Water passes either directly into the subdermal cavity or via short epithelial-lined canals into the lacunae and from there into the choanosome (Fig. 1.2). The choanosome consists of canals and chambers together with spaces that may exist around each chamber. The structure of chambers and the way they are attached to each canal are characteristic of genera and families of sponges (Boury-Esnault *et al.*, 1990).

Like the subdermal tissues, the subatrial tissues may have lacunae in which the excurrent water collects before venting out of the osculum, or they may simply consist of converging excurrent canals, as they increase in diameter before merging at the base of the osculum. The excurrent canals and lacunae may be important for monitoring and adjusting the rate of flow of water through the animal, either by contracting or by providing a reservoir for water before and after the feeding chambers.

2.3. Cells, tissues, and regionalization

The sponge body has functional regions defined by specific tissues and the cell types in them. Tissues are defined after Hyman (1940) as being formed by one or more type of cell and organized so as to form a functioning unit (whole). Most sponge larvae also have well-differentiated regionalized tissues, and in the larva, there is the additional function of motility and direction finding, for which regions are specialized (Maldonado and Bergquist, 2002), but the types of tissues and cells present in the larva for the most part take their names (and we infer their function) from those in the adult. In the adult, sponge tissues include the choanocyte epithelium (choanoderm), including cells supporting the choanocyte chambers; the pinacocyte epithelium (pinacoderm), including dermal tissues, incurrent, and excurrent canals; the skeletal and collagenous support tissues; and the osculum. Here, we focus on the structure and formation of two epithelial tissues only, the choanoderm and pinacoderm. We do not address skeletal tissues or cells of the mesohyl.

3. The Choanoderm Epithelium

3.1. Overview of the aquiferous system

Flow through the sponges is generated by the beating of many thousands of flagella per cubic millimetre of choanosomal tissue. The beat of the flagellum generates a low pressure at its base, drawing water toward and through the collar and from there up along the length of the flagellum away from the cell body (Larsen and Riisgård, 1994). Many choanocytes in a choanocyte chamber, with collars and flagella facing toward the exit of the chamber, generate a current of water through the collar into their bases, and from there into the chamber and out of the chamber exit. The manner in which choanocytes are organized into chambers (how many there are, how many openings there are between them, what the dimensions of the collar slits are) and the manner in which chambers are organized at the end of incurrent canals have been studied in many sponges (e.g., Langenbruch, 1983, 1988; Langenbruch *et al.*, 1985; Langenbruch and Scalera-Liaci, 1986; Langenbruch and Weissenfels, 1987; Langenbruch and Jones, 1990; Boury-Esnault, 2006). The dimensions of the fine incurrent and excurrent canals are features that determine the precise resistance over the collar slit in the choanocyte chamber (Larsen and Riisgård, 1994) (Fig. 1.3). The pressure drop must slow the flow sufficiently for choanocytes (or pinacocytes at the entrance to chambers) to phagocytose food which is primarily bacteria 1/10 to ½ the size of the choanocyte.

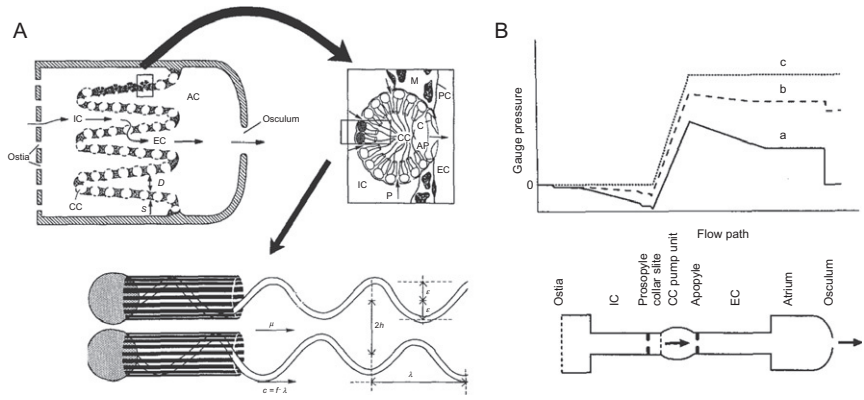

Figure 1.3 (A) Diagram illustrating the basic sponge design with pumps (choanocyte chambers) in parallel. Water enters through ostia to incurrent canals, through the choanocyte chambers and out excurrent canals to the osculum. (B) Schematic of the pressure head variation along a flow path from ostium to osculum, illustrating the pressure at low (a), medium (b) and high (c) resistance along the path. From Larsen and Riisgård, 1994.

3.2. Choanocyte structure

Choanocytes are very small cells (2–10 μm in diameter) with a flagellum that is surrounded by a 'collar' of microvilli, finger-like actin-filled extensions that arise from the cell membrane (Fig. 1.4). Bidder (1895) described choanocytes from Calcarea (*Sycon raphanus* and *Sycon compressa*) as being barrel-shaped cells, like 'full corn-sacks standing side by side in a granary', with a collar that is almost a perfect cylinder. Later, their structure and function were studied in live specimens and in ultrathin sections of spongillids using electron microscopy by Kilian (1952), Rasmont *et al.* (1958), and Rasmont (1959), and each account has confirmed the presence of common components including a fibrillar material that links microvilli of the collar.

The collar microvilli rise about 2/3 the height of the flagellum. Choanocytes may be squat or tall and may lie on a thin or a thick collagenous mesohyl, appearing in the former case to be suspended in the inhalant canal system, and in the latter to be embedded in the mesohyl (see Section 3.3). That choanocytes adhere to one another or to endopinacocytes with sealing junctions should be considered the typical situation because tissues fixed with ruthenium red showed no tracer passes between choanocytes or between pinacocytes and choanocytes into the mesohyl (Adams, 2010).

Between choanocyte microvilli stretch fine strands of proteoglycan ('glycocalyx') mesh (Fig. 1.4G, H, I). Already in the late 1800s, Bidder (1895) saw in sections of osmium-fixed tissues a 'film of some other substance' uniting the collar microvilli; later in ultrathin sections of spongillids, Rasmont (1958) refers to 'bridge-like structures' between the microvilli. The mesh is

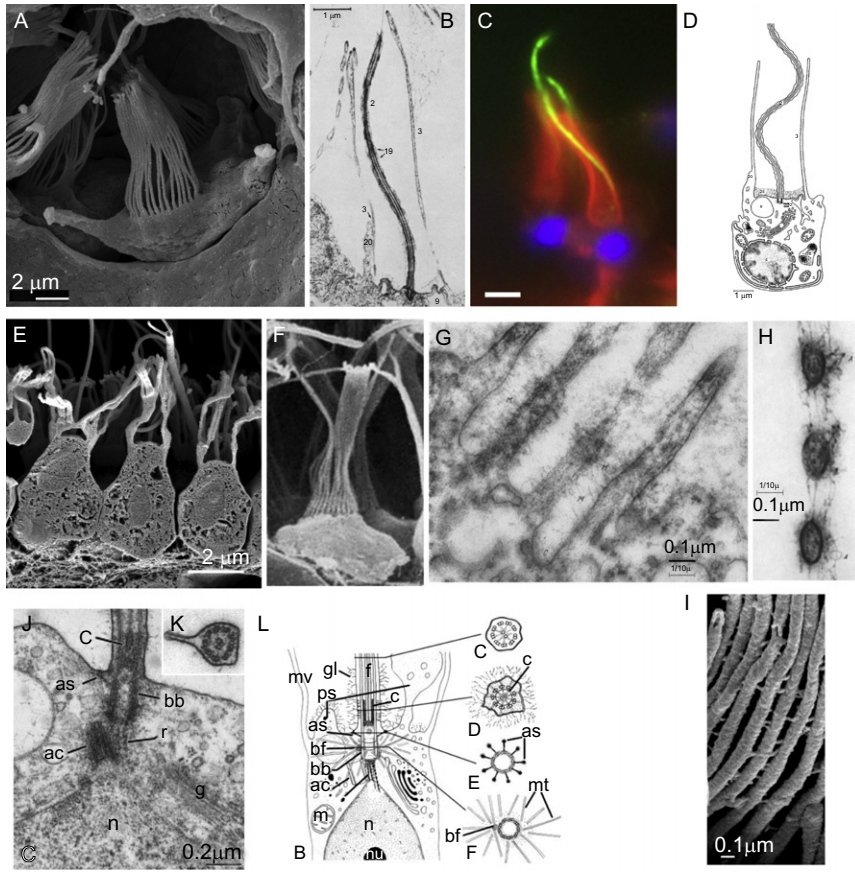

Figure 1.4 Choanocyte structure. (A) Choanocyte from *Ephydatia muelleri* (SEM); (B) Longitudinal section through the flagellum of *E. fluviatilis* TEM; (C) Fluorescence image of two choanocytes from *Ephydatia muelleri* showing actin labelled with Bodipy Fl phallacidin (green) and tubulin labelled with anti-alpha tubulin (red); nuclei are labelled with Hoechst 33342 (blue); (D) Diagram of a choanocyte from Brill, 1973); (E) Choanocytes in *Sycon coactum*, Calcarea; (F) Choanocyte from *Oscarella lobularis*, Homoscleromorph; (G–I) Fibrillar proteoglycan mesh between microvilli of *Spongilla lacustris*, G and H, and between microvilli of *Sycon coactum*, I. (J–L) Structure of the flagellum and basal body of a choanocyte in *Halisarca dujardini*. (A, C, E, Leys unpublished; B, D, Brill, 1973; F, Boury-Esnault et al., 1984; G, H, Fjerdingstad, 1961; J–L, from Gonobobleva and Maldonado, 2009).

better preserved by glutaraldehyde/osmium fixation and has subsequently been found both outside and inside the collar in many sponges. Electron micrographs show a mesh on the collar in *Spongilla lacustris* (demosponges, Fjerdingstad, 1961), *Sycon* (Calcarea, Ledger 1976 in Simpson 1984), and *Farrea occa* and *Rossella racovitzae* (hexactinellids, Reiswig and Mehl, 1991;

Koestler in Leys *et al.*, 2007). The mesh forms spaces of approximately 40 square nanometres, and importantly, this is the smallest space (and greatest surface area) through which the water actually passes in the sponge.

In freshwater demosponges, a layer of glycocalyx also forms what looks like a gasket—a structure filling in the space between neighbouring collars approximately 2–3 μm above the cell's surface (Weissenfels, 1992). In some marine demosponges (e.g. *Haliclona mollis*), a set of cells forms a layer similar to that gasket, sealing the spaces between each of the collars (Adams, 2010) (Fig. 1.5A–C); it is possible this layer of cells is what is referred to as Sollas' membrane (Bidder, 1895), an enigmatic structure whose existence has been difficult to confirm. This gasket is well known in glass sponges (Hexactinellida) where it is called the 'secondary reticulum', a layer of syncytial tissue that rises from the primary reticulum to surround the collar of each collar body (Mackie and Singla, 1983). The secondary reticulum may play a role in channelling flow through the collar, as inferred from a study that watched the passage of micorospheres through sponge fragments using video microscopy (Wyeth *et al.*, 1996; Wyeth, 1999). In *Ephydatia fluviatilis*, it is suggested to function as a 'one-way valve' to prevent back flow of water (Langenbruch and Weissenfels, 1987), so in both cases, perhaps it has a similar function.

3.3. Organization of choanocyte chambers—Terminology

There are some 50–200 choanocytes in demosponge chambers (Reiswig, 1975; Rasmont and Rozenfeld, 1981), 200–300 in a hexactinellid chamber (Leys et al., 2011), and up to 1000 in a calcareous syconoid chamber (*Sycon*, Leys, personal observation). Openings to choanocyte chambers are called 'pyles' (orifice Gr.), and, therefore, entrances are called prosopyles (L. forward of) and exits, apopyles (Gr. away from). Prosopyles can be formed by pseudopodial extensions of choanocytes arranged so as to create a hole

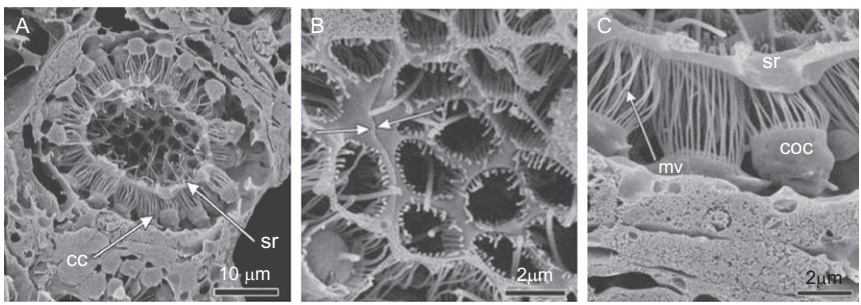

Figure 1.5 Gasket of cells surrounding the collars in choanocyte chambers of *Haliclona mollis*. (Adams, 2010). (A) Low and (B,C) high magnification views of the cells that envelop the collar microvilli.

(choanocytic prosopyles), or by pinacocytes that contact the choanocytes and form something like a porocyte (pinacocytic prosopyle). Apopyles are larger and, therefore, formed by several cells, called either 'cone' cells or apopylar cells (De Vos *et al.*, 1990), which are interpreted to arise from choanoblasts during early chamber formation (Weissenfels, 1981). Unlike choanocytes, however, which are flagellated, cone cells are ciliated and the cilium beats in a slow whip-like fashion away from the chamber (Fig. 1.6) (see Section 4.2). Some sponges lack cone cells and instead several endopinacocyte-like cells form a sieve plate at the exit of the chamber (e.g. *Tethya wilhelma* Nickel, personal communication; SPL, unpublished data).

3.4. Choanocyte function—Feeding

The action of the choanocyte flagellum in generating a low pressure to draw water through the collar is well described by Simpson (1984) from Van Tright (1919) and Kilian (1952). Though the basics are quite clear, exactly how the water moves through the collar and chamber is not actually known. For example, while it is understood that water follows a pressure gradient generated by the choanocyte, as noted above, it is suggested that there must be one-way valves to prevent water from flushing the other way under changes in external pressure (Vogel, 1978). Though potential one-way valves (apopylar cells and a glycocalyx mesh between the collars) have been identified by Langenbruch and Weissenfels (1987), it would be difficult to test how they work. Also, whether all water must pass through the collar filter or whether there are bypass canals is likewise not known. Some authors suggest that bypass structures exist, for example, to compensate for pressure changes that occur during rhythmic contractions (Nickel *et al.*, 2006), and other authors, using corrosion casts to study the aquiferous system, also find links between incurrent and excurrent canals (Bavestrello

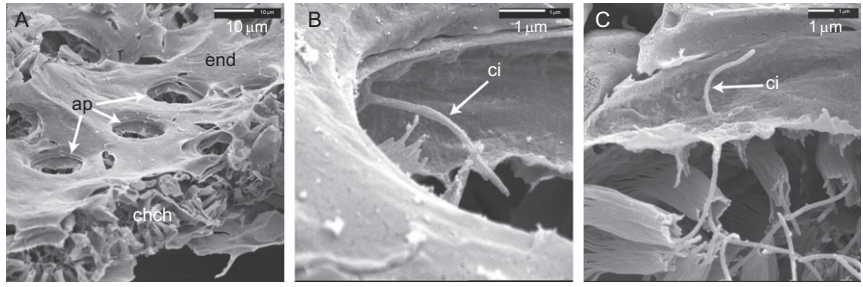

Figure 1.6 Apopyles and apopylar (cone) cells. (A) Several apopyles (ap) open into an excurrent canal in *Ephydatia muelleri* (end, endopinacoderm; chch, choanocyte chambers). (B, C) Apopylar cells with cilia (ci) which are approximately 4 µm long and show the characteristic bending of ciliary motion. (Leys, unpublished).

et al., 1988). However, since using corrosion casts may cause tearing or rupture of the canal system, doubt remains as to whether such bypasses exist, especially when it is considered that these would generate leakage in the pressure system that the choanocyte filter depends upon. Feeding studies which show greater than 95% retention of bacteria < 1 μm by the sponge do not support the presence of bypass canals in all sponges (Pile *et al.*, 1996; Yahel *et al.*, 2003, 2007).

Food capture occurs at choanocytes, prosopyles, and incurrent canal epithelia (Kilian, 1952; Weissenfels, 1976; Imsiecke, 1993; Leys and Eerkes-Medrano, 2006); even exopinacocytes have been shown capable of uptake of latex beads (Willenz and Van de Vyver, 1982), so epithelial cells in general must be capable of phagocytosis. Generally however, bacteria and other picoplankton are captured by pseudopodia at the choanocyte, and once in that cell, are transferred to amoebocytes for digestion and transfer of food to other cells in the sponge. The transfer of food between choanocyte and amoebocyte has been shown by video microscopy by Imsiecke (1993), and between amoebocyte and other cells in a suite of studies by Willenz and colleagues (Willenz and Van de Vyver, 1984, 1986; Willenz *et al.*, 1986).

3.5. Choanocyte differentiation and turnover

Choanocytes are one of the better-understood cells in sponges in terms of their origin, function, and differentiation potential. They generate flow, capture food, and can differentiate into other choanocytes, spermatocytes, and even oocytes. Other studies suggest that they can even become archaeocytes, and in this sense, they are a type of stem cell (Funayama *et al.*, 2010). In cases where sperm arise from choanocytes, usually whole populations of choanocytes in chambers undergo this modification, multiplying in number, losing the collar, packaging the nucleic material, and modifying the cell body to form spermatic cysts (Boury-Esnault and Jamieson, 1999; Maldonado and Riesgo, 2008). Therefore, choanocytes are at the same time dispensable cells and have the potential to re-differentiate into gametes.

In sponges hatched from gemmules or from aggregates of dissociated cells, choanocytes develop from archaeocytes (Buscema *et al.*, 1980), but during development from larvae, they arise from ciliated epithelial cells (Amano and Hori, 1996; Leys and Degnan, 2002). In each instance, the collar develops secondarily: when choanocytes develop from archaeocytes, they first develop the flagellum and then later the collar (Weissenfels, 1981); when choanocytes develop from ciliated epithelial cells of the larva, the cilium is withdrawn into the cell (engulfed), its axoneme is disassembled into tubulin dimers, and then it is reassembled as a flagellum and the collar is formed (Leys and Degnan, 2002) (Fig. 1.7). In larvae that already have choanocytes (or in glass sponges, collar bodies rather than choanocytes), the collar is lost at metamorphosis and is only formed again after the choanocytes of the small existing chambers move to new positions and form new chambers

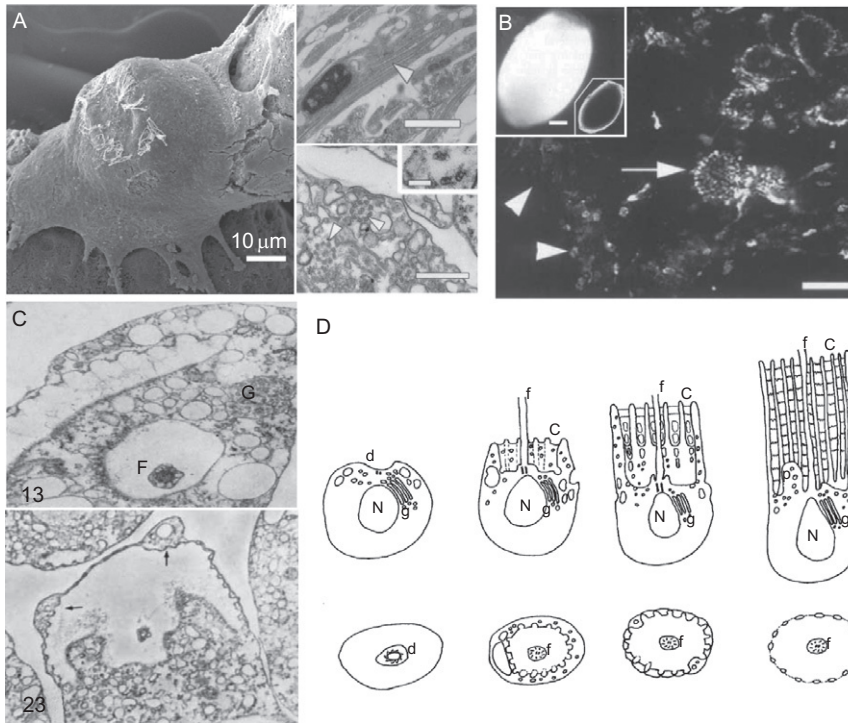

Figure 1.7 Differentiation of choanocytes. (A) Loss of cilia from the metamorphosing larva of *Amphimedon queenslandica* and their disassembly in cells of the post-larva. (B) Fluorescently labelled ciliated cells of the larva transdifferentiate into choanocytes (arrows) of the juvenile sponge. (C, D) Collar formation in *Tetilla serica*: a tube becomes gradually separated into microvilli. (A, B, Leys and Degnan, 2002; C, D, Watanabe, 1978b).

(Leys, personal observations). An excellent account of collar formation comes from Watanabe (1978a) who showed that in *Tetilla serica*, new choanocytes form the collar as a membranous tube (essentially a pseudopodial extension) which, once it reaches a 2–3 μm in height, divides into the fingers that become microvilli (Fig. 1.7). Choanocytes can, therefore, dedifferentiate and re-differentiate easily—they are fairly plastic.

Funayama et al. (2005a) took advantage of the feeding behaviour of choanocytes to find a molecular marker for the choanocyte cell lineage. They fed the sponge fluorescent plastic microspheres (latex beads), and once these were captured by the sponge, they dissociated the sponge tissue and via fluorescent cell sorting isolated the cell fraction that contained the fluorescent beads. A gene that was expressed highly in mRNA isolated from that fraction was *annexin,* and via *in situ* hybridization its expression in choanocytes and choanocyte-committed archaeocytes was confirmed (Funayama et al., 2005a). *Annexin* has many roles in cell adhesion, cell

motility, endocytosis, and vesicle transport, and so its specific expression in choanocytes may reflect the role of those cells in nutrient uptake and transport (Funayama et al., 2005a).

The high turnover rate of choanocytes has been known for some time, and in one instance, it has been suggested that spent choanocytes are sloughed off several times a day (De Goeij *et al.*, 2009). Measurements of the choanocyte cell cycle range from 20 h for *Hymeniacidon synapium* (Shore, 1971) to 15 h for *Baikalospongia bacillifera* (Efremova and Efremov, 1979) and 5.4 h for *Halisarca caerulea* (De Goeij *et al.*, 2009). The latter estimate based on the number of BrdU positive cells in sections of labelled sponges may be on the short side; mammalian intestinal epithelial cells turnover every 3–60 days (Karam, 1999). Regardless of the precise rate, clearly, choanocytes are replaced frequently compared to other cells in the sponge body, and this has important implications. Firstly, it means there is a population of stem cells that replaces them and this appears to be archaeocytes committed to the choanocyte lineage. In contrast to pinacocytes, choanocytes are continually dividing, as can be shown by BrdU staining of dividing nuclei (De Goeij *et al.*, 2009; Funayama *et al.*, 2010) and the labelling of archaeocytes that are committed to be choanocytes as well as choanocytes themselves with the stem cell marker Piwi (Funayama *et al.*, 2010). Secondly, it implies that either choanocytes are cheap to make or that pumping is a costly activity and that these cells are destroyed or are easily damaged. The small size of choanocytes might reflect the need to be able to replace them rapidly at a minimal expense. Rasmont and Rozenfeld (1981) determined that based on the volumes of each cell type, in *E. fluviatilis*, a single archaeocyte could divide into at least 30 choanocytes.

Determining the cost of production of a choanocyte would greatly help us understand the exact cost of pumping for the sponge. It has been estimated that the cost of pumping amounts to 1% of metabolism (Riisgård *et al.*, 1993; Larsen and Riisgård, 1994). However, recent measurements of oxygen consumption in a sponge that went from not pumping to a maximum pumping rate suggest that the cost of pumping is up to 30% of metabolism (Hadas *et al.*, 2008), and a new study of filtration in glass sponges (Leys et al., 2011) suggests this may be due to the resistance generated by the fine dimensions of the glycocalyx mesh on the collar, which was not taken into account earlier. More data on the dimensions of passages through sponges, and of oxygen consumption in a variety of sponges, will greatly help refine our understanding of energy expenditure and the cost of filtration for sponges.

3.6. Control over flow

Sponges can control the flow through their aquiferous system, but they do this in different ways. In glass sponges (Hexactinellida), the flagella stop moving ('arrest') when the sponge is irritated by sediment or there is damage

to the tissue. This happens by the spread of an action potential through the syncytial tissues (see below); the flagella only start to beat again after the membrane potential has reset. Other sponges are cellular and as far as we know are not able to spread action potentials and changes in flow never occur within the timeframe that would suggest electrical activity. In those sponges, the flagella beat without arrest for hours on end or until 'conditions are unfavourable' (Grant, 1825).

Grant was probably one of the first to observe this incessant pumping of water and gives a nice account of using a microscope and the reflection of candle light to watch the flow pour from the osculum for up to 5 h without change in rate (Grant, 1825). Cellular sponges can contract their canals and close their ostia and oscula, so flow can be slowed and stopped gradually, but in general, it is understood that the flagella of choanocytes—as do cilia—beat relentlessly, even if each flagellum might beat at a slightly different rate than the others. Observations of flagella beating in intact living tissues (damaged dissociated choanocytes have a highly variable beating pattern) indicate that the flagella beat in sinusoidal waves in a single plane and each choanocyte flagellum in a chamber beats in a slightly different plane to the others (Jones, 1964; Kilian and Wintermann-Kilian, 1979; Wyeth *et al.*, 1996; Wyeth, 1999). Jones (1964) notes that in the calcareous sponge, *Leucosolenia*, the beat varies in frequency but is usually too fast to be measured; the same is true for freshwater demosponges (Kilian and Wintermann-Kilian, 1979); the beat eventually slows and stops after some hours (Kilian, 1952; Jones, 1964). According to Kilian and Wintermann-Kilian (1979), however, in *E. fluviatilis*, some flagella do occasionally stop beating. While slowing and arrest after several hours of observation can be attributed to damage (light, tearing, lack of oxygen or flow under the microscope slide), it is unclear what the mechanism of arrest of an individual flagellum would be.

So far, only glass sponges (hexactinellids) are known to be able to arrest the flagella in an instant (< 30 s), and they can do this because they have syncytial tissue which, upon a mechanical or electrical stimulus to the sponge (a strong prod, or damage to the tissue), propagates electrical signals throughout the entire sponge body (Leys and Mackie, 1997). The action potential would cause calcium to enter the collar bodies, and the rise in intracellular calcium would arrest the flagellum (Leys *et al.*, 1999). The flagella beat resumes only once calcium is pumped out of the cell body. In demosponges, homoscleromorphs, and calcareous sponges, no electrical (rapid) signalling mechanism is known. If flagella were to stop, as they do in hexactinellids, this implies that there must be control over calcium levels in individual choanocytes and it is unclear how that would happen except during contraction of the choanosome. All cellular sponges have been known to be able to contract their tissues slowly in response to poor water quality, mechanical or chemical irritation, or as a spontaneous rhythm (e.g. Parker, 1910; McNair, 1923; Prosser *et al.*, 1962; Emson, 1966; Prosser, 1967; De Vos and Van de Vyver, 1981;

Weissenfels, 1990; Nickel, 2004; Elliott and Leys, 2007), and it has now also been shown that the lyssacine hexactinellid *Oopsacas minuta* can also carry out very slow contractions of the entire body (Nickel, 2010). Contractions are thought to be coordinated by calcium waves that travel through the tissues because contraction amplitude is reduced in the absence of calcium and abolished in the absence of calcium and magnesium (Elliott and Leys, 2010). In other animals, calcium waves are propagated by a repeated cycle in which a trigger (e.g. mechanical irritation) causes a rise in intracellular calcium $[Ca^{2+}]_i$ which causes release of molecules (e.g. glutamate, NO, ATP, or cAMP; Elliott and Leys, 2007; Elliott and Leys, 2010) that bind receptors on neighbouring cells triggering a rise in $[Ca^{2+}]_i$ in those cells, and so on until all the cells in a region have experienced a calcium rise (reviewed in Nickel, 2010). During a strong contraction, even whole choanocyte chambers contract and choanocyte collars and flagella are so compressed that they project out of the exit opening (apopyle) of the chamber (Elliott, 2009); at that point, the motion of the flagella is also noted to have stopped (Weissenfels, 1990). Water may also be slowed from moving through canals by the contraction of sphincters formed by cells in canals. Reiswig (1971) showed that reduced oscula diameter correlated with reduced pumping velocity in *Tethya crypta*, and constriction of atrial canals corresponded with cessation of pumping in *Verongia gigantea*.

Control over the flow may be necessary to prevent collapse of the canals due to unequal pressure in the incurrent and excurrent canal system. If the incurrent canals clog, then the choanocyte pumps will be unable to bring water into the chambers, or if the osculum is torn or any part of the excurrent or incurrent canal system is opened by wounding, the pressure drop across the choanocytes in the chamber presumably will be lost. The sponge must have mechanisms to sense fine changes in the pressure across the entire system and adjust the dimensions of canals by contractions or remodelling. To repair the system, changes must occur in the space of minutes. We might, therefore, expect sensory cells in different regions of the aquiferous system, as well as the ability of the sponge tissues, to have positional information to determine which regions are incurrent and which are excurrent so as to be able to consistently remodel according to this directional body plan.

4. The Pinacoderm Epithelium

4.1. Pinacoderm description and overview of function

The main function of an epithelium is compartmentalization, to control the secretion and absorption of water and the transport of chemicals, proteins and ions between body regions. Epithelia also detect and ward off infection (Van de Vyver, 1970; Müller and Müller, 2003), and provide structural support (Teragawa, 1986), especially when out of water as would be the

case for intertidal sponges. In Hexactinellida, epithelia are syncytial: a single unbroken 1-μm-thin sheet of tissue called the trabecular reticulum covers the entire sponge. As these were described in some detail previously (Leys *et al.*, 2007), they will not be covered here. In 'cellular' sponges (demosponges, calcareous, and homoscleromorph sponges), epithelia are formed by a single layer of squamous (flat) cells that adhere to one another with a short overlap of their edges (Fig. 1.8). The slight nature of this epithelium coupled with the idea that cells can move readily into and out of the epithelium has led to the view that sponge epithelia are tenuously held together; but imaging of sponge epithelial cells *in vivo*, physiological experiments on their sealing and secretory ability, and studies of the molecules

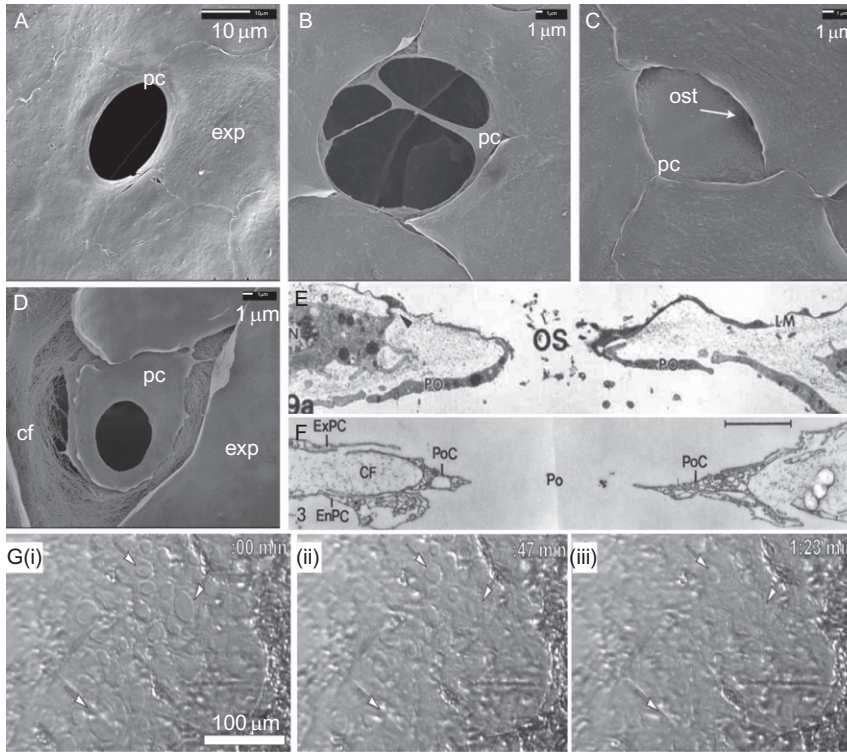

Figure 1.8 Dynamics of opening and closing of ostia. (A–D) Porocytes in *Ephydatia muelleri* showing A, B, open and C, closed and largely hidden under the exopinacocytes. (D) Contraction of exopinacocytes during fixation reveals the collagen layer on which pinacocytes lie and to which pinacocytes attach (pc, porocyte; exp, exopinacocyte; ost, postium; cf, collagen fibers). (E, F) Cross-sections through porocytes show a similar structure in E, *Dysidea etheria* and F, *Haliclona elegans*. (G, i–iii) video images of the ostia closing in the dermal membrane of *Ephydatia muelleri*. (A–D, Leys, unpublished; E, Teragawa, 1986; F, Langenbruch and Scalera-Liaci, 1986; G, Elliott and Leys, 2007).

present in sponges shows that despite their apparent simplicity, established epithelia in sponges can and do form effective barriers (Adams *et al.*, 2010).

4.2. Pinacocytes—Terminology

The sponge surface is formed by a flat cell type called a pinacocyte (literally 'pavement' cell and pinacoderm, a pavement epithelium). A good description of the pinacoderm is given by Simpson (1984) and De Vos *et al.* (1991). Pinacocytes are thin (often 1 μm thick except at the nucleus) broad cells (up to 20 μm in diameter) that form a squamous sheet. In many sponges, pinacocytes are T-shaped, with the nucleus in a cell body that projects down into the collagenous middle layer. Pinacoderms line the sponge surface and canals and also line the internal cavity and canals of the larvae of freshwater sponges.

At least six types of pinacocytes can be identified by morphological or positional differences. Pinacocytes forming the outer surface of the sponge are exopinacocytes, those on internal surfaces (on canals or lacunae) are endopinacocytes, and those that form the base of the sponge and provide the attachment to a surface and which can secrete a massive calcareous skeleton (e.g. Gilis, 2011) are distinguished as basopinacocytes. Furthermore, the polarity of the aquiferous system defines the prefixes given to pinacocytes before (pro) and after (ap) the feeding chambers, hence prosopinacocytes and apopinacocytes (Boury-Esnault and Rützler, 1997). As more gene expression data become available, with genes labelling particular regions of a sponge, it is increasingly important to use terminology that accurately defines location. Differences might be expected, for example, in the populations of potassium channels or glutamate receptors on incurrent and excurrent canal epithelia.

4.3. Cilia and flagella—Function and location in the sponge

An interesting and little discussed feature of sponge pinacocytes is that they can be ciliated (Boury-Esnault and Rützler, 1997); Simpson refers to the cilia as flagella (Simpson, 1984, p. 52). There is little (if any) difference in the ultrastructure of cilia and flagella and often both organelles are referred to by a single term (usually cilium) (Lindemann and Lesich, 2010). However, cilia and flagella have fundamentally different patterns of movement—cilia beat in a whip-like manner with active and recovery strokes (or are non-motile, sensory, organelles), whereas flagella beat in sinusoidal waves, and this has been traced to differences in the domains of proteins involved in forming the axonome (Linck, 1973). Sponges are one of the few animals that have somatic cells (choanocytes) with flagella; flagella are also found in the flame cells of Platyhelminthes, but it seems that most other metazoans have cilia rather than flagella on somatic cells. Choanocytes can differentiate into sperm, which also possess flagella. In contrast, the sponge larval epithelium

is formed by ciliated cells, which beat with a whip-like motion in metachronal waves, like the ciliated epithelia of other invertebrate larvae; at metamorphosis of the larva, however, these ciliated cells dedifferentiate, resorb or lose the cilium, and migrate inwards, where they re-differentiate into choanocytes with a flagellum and a collar (Amano and Hori, 1996; Leys and Degnan, 2002). Not all sponge larvae retain their ciliated cells for use as choanocytes. In *E. fluviatilis*, the ciliated epithelial cells are engulfed by amoeboid cells which then spread out to form the new pinacocytes (Wielsputz and Saller, 1990), but since choanocytes are already formed in freshwater sponge larvae presumably, the ciliated epithelial cells are no longer necessary to make new choanocytes at metamorphosis. It is interesting that only freshwater sponges and hexactinellids have choanocyte chambers in the larva; in both cases, these are ready to be used once the osculum has formed after metamorphosis. The two types of sponges share little in common except perhaps living in low nutrient habitats. If nutrients are limited, perhaps it is more advantageous to be equipped to feed immediately upon metamorphosis rather than use up energy to reshuffle epithelial cells and organelles into new locations.

As the sponge forms new canals after metamorphosis, ciliated pinacocytes arise on newly formed canal epithelia (SPL personal observation). Therefore, pinacocytes must be able to differentiate cilia, but whether all pinacocytes can do this, or whether certain archaeocytes are committed to the ciliated lineage and differentiate into ciliated pinacocytes is not yet known. In demosponges, endopinacocytes in the canals may have one cilium or a pair of cilia, for example, in *Hippospongia communis* and *Tethya aurantium* (Simpson, 1984, p. 52; Vacelet *et al.*, 1989; Boury-Esnault *et al.*, 1990, figs. 23 and 24) or *Ephydatia muelleri* (Elliott, 2009), but all endopinacocytes that form the inner lining of the osculum are ciliated (in all sponges observed so far) with one to four cilia per pinacocyte (Ludeman, 2010; Leys, personal observation). Cilia also occur on the apopylar cells which form a 'gasket'-like exit to chambers (see Section 3.3). Many authors refer to these as flagella, but in *E. muelleri*, where chambers and canals can be observed live in thin areas around the periphery of the animals, while the flagella of choanocytes beat in sinusoidal waves, the organelles on the apopylar cells beat like cilia in a whip-like manner (SPL personal observation). Furthermore, using the fluorescent vital dye FM 1-43, which is taken up by channels in the cilium membrane, and using phase contrast microscopy to watch their motion cilia in the canals and osculum can be located and watched, and these do not 'beat' at all but simply vibrate as if in the flow of water (SPL and D. Ludemann, unpublished observations).

In homoscleromorph sponges, all endopinacocytes are ciliated, each with one cilium, and in most species so are exopinacocytes (Boury-Esnault *et al.*, 1984; Maldonado and Riesgo, 2007). But no study has examined whether these are motile or non-motile, nor if motile, how they beat. In Calcarea, no

ciliated pinacocytes have ever been reported, and no cilia have been found on any part of the syncytial trabecular reticula or dermal membranes of hexactinellids.

4.4. Pinacoderm: Role in sealing and osmoregulation

In his studies of contraction mechanisms in sponges, Prosser (1967) found that marine sponges have an ionic gradient across the tissues, inward sodium, and outward potassium, such that he anticipated the membrane potential would be −30 to −65 mV. Freshwater sponge pinacocytes have a low osmolarity (12–15 MOsM) and possess contractile vacuoles which actively excrete water to the outside as external solute concentration falls (Brauer, 1975). These data together suggest that for both marine and freshwater sponges, pinacocytes differ in ion content from the external environment (salt or freshwater) and the pinacoderm controls the ion content of the mesohyl.

Sponge pinacoderms are thin and elastic and are coated with a proteoglycan matrix which has so far prevented intracellular recordings of true membrane potential. But it is still possible to determine the ability of the pinacoderm to seal by studying the transport of ions across the whole epithelium in culture. Adams and colleagues cultured sponge tissues in small cups with a permeable membrane bottom and recorded the ionic resistance across the sponge epithelium (Adams *et al.*, 2010). Using miniature cell cultures, they simultaneously recorded current in the medium above and below the tissue. Cultures that formed a confluent (continuous) sheet covering all of the membrane in the cup had a transepithelial resistance similar to that of epithelia in other metazoans (>2 MΩ). Furthermore, for a culture with high resistance, there was no passage of a small molecular weight tracer across the tissue; if the tissue in the cup was torn, then the tracer was able to pass across the tissues (Adams *et al.*, 2010). The authors considered the resistance was generated across the basopinacoderm (the tissue that was directly in contact with the cup membrane), but the endopinacoderm would also contribute to the resistance to passage of ions across the fine mesohyl and between pinacocytes. Therefore, it can be concluded that the pinacoderm (baso- and endopinacoderm at least, and likely also the exopinacoderm) provides good resistance to the passage of ions and controls the passage of small molecules across it.

In the context of being able to control cell–cell signalling via the release of small molecules, a sealed and stable pinacoderm makes sense. Images of the exopinacoderm labelled with a vital fluorescent dye (FM 1–43) also show that contacts between neighbouring pinacocytes remain stable for at least 1 h (Leys *et al.*, 2009; Adams, 2010). So why is it thought that sponge epithelia are transient? Statements about the looseness of sponge pinacocytes appear to stem from observations of highly mobile cells filmed at the periphery of explants of sponge tissue (e.g. Bond, 1992), and from sections and scanning electron

micrographs of fixed tissues that show cells not adherent to one another at the choanocyte chambers (e.g. fig. 6 in Simpson, 1984), and the supposed entry of bacteria through endopinacocytes into the mesohyl (Weissenfels, 1976). Whereas the motility of cells in explants is to be expected because these cells are actively seeking new unexploited areas to establish tissues in, the direct openings to the mesohyl from incurrent canals are not (Weissenfels, 1976, 1983). The images showing openings to those areas were subsequently acknowledged to have been caused by damage (Langenbruch and Weissenfels, 1987). Tissues that are poorly preserved often reveal openings to the mesohyl, but careful preservation and handling shows the cells in contact. The fact that sponge epithelia require such careful handling in order to preserve well, however, may be taken as some indication of the tenuous adhesion of sponge junctional molecules.

4.5. Cell adhesion and cell junctions

Sponges are almost best known for their ability to be disassociated—usually by being forced through fine mesh—and for the individual cells to re-associate in a species-specific manner (Wilson, 1907). Carbohydrate molecules, and specifically large sulphated proteoglycans originally known as 'aggregation factors', carry out species-specific adhesion (Fernandez-Busquets and Burger, 2003; Carvalho de Souza *et al.*, 2009; Vilanova *et al.*, 2009). The adhesion occurs at moieties on glycan fingers that extend from massive proteoglycan molecules attached loosely to the cell surface. Both the attachment to the cell and the species-specific recognition with other cells are calcium dependent. Carbohydrates are unusual cell-adhesion molecules, not commonly found in other phyla, although perhaps this mechanism of adhesion has not been adequately explored in other metazoans (Fernandez-Busquets and Burger, 2003), but they have a remarkable capacity for adaptation, and are able after one interaction to modify the types of ligands so as to recognize second encounters, that is they are polymorphic (Fernandez-Busquets and Burger, 1999). This type of adhesion has been considered a potential 'first' adhesion between early unicellular animals (Fernandez-Busquets and Burger, 1999; Vilanova *et al.*, 2009).

4.5.1. Molecules in sponge cell junctions—Overview

From the genome of the demosponge *A. queenslandica*, we know that sponges have molecules that are involved in cell polarity, adhesion and sealing, and in attachment to and communication with the extracellular matrix (Fahey and Degnan, 2010). Transcriptome data from several other sponges are also beginning to provide a rich picture of potential epithelial genes in sponges. Therefore, in addition to the carbohydrate type adhesion described above which is involved in species-specific aggregation, sponges produce membrane-associated proteins that are used in cell–cell adhesion. Junctions in

sponges differ from those in other invertebrates and vertebrates, but the details of position of the junction, molecules, and proteins involved in each junction type are only just being examined (e.g. Leys and Riesgo, 2011).

4.5.2. Adherens junctions

Adherens junctions hold cells together; they usually occur on one side of a sealing junction and their presence around the apex of the cell is considered an indication of tissue stability. While many authors refer to adhering junctions in sponges as desmosomes, strictly speaking desmosomes (macula adherentes) have desmosomal cadherins (desmogleins, desmocollins, and plakophilins) and plakoglobins, which are innovations of vertebrates. Invertebrates usually have zonula adherens, a belt-form junction, but in sponges, adherens junctions are usually spot-form junctions, appearing as dense plaques of actin between cells (Bagby, 1965; Pavans de Ceccatty, 1981; Boury-Esnault *et al.*, 2003; Elliott and Leys, 2007). Focal adhesions (spot desmosomes) have also been found on the basal epithelial layer of *E. muelleri* (Pavans de Ceccatty, 1986) and perhaps are the only real desmosomal adhesive junction sponges have. However, since none of the proteins that make up any of these adherens junctions have been studied, the true nature of sponge adherens junctions remains to be explored.

The principal component of adherens junctions is *cadherin*, a transmembrane molecule with extracellular cadherin (EC) repeats, EGF, and LAMG repeats (altogether called Fat cadherins). Sponges have 17 cadherins (Fahey and Degnan, 2010), and one of these (first reported by Abedin and King, 2008) is a classical Fat cadherin, which is usually associated with adherens junctions. In choanoflagellates, *cadherins* have binding sites for tyrosine phosphorylation and are thought to be involved in cell signalling (Abedin and King, 2008). In metazoans (including sponges), *cadherins* also have a conserved cytoplasmic domain, which has a binding site for *β-catenin* that is involved in signalling via the Wnt pathway. Therefore, adhesion and cell–cell signalling likely went hand-in-hand in the earliest metazoans. One *cadherin* in *A. queenslandica* has a *hedgehog*-like domain (Adamska et al., 2007b), and it is suggested that its expression around the pigmented posterior pole of the sponge larva reflects a role in signalling during the development of polarity in that sponge (Adamska et al., 2007b, 2010) (see Section 7).

Other adhesion molecules present in the sponge genome belong to the MAGUK family of transmembrane proteins (Sakaraya *et al.*, 2007; de Mendoza *et al.*, 2010; Fahey and Degnan, 2010). There are 10 types of MAGUKs (de Mendoza *et al.*, 2010), some of which generate the scaffold that anchors post-synaptic molecules in their place in nerve cells and others that generate tight junctions in vertebrates. Sponges have neither nerves nor tight junctions, but given the presence of members of the DLG, MPP, and MAGI super families of MAGUK proteins in sponges, we might expect these to be involved in cell–cell adhesion and/or signalling at cell junctions.

In situ hybridization of MAGI RNA in 'primorphs' (small sponges grown from dissociated and reaggregated cells) suggested expression in both the dermal membrane (exopinacoderm) and the choanoderm of sponge epithelia (Adell *et al.*, 2004). Similar hybridization experiments with DLG and several of the post-synaptic scaffolding genes in the *A. queenslandica* larva suggest a varied expression with some label in the inner cell mass, some in the sub-epithelial layer, and some specifically in a type of globular cell (also called a mucous cell) that populates the larval epithelium (Sakaraya *et al.*, 2007).

4.5.3. Occluding junctions

Occluding junctions in sponge epithelia are both 'parallel membrane' junctions (Green and Bergquist, 1979, 1982; Pavans de Ceccatty, 1981; Adams, 2010) and septate junctions (tight junctions are considered a chordate innovation). The former, in which the plasma membranes of two cells lie parallel to one another for about 500 nm with only 10–20 nm separation, occlude the passage of small molecular weight tracers (^{3}H-inulin, 5KD, and ruthenium red, 0.85KD) (Adams *et al.*, 2010). In these junctions, faint septae can sometimes be seen in transmission electron micrographs, but generally the occluding region occurs either at the parallel junctions or at regions adjacent to them in which the membrane of one cell appears merged (fused) or in tight contact with the other cell. Septate junctions have been reported in all sponges (see review by Leys *et al.*, 2009), but only in calcareous sponges are the septae very clear with 10 nm spacing between cells and between septae (Ledger, 1975; Green and Bergquist, 1979).

Septate junctions have been best characterized in *Drosophila* where two types are known. Pleated sheet SJs (pSJ) are found in the epidermis, salivary gland, and photoreceptors, while smooth SJ (sSJ) occur in the mid-gut and malpighian tubules. The ultrastructure of each shows that pSJ have distinct septae and sSJ lack them. Both junctions have the molecules neuroglian and contactin and both molecules are needed for barrier function, but only pSJ have *neurexin IV* (a contactin-associated protein, or Caspr) (Baumgartner *et al.*, 1996; Genova and Fehon, 2003; Faivre-Sarrailh *et al.*, 2004), so this molecule seems to be associated with the presence of septae but is not necessary for barrier function (Baumgartner *et al.*, 1996; Baumann, 2001). sSJ are instead characterized by *fasciclin III*, *ankyrin*, and *α,β-spectrin*.

Although the composition of the sponge septate and parallel membrane junctions is not yet known, the *A. queenslandica* genome does not seem to have clear orthologs of *neuroglian*, *neurexin IV*, *fibronectin III*, or *contactin* (Fahey and Degnan, 2010). *Neurexin IV*, however, was reported in *Oscarella carmela*, a homoscleromorph (Nichols *et al.*, 2006), and *A. queenslandica* does have a *claudin* gene ortholog which aligns well with sequences from two other sponges as well as *Drosophila*, nematode, and human sequences (Leys and Riesgo, 2011). *Claudins* are known from tight junctions, but in the fly, these proteins are localized to septate junctions and have been shown to play a role

in occlusion (Asano *et al*., 2003). *Claudins* are also present in the genome of *Hydra* (Chapman *et al*., 2010) and recent work using GFP-claudins shows labelling around the apex of each cell (Salvenmoser, 2011). It is, therefore, quite possible that the demosponge *claudin* molecules may also function in occlusion, and that in sponge parallel and/or septate junctions, perhaps *claudins* rather than *neurexins* perform the sealing role (Leys and Riesgo, 2011). To better understand how the sponge junction works, antibody localization and gene knockdown is needed, as well as further more detailed exploration of other sponge genomes as they become sequenced.

Other molecules found in the *A. queenslandica* genome and which may be involved in establishing polarity of the epithelium, or in localizing junctions to their polarized position in the epithelium, include *Scrb*, *Par*, *Crumbs*, and *Patj* (Fahey and Degnan, 2010). In other invertebrates, *Scrb* and *Par* are localized to septate junctions. Given the very narrow region which sponge epithelial cells make contact with one-another, it is conceivable that these molecules are also present at the sponge junctions, but testing this will require antibody localization using immunogold to confirm the position at the ultrastructural level.

4.6. The basement membrane: Differences among sponge epithelia

The integrity of the epithelium is achieved not only by adhesion between cells but also by the support of the extracellular matrix below. Depending on the location in the sponge body, epithelia have some sort of basal support provided by collagen, and most also have an upper cuticle. There are typically two components to the basement membrane—the lamina reticularis (a sheet of collagens I, II, and V), and the basal lamina (a 50–100 nm layer of type IV collagen together with laminins and proteoglycans such as nidogen/entactin and perlecan) that lies immediately below, and follows every contour of, the cell membrane. Electron micrographs frequently show a dense mat of collagen underlying the sponge epithelium, which may be the equivalent of a lamina reticularis. In electron micrographs of homoscleromorph sponges, there is also a fine basal lamina below both the pinacoderm and choanoderm and antibodies to type IV collagen raised from that sponge recognize a layer that is beneath the epithelium (Boute *et al*., 1996). Recently, type IV collagen that corresponds well to alpha and beta chains has also been found in the calcareous sponge *Sycon coactum* and another homoscleromorph, *Corticium candelabrum* (Leys and Riesgo, 2011). The finding of type IV collagen in *Sycon* is all the more unusual since electron micrographs do not show a basement membrane structure in any calcareous sponge.

The *A. queenslandica* genome does not have either type IV collagen or the proteoglycans nidogen or perlecan, and though there are several laminin-related genes, only one (*Lam* γ-like) has a domain architecture sufficiently

comparable to laminins from bilaterians to be considered proper laminins (Fahey and Degnan, 2010, 2012). Type IV collagen is necessary for basal lamina formation since its globular heads link to form a mesh that is attached to the cell surface by laminins. Although no other demosponge genomes are available, searches of other sponge transcriptomes and use of degenerate PCR has not found conventional type IV collagen in other demosponges. However, it has been shown that demosponges have a related form of this molecule called 'spongin short chain collagen' (SSCC), which shares very similar NC1a and NC1b domains with type IV collagen and possesses four of the six conserved cysteine molecules typical of type IV collagen (Aouacheria *et al.*, 2006). SSCC is also predicted to have the same three-dimensional structure and therefore to be capable of forming the same interactions with the epithelium as type IV collagen (Aouacheria *et al.*, 2006). SSCC is expressed by cells of the basal pinacoderm (Exposito and Garrone, 1990; Exposito *et al.*, 1991). Further support for the idea that the three-dimensional structure of SSCC may be similar to type IV collagen comes from a study by De Goeij *et al.* (2009) who showed that polyclonal antibodies to type IV collagen from rabbit also recognize a layer below the choanoderm in the demosponge *H. caerulea*. It is possible that these antibodies recognize a conserved 3D structure in the protein despite what would be considered to be a little conserved amino acid sequence.

4.7. Pinacoderm function: Biomineralization

Biomineralization in sponges—the production of the skeleton—is the subject of several excellent reviews (Uriz *et al.*, 2003; Uriz, 2006; Sethmann and Wörheide, 2008; Wang *et al.*, 2010) and is also covered by Wang and co-authors in this issue. Most secretion of mineral and organic skeletons in sponges takes place in the mesohyl, but here, we alert the reader to two instances in which the pinacoderm functions in biomineralization. The first is the observation that some spicules are formed by pinacocytes in the homoscleromorph sponge *Corticium candelabrum* (Maldonado and Riesgo, 2007). This observation may not be so unusual if pinacocytes in homoscleromorphs give rise to sclerocytes as they are said to do in Calcarea (Simpson, 1984, p. 147), and also to microsclerocytes as they are said to do in some demosponges (Simpson, 1984, p. 178). Maldonado and Riesgo (2007), however, do not think this is the case in *C. candelabrum* and therefore this points to an unusual secretory ability of the pinacoderm in that homoscleromorph sponge. The epithelium also plays a role in biomineralization in hypercalcified sponges, both demosponges (e.g. *Acanthochaetetes wellsi*, *Astrosclera willeyana*) and Calcarea (e.g. *Petrobiona massiliana*). The precise mechanism of mineral uptake, transport and secretion is not yet fully understood, but histology, histochemistry, and many types of energy X-ray analyses of the basopinacoderm from both demosponges and calcareous sponges

suggest that minerals (magnesium and/or calcium) are sequestered into vesicles in basopinacocytes and secreted as an amorphous material on the basal side of the epithelium (Reitner and Gautret, 1996; Wörheide, 1998; Gilis, 2011; Gilis *et al*., 2011). Polymerization (crystallization) of the material below the basopinacoderm is promoted by the prior or concurrent secretion of an organic matrix, which is shown by staining with alcian blue, ruthenium red and acridine orange, to contain highly sulphated or acidic polysaccharides (e.g. glycosaminoglycans) (Gilis, 2011). Together, these materials allow for a biologically controlled crystallization of the minerals moulding them into a basal skeleton and incorporating into it spicules also produced by the sponge. In *Astrosclera,* biomineralization has also been shown to involve a type of mesohyl cell, a large vacuolar cell (LVC) (Wörheide, 1998), which is found in the choanosome and ectosome above the calcified skeleton. Demonstration that these cells express the enzyme carbonic anhydrase required for catalyzing HCO_3^- (Jackson *et al*., 2007) suggests that LVCs are involved in uptake of calcium and its transport to basopinacocytes where secretion of the matrix occurs.

4.8. Pinacoderm development

The origin of pinacocytes is, after more than a century of study, still unclear. Earlier research using a variety of models, such as dissociated tissue forming reaggregates (Wilson and Penney, 1930), dissociated larvae forming juvenile sponges (Borojevic and Lévi, 1964, 1965), and explants of adult sponges (Simpson, 1963; Bagby, 1972), all from different species, concluded that archaeocytes differentiate into collencytes which become pinacocytes. Galtsoff (1925) who was the first to use aggregates for this, thought that pinacocytes remained differentiated upon dissociation of a sponge, and simply 'sorted' out to reform the pinacoderm. Wilson and Penney (1930) concluded that a type of cell called a 'grey cell' with grey granular inclusions, formed the pinacocytes. Bagby (1972) determined that since there were no grey cells in *Microciona* archaeocytes must become the pinacocytes. The only 'live cell' study of this problem was by Simpson (1963), who showed that in explants pinacocytes did not divide to form the pinacoderm, but rather they arose from mesohyl cells which differentiated and moved into the pinacoderm. Study of the metamorphosing larva of *E. fluviatilis* supports the view that archaeocytes migrate up into and form the pinacoderm of the juvenile sponge (Boury-Esnault, 1976; Wielsputz and Saller, 1990). So in sum, new pinacocytes arise by differentiation of archaeocytes from the mesohyl.

Curiously, a recent study of sponges differentiated from gemmules of *Suberites domuncula* suggests the opposite that the expression of genes in the pinacoderm triggers the differentiation of pinacocytes into sclerocytes and mesohyl cells (Schroder *et al*., 2004). After stimulation of canal formation using increased temperature and Fe^{3+}, pinacocytes were the first cells to

express *noggin* and *silicatein*, and subsequently, these genes were expressed in cells in the mesohyl (Schroder *et al.*, 2004). The authors concluded that a subpopulation of pinacocytes is able to differentiate into other cell types; but it is also possible that genes expressed by pinacocytes could induce the differentiation of cells in the mesohyl into other cell types. Whether cells from the pinacoderm can differentiate into other cells might be studied by using a fluorescent cell-tracker such as CMFDA which incorporates only into cells it has direct contact with; therefore, using a bath application mesohyl cells would not be labelled. So far, two studies have used labelling of new nuclei using 5-bromo-2′-deoxyruridine (BrDU) to identify stem cells, one in adult specimens of the crevice-dwelling marine sponge *Halisarca* (De Goeij *et al.*, 2009) and the other in the freshwater sponge *E. fluviatilis* differentiating from gemmules (Funayama *et al.*, 2010). Although both groups found incorporation of the label into choanocytes and archaeocytes within the 10-h time frame of exposure to the drug, neither found the label in pinacocytes.

In sum, pinacocytes do not appear to divide, although this does not mean they are terminally differentiated. It is possible that some pinacocytes can differentiate into other cell types, but it is perhaps more likely that they trigger/stimulate the differentiation of mesohyl cells into other cell types. The latter role is important in the formation of the canals and differentiation of the sponge body plan. Pinacocytes are, if not the first, certainly among the first cells to differentiate in the early development of a sponge from a larva or from a gemmule and during aggregation after dissociation of the adult. Pinacocytes line the incipient canal epithelia and are responsible for controlling ion exchange across the epithelium into the mesohyl.

5. The Aquiferous System

5.1. Differentiation of porocytes and canals

Ostia—incurrent openings into the canal system—are formed by porocytes, either singly, by having a large ‘hole’ in the middle of the cell, or possibly by several meeting up around an ostium to form the pore (Simpson, 1984). Evidence for the latter, however, comes from electron micrographs that show several pinacocytes converging on an ostium in a freshwater sponge exopinacoderm. Given that the porocyte lies below the exopinacocytes and between the two pinacoderm epithelia, when the pore is open, the cell body may actually be completely hidden by the two pinacoderms (Fig. 1.8).

Porocytes have been seen to form in as little as 10 min in *E. fluviatilis* (Weissenfels, 1980) and within 10 h in *Leucosolenia variabilis* (Jones, 1961). The former observation might be explained by a porocyte having been hidden by pinacocytes and opening to reveal itself since videos of opening and closing ostia show this sort of time frame (Elliott and Leys, 2007)

(Fig. 1.8), but the latter suggests that—if cell division takes 10–20 h—they probably arise from mesohyl cells, rather than dividing from other pinacocytes. Oscula are also thought to arise initially from a single porocyte (Weissenfels, 1980), but how they coordinate with other porocytes to form a larger osculum is still unclear. In freshwater sponges hatched from gemmules, the region in which the osculum will differentiate appears first as a bulge, which according to Brien (1932) is caused by pressure from water pushed by the beat of newly formed chambers; but one wonders how that pressure is dissipated if there is still no vent or opening that is the osculum. Nevertheless, the movement of fluid over the tissues to generate signals for differentiation of tissues is a common theme in animal development, even in, for example, the nodal cilia.

Much of our understanding of canal formation comes from the observations by Wintermann (1951) and Mergner (1964; 1966) and later by Weissenfels (1981), all of whom found that the excurrent canal system arises first, chambers plug into the canals, and the incurrent canal system joins up last (and is most plastic). The canals themselves arise from lacunae, small epithelia-lined cavities within the sponge mesohyl, which move around, often in a clockwise centripetal manner, until they merge with others to form canals (Leys, personal observation). Hans Mergner first experimented with the role of the osculum in canal formation and found that if he removed the osculum (from a small freshwater sponge hatched from gemmules), it remained differentiated and formed a small ball of exo and endopinacoderm, which he called the 'oscula ball'. He found that if he inserted this oscula ball anywhere in the sponge canal system it caused a new osculum to form in that spot (Mergner, 1964), and surmised that a substance ('Induktionstoffes') from the osculum tissue caused the differentiation of canal epithelia and formation of an osculum.

Recent experiments support the idea that osculum sends out a factor controlling canal formation. Windsor and Leys (2010) used Mergner's (1964) experimental approach to remove oscula from the freshwater sponge *E. muelleri* and place them on the surface of a different sponge, one hatched from the same batch of gemmules. They found that the transplanted osculum not only attached to the surface of the sponge but also linked up with the excurrent canal system and in fact drew the entire aquiferous system towards it, causing the original osculum to cease to function (Windsor and Leys, 2010). This seemed to confirm Mergner's (1966) idea that a substance secreted by the oscula tissues induces canal formation, competing with gradients secreted by the original host osculum. An alternative hypothesis, however, is that the secreted factor stimulates regeneration, and regeneration triggers the release of factors that result in oscula formation. Windsor and Leys (2010) proposed that the secreted factor is Wnt protein because soaking the developing sponge in Wnt activators caused a phenotype of multiple oscula and disruption of the aquiferous system.

5.2. Role of *Wnt* in canal differentiation and polarity in sponges

To date, *Wnt* pathway genes have been isolated from at least 10 different sponges—*Amphimedon queenslandica* (Adamska *et al.*, 2010; Srivastava *et al.*, 2010), *Suberites domuncula* and *Lubomirskia baicalensis* (Adell *et al.*, 2007), *Ephydatia muelleri* (Windsor and Leys, 2010), *Oscarella lobularis* (Lapébie *et al.*, 2009), and *Sycon ciliatum* (Adamska, unpublished); *S. coactum, Pseudocorticium jarrei, Spongilla lacustris* and *Petrosia ficiformis* (Ana Riesgo, Pamela Windsor and Sally Leys, unpublished). Expression of *Wnt* genes has been studied in larvae, young adults and adult sponge tissue, but not in the different developmental stages (larvae, postlarva and adult) of any one species. Our understanding the role of *Wnt* in sponge development and body plan formation is therefore still patchy.

The most complete studies to date of *Wnt* gene and ligand expression have been carried out in the *A. queenslandica* sponge larva (Adamska *et al.*, 2010) where it has been shown that one of the three *Wnt* genes (*WntA*) has a polarized expression at the posterior pole of the larva together with pigment cells that form a ring around the posterior pole. *WntA* expression is juxtaposed with expression of *TGFβ*, which labels the entire anterior portion of the larval ciliated epithelium (Adamska *et al.*, 2010). Some *Wnt* ligands also have a polarized expression (e.g. LRP) while the expression of others is localized more to the outer, middle or inner layers of the larva (Adamska *et al.*, 2010). Since LiCl experiments have so far not been successful in *A. queenslandica*, it is unknown what function Wnt has except for a probable role in guiding anterior and posterior axis formation in the larva.

In the cnidarians *Hydra* and *Nematostella*, artificial activation of the *Wnt* pathway causes the formation of ectopic tentacles and multiplication of the 'head' end (Broun *et al.*, 2005), and gradients of *Wnt* gene expression mark the anterior to posterior poles of the polyp (Kusserow *et al.*, 2005). To determine whether the sponge canal system might develop in a polarized manner by gradients of *Wnt* gene expression, Windsor and Leys (2010) hatched sponges in a growth medium with drugs that activate the *Wnt* pathway (lithium chloride and alsterpaullone) and found that with both drugs the sponges had many more oscula than normal sponges, and that the aquiferous system was completely malformed and non-functional. Furthermore, in the treated sponges, there were either fewer ostia and porocytes, or they were completely absent. A similar approach was used in *Oscarella lobularis* by Lapebie *et al.* (2009). Incubation in azakenpaullone caused the sponge to form many more ostia; oscula were not studied. These results suggest that secreted Wnt protein may be Mergner's 'induktionstoffe' (Mergner 1966) which polarizes the epithelia during development to determine where ostia and incurrent canals or oscula and excurrent canals form. In *E. fluviatilis*, there is also evidence that the *Wnt* pathway may be involved independently in radial organization of the

skeleton because sponges grown in BIO or azakenpaullone have a disorganized skeleton (Nakata and Funayama, 2011). If production of the skeleton were necessary for proper organization of aquiferous system, these results would make sense together, but since it is also known that normal sponges form in the absence of materials to construct spicules (Weissenfels and Langenbruch, 1985), the simplest explanation is that the *Wnt* pathway has multiple roles in sponge development.

Other genes that may have a role in canal formation include the homeobox genes *Iroquois* and *NK* class. Both show expression around canals or on the outer epithelium (Perović *et al.*, 2003; Gazave *et al.*, 2008, see Section 7), but experimental approaches will be needed to determine what role these genes might have in actual canal formation.

6. Epithelia as Sensory and Contractile Tissues

6.1. Overview of sensory and coordinating tissues

Sponges lack nerves in the conventional sense; that is, they do not possess a defined cell type that transmits signals using electrical impulses that travel along the cell membrane and communicate to other cells via a chemical synapse. This does not mean, however, that sponges do not send either electrical or chemical signals, but rather that the speed at which the signals propagate through the sponge is slower than conventional electrical signalling. Furthermore, the means by which signals propagate is not necessarily by electrical impulses, as generally is the case in other animals. Glass sponges propagate electrical signals that shut down their feeding current (Leys and Mackie, 1997; Leys *et al.*, 1999; reviewed in Leys and Meech, 2006; Leys *et al.*, 2007), but so far, there is no evidence of electrical signalling in cellular sponges. However, at least one species (*Hymedesmia*) is capable of dropping a tissue flap over its ostial pore fields in under 1 s (Reiswig, 1979), which indicates that localized, rapid (perhaps electrical) signalling may occur in some species. Signals that are not electrical impulses would presumably be waves of calcium (propagated, localized increases in calcium concentration that spread through the cell), triggered by stimuli to the cell membrane, either via receptors on the cell surface or via channels in the cell membrane. Signals could propagate through cells of the epithelium or cells of the mesohyl, but in recent years, the epithelium has become recognized as the primary conduit of coordination in sponges.

In the 1970s, it was already known that signal transduction largely occurred through the epithelia of all sponges, although thick walled sponges were thought to have single contractile cells or bundles of cells in the mesohyl which were considered to play a role in contraction (Pavans de Ceccatty, 1974a,b). Viewed live by cine-film or time-lapse digital microscopy, amoebocytes in the mesohyl of thin-walled sponges are very active,

some of the cells move in a highly directional manner, others travel almost in circles (Pavans de Ceccatty, 1969, 1976; Adams, 2010). Collencytes leave a trail of collagen in their wake, sclerocytes sit stationary and take minutes to hours to develop a single spicule, lophocytes cluster around spicules, cells with inclusions appear stationary, archaeocytes are sluggish and tend to stay near choanocyte chambers.

It is not possible to film cell activity in thick walled sponges, and certainly mesohyl cells may not be equally active in all sponges, or perhaps the activity of cells in the mesohyl depends on the thickness or density of collagen. Ultrastructural studies in the 1960s and 1970s showed cells stretching across the mesohyl and in contact with epithelia at either end, interactions that are suggestive of cell–cell communication (Pavans de Ceccatty *et al.*, 1970; Pavans de Ceccatty, 1974a,b, 1986). The presence of actin microfilaments in these cells was taken to indicate they might be contractile; therefore, they were termed myocytes (Bagby, 1965, 1970). The general view was that where sponge tissues were thin, it was more likely that pinacocytes were contractile, while in thick tissues, it was more likely that mesohyl cells (myocytes) were contractile. In very thick walled sponges, sphincters around canals contract more rapidly than the whole sponge can and reduce the diameter of canals; the contractile cells in sphincters are pinacocytes. In recent years, pinacocytes have come to be considered the primary contractile cell (Nickel *et al.*, 2006; Nickel, 2010; Nickel *et al.*, 2011), and actinocyte substituted as a more generic term (Boury-Esnault and Rützler, 1997), even though strictly speaking all cells would be expected to have actin. Although it is not possible to view mesohyl cells in thick sponges *in vivo*, estimates from area projections of contracted and relaxed sponges using microCT scanning suggest that even where the mesohyl is substantial, the epithelium is the primary contractile and conductive pathway (Nickel *et al.*, 2011). Nevertheless, even in thin-walled sponges like *Ephydatia* in which periodic contractions have been studied by time-lapse imaging since the mid-20th century (Pavans de Ceccatty *et al.*, 1960; Pavans de Ceccatty, 1969; De Vos and Van de Vyver, 1981; Weissenfels, 1990), it is still unclear whether both pinacoderm and mesohyl cells are involved in contractions, but it is likely that the epithelium is the principal conduit for signal propagation through the sponge.

6.2. Molecules involved in coordination and signal transduction

One of the most central questions in the evolution of animals is *when did nervous tissues first arise*? Because sponges show propagated signals that translate into slow but coordinated behaviour, they appear to bridge the 'gap' between non-neural and neuronal states. Thus, it would seem that genomics and transcriptomics promise to shed much light on the topic of

neuron origins by revealing what aspects of ion channels, transmitters and synapses are present in extant species. Molecules identified to date, however, seem to simply support the current idea that sponges have an 'almost nervous system' of the type discussed by Pavans de Ceccatty (1974a,b, 1979). Nonetheless, it is not yet clear if sponges never fully went down the path to conventional nerves, or if they have in fact lost some of the key molecules involved in conventional nervous systems. For example, the *Amphimedon* sponge genome has genes for calcium and potassium channels but no sodium channels (Liebeskind *et al.*, 2011), yet sodium channels seem to be present in choanoflagellates, the unicellular protistan lineage sister to the Metazoa. The absence of sodium channels in sponges may make sense given that physiological experiments on glass sponges (Hexactinellida) suggest they use calcium/potassium impulses (depolarization of the membrane by calcium influx and repolarization by potassium) rather than sodium/potassium as do most other animals (Leys *et al.*, 1999). Calcium impulses are temperature sensitive and have been put forward as one of the reasons that glass sponges are restricted in their range to deep, cooler waters (Leys *et al.*, 2003). The presence of a functional sodium channel in choanoflagellates, however, prompts speculation that sponges may have lost aspects of a conventional nervous system. The *Amphimedon* genome has both inward rectifying (K_{ir}) and putative votage gated K^+ channels (Sakaraya *et al.*, 2007; Alie and Manuel, 2010), and expression of K_{ir} channels in the frog oocyte system has shown that sponge K_{ir} channels are strong rectifiers (Tompkins-MacDonald *et al.*, 2009), which indicates that the membrane potential can be quickly returned to normal should it be depolarized. These data suggest that *Amphimedon* cells might normally depolarize, as if for electrical signalling.

Most research has focused on seeking evidence for synapses or synaptic molecules in sponges. There is now abundant evidence that many molecules known from the post-synaptic scaffold (PSD) are present in *Amphimedon* (Sakaraya *et al.*, 2007; Alie and Manuel, 2010; Srivastava *et al.*, 2010), but no work has convincingly shown the presence of a synapse. *In situ* hybridization of *PSD* genes from *Amphimedon* (*GKAP*, *GRIP* and *HOMER*) is confusing, showing potential expression in a globular cell in the sponge epithelium, but also in many other regions of the sponge larva (Sakaraya *et al.*, 2007). Can these all be post-synaptic signaling sites, even in the supposed receptor cell (the globular cell)? Perhaps the best morphological evidence for a synaptic-like structure is still found in the electron micrographs of Pavans de Ceccatty (1959). Because of the lack of recognizable landmarks in a sponge, gene expression data are very difficult to interpret. What is needed now is a merging of the two techniques by making antibodies to the proteins coded by the genes involved in the post-synaptic density for visualization by immunogold electron microscopy to determine whether sponges in fact possess a discrete 'pseudo-synapse'

as often suggested, or whether these proteins are distributed more widely around the sponge cells.

More progress has been made recently by physiological studies of sponge responsiveness. Molecules found to be present in the *Amphimedon* sponge genome do appear to have a specific role in signaling along sponge epithelia. The range of studies that have demonstrated the responsiveness of sponges to different stimuli was recently reviewed by Nickel (2010). Since the early 1900s, chemicals as well as mechanical and electrical stimuli were applied to sponges to determine the speed at which they could respond (Parker, 1910; Emson, 1966; Prosser, 1967; Ellwanger and Nickel, 2006). Rates of contraction were fastest in the osculum (McNair, 1923), but all regions of the sponge have now been shown to contract periodically (Nickel, 2010).

Since the *A. queenslandica* genome contains several GABA receptors, at least seven metabotropic glutamate receptors, serotonin, and a host of other transmitters known from neuronal systems (Sakaraya *et al.*, 2007; Srivastava *et al.*, 2010), it is expected that demosponges are fully capable of responsiveness and signaling. In recent years, it has been shown that contractions can be triggered by bath application of glycine, cAMP and the neurotransmitters adrenaline and serotonin, while nitric oxide appears to modulate contraction intensity (Ellwanger *et al.*, 2004; Ellwanger and Nickel, 2006). Glutamate seems to be particularly important. It is able not only to trigger small contractions, but at the right concentration it also initiates the propagation of a coordinated inflation and contraction (I/C) behaviour, termed a 'sneeze' because clogging by dye particles also triggers this behaviour, which through several such I/C events manages to rid the chamber of the particles (Elliott and Leys, 2007). It has also been shown that GABA acts antagonistically to glutamate to prevent sneezes, which importantly shows that even without conventional nerves, sponges have and use glutaminergic signalling systems (Elliott and Leys, 2010). Antibodies to GABA receptors recognize pinacocytes in *Chondrosia reniformis* (Ramoino *et al.*, 2007), but given that those antibodies were not raised against sponge molecules, the possibility of cross-reactivity with other cell surface molecules cannot be ruled out. Use of antibodies raised to specific sequences of glutamate, GABA and other receptor and signalling molecules from the sponges themselves will help determine whether sponge incurrent and excurrent endopinacoderms have different receptor populations, or whether all pinacoderms present a uniform population of receptors.

6.3. Gene expression as an indicator of sensory epithelia

Two sets of genes have been suggested as indicators of sensory cells and tissues in sponges. An *Antennapedia* (*ANTP*) *NK* class homeobox transcription factor (*HomoNK*) has been isolated from *Oscarella lobularis* and, based on phylogenetic analysis, likened to *NK6* and seven genes which in

vertebrates are expressed in neurosensory cells. In *O. lobularis HomoNK* is expressed in the choanocytes of the chambers in both reproductive and non-reproductive adult tissue, and these, therefore, were tentatively suggested to have a sensory function (Gazave *et al.*, 2008). Choanocytes were also suggested as potential mechanosensory cells by Jacobs *et al.* (2007), but functional data to support this idea still needs to be obtained. *A. queenslandica* larvae also express genes of the basic Helix loop Helix (*bHLH*) family (Simionato *et al.*, 2007), and *bHLH* as well as the *Notch* receptor and *Delta* ligand are expressed in globular cells of the outer epithelium (Richards *et al.*, 2008; see also Section 7.4). Phylogenetic analysis shows *A. queenslandica bHLH* is ancestral to the *Atonal/neurogenin* family, and functional studies demonstrate that the sponge gene can substitute for the native gene to trigger neuronal differentiation in *Xenopus* as well as development of sensory bristles in *Drosophila*. Given this finding, the authors speculate that the globular cells themselves might be a type of sensory cell in the sponge larval epithelium (Richards *et al.*, 2008). However, it should perhaps be considered that the globular cells play a role in causing the differentiation of other, neighbouring cells—perhaps the flask cell—into sensory cells in the epithelium. It would be useful to find a marker for sensory cells in sponges in general and to be able to test this hypothesis using knock down by RNAi.

7. Tissue Formation During Sponge Development

7.1. Overview of embryogenesis and larval morphogenesis in sponges

Mechanisms of sexual reproduction and larval development are highly varied in sponges (see Leys and Ereskovsky, 2006). Gametes are formed from archaeocytes or choanocytes, and whereas sperm are released into the water column, in many cases, oocytes are retained and fertilized in the parent sponge developing into a swimming larva that is released. In fewer known instances, both eggs and sperm are released into the water column, and in these cases, fertilization is usually (but not always) external. The different types of development and different types of larvae are reviewed elsewhere (Ereskovsky, 2004, 2005; Leys and Ereskovsky, 2006), but the most studied and probably the best understood modes of development involve brooded larvae. See Ereskovsky (2005) and Leys and Ereskovsky (2006) for a more comprehensive review of sponge development.

Eight types of larvae have been described in sponges (Maldonado and Bergquist, 2002), and these fall into four categories: parenchymella, coeloblastula (hollow blastula), amphiblastula, and trichimella. There are other unusual

types of larvae which differ in construction of cell layers, skeleton, polarity, regionalization, for example, the direct developer of *T. serica* (Watanabe, 1978a,b), the solid larva of *Chondrosia* (Lévi and Lévi, 1976), the armoured larva of *Alectona* (Vacelet, 1999), and while these lack specific names, they are each very different in morphology from the other four categories.

Sponges develop through cleavage to form a solid or hollow blastula. Cellular differentiation begins after approximately the 64 cell stage to form micromeres and macromeres which sort, often by cellular migration, to position micromeres on the outside and macromeres on the inside, or by centrifugal movement of all cells to generate a hollow larva. Other mechanisms of sorting include inversion of a hollow blastula in syconoid Calcaronea. The result is a layered and/or polarized larva. For the parenchymella, embryogenesis results in a layered larva in which there is an outer pseudostratified ciliated epithelium (the nuclei lie at different heights in the epithelium), an inner cell mass with amoeboid cells and often also with spicules, and a thin sub-epithelial layer of cells that lie perpendicular to the other layers thereby forming a sort of belt or girdle around the larva. The coeloblastula is a hollow larva that has a single layer of ciliated cells, and within that layer some other cells either at the middle of the larva (e.g. the cinctoblastula of homoscleromorphs) or at the posterior pole (e.g. calcinean calcareous sponge larvae). The amphiblastula is also formed by a single layer of cells, but only the anterior half is ciliated, while the posterior half large has large globular cells. Amphiblastulae larvae also have some maternal cells which migrate in from the parent mesohyl at the time of inversion. The trichimella larva is somewhat unusual in being syncytial. Blastomeres fuse during early development to form the future trabecular reticulum and that tissue envelops cellular regions. The trichimella has precociously formed flagellated chambers and multiciliated cells whose cilia pierce the smooth syncytial outer tissue.

The sorting of cellular material that occurs during sponge embryogenesis has been likened to gastrulation (Leys and Degnan, 2002) although by definition gastrulation involves the formation of the feeding epithelium, and in sponges, this is, therefore, a two-step process, the first involving positioning the cells that will do the feeding (that is those that will generate the feeding current and do much of the particle capture) into a tissue, and the second involving moving those cells to a region in which they can feed; the latter step usually occurs at metamorphosis (Leys, 2004).

7.2. Regulatory genes in development

The 'genetic toolkit' present in sponge genomes that is presumably responsible for the organization of sponge development is largely absent from other non-metazoan genomes including choanoflagellates. This finding supports the notion that ontogenetic development originated at the base of the metazoans and that sponge genetics will play an important role in our

understanding of metazoan development in general (Srivastava *et al.*, 2010), and sponge developmental processes in particular. Two major groups of gene families are generally considered when discussing the genetic programming of animal development and include the molecules involved in signal transduction pathways and the host of factors that influence transcription of developmentally important genes.

Generally speaking there are seven major cell–cell signalling pathways that control the developmental programmes of bilaterian animals: *Wnt*, *TGF-β*, *Hedgehog*, *receptor tyrosine kinase*, *nuclear receptor*, *Notch*, and *JAK/STAT* (e.g. reviewed in Barolo and Posakony, 2002). Sponge genomes are known to possess ligands and receptors for the majority of these pathways. The components of arguably the most prominent developmental control pathways, *Wnt*, *TGF-beta* and *Notch-delta* appear to be complete in *A. queenslandica* (Adamska *et al.*, 2011), while important components of the *Hedgehog*, *receptor tyrosine kinase*, *nuclear receptors* and *JAK/STAT* signaling pathways are also found in Porifera (Müller, 2003; Wiens *et al.*, 2003; Larroux *et al.*, 2006; Nichols *et al.*, 2006; Adamska *et al.*, 2007b).

Many transcription factor genes that play important roles in developmental processes in animals are present in sponges. Here, we will confine our discussion to those transcription factors and signal pathway genes for which we have some expression or functional data in tissue formation or function. A more complete description of classes of sponge transcription factors can be found elsewhere (e.g. Larroux *et al.*, 2008; Degnan *et al.*, 2009; Srivastava *et al.*, 2010).

7.3. Gene expression during early embryogenesis

Little is presently known about the earliest molecular factors that set the developmental programme in fertilized eggs leading to proper cleavage and blastula formation in sponges. This is partially due to the difficulty of obtaining eggs, sperm, and/or early embryos in most species. A host of genes are known to be expressed in cleavage and/or blastula stage embryos of *A. queenslandica*, and these include components of the *Wnt* signal transduction pathway: *WntA*, *Frizzled* (*FzdA*), *Secreted Frizzled Related Proteins* (*SFRPA, C, D*), *Lrp5/6*, *GSK3*, *APC*, *Axin*, *Dishevelled*, *β-catenin*, *Tcf* and *Groucho*. In most cases, the expression patterns of these genes appear in subsets of micromeres distributed throughout the embryo (including the follicle envelope) with some genes demonstrating apparently higher expression levels than others. *Secreted Frizzled Related Protein*, *SFRPD*, however, seems to be a marker for the follicle membrane surrounding embryos and is not expressed in the embryo proper. Interestingly, another *Secreted Frizzled Related Protein*, *SFRPC*, is expressed only in the inner part of the blastula embryo (and is excluded from the follicle layer). *Lrp5/6* is also expressed in the follicle layer of early embryos and does not become expressed in

the embryo until movements of gastrulation (Adamska *et al.*, 2010). Another signalling pathway gene, *TGF-β*, is expressed in cleavage stage embryos of *A. queenslandica* in small micromeres that are distributed uniformly throughout the embryo; expression is subsequently confined to the outer region of the blastula (Adamska *et al.*, 2007a).

Several transcription factors are known to be expressed in *A. queenslandica* embryos: these include *PaxB*, *POU*, *Lim3*, *Bsh*, *Prox2*, *NK2*, *Sox* (B, C and F), *Fox* (J and L1), *Tbx* (A and B), *Mef2*, *HNF4*, *Ets* (Larroux *et al.*, 2006) and *NF-κB* (Gauthier and Degnan, 2008). Two of those genes, *Bsh* and *NF-κB*, are expressed in subsets of micromeres at cleavage; *NF-κB* expression, however, is strongest in granular cells of the post-cleavage embryo (Larroux *et al.*, 2006; Gauthier and Degnan, 2008). Given the fact that most of the genes studied thus far in embryos are from one species (*A. queenslandica*) and that most reveal expression patterns in 'subsets of micromeres', it is difficult to draw any conclusions about the role of these molecules in early development. For two of these transcription factors, *PaxB* and *Six1/2*, expression in embryos and larvae has been partially evaluated in the demosponge *Chalinula loosanoffi*. *PaxB* expression levels during early developmental stages (embryos through to metamorphosing larvae) are very low. *PaxB* transcripts could not be detected by *in situ* hybridization in pre-release larvae for this species (Hill *et al.*, 2010). These data are consistent with what has been observed for *A. queenslandica PaxB* (Larroux *et al.*, 2006). *Six1/2* transcripts are evident throughout all developmental stages. In blastula/early gastrula stage embryos, *Six1/2* expression is restricted to the outer epithelial layer (Hill *et al.*, 2010). Interestingly, *Six1/2* expression is found in the choanosome of adult tissue in this species. These data might fit well with what is known of demosponge larval metamorphosis where the ciliated outer epithelial cells of the larvae end up migrating inward to form choanocytes (e.g. Leys and Degnan, 2002). In this scenario, *Six1/2* could be a marker of that process. More detailed analysis of gene expression during sponge embryogenesis from more species as well as experimental manipulation of gene expression at early developmental stages will be necessary.

7.4. Gene expression during gastrulation and formation of larval layers

In *A. queenslandica*, *Wnt* expression patterns during gastrulation and formation of larvae indicate a role in anterior–posterior axial polarity as *Wnt*-expressing cells become progressively confined to the posterior pole during gastrulation. The expression of *Wnt* later coincides with the region where pigment cells will form a spot and ring at the posterior end of the larvae (Adamska *et al.*, 2007a). Other members of the *Wnt* pathway are also expressed during gastrulation and larval development; this work is detailed

in Adamska *et al.* (2010). The expression of the genes encoding *Wnt* pathway receptors, cytoplasmic domains and nuclear components of the pathway discussed earlier (e.g. *Frizzled*, *GSK3*, *β-catenin*) becomes localized to the outer layer of the early gastrula stage embryo, and it is suggested that the *Wnt* pathway is activated in a gradient from posterior to anterior (Adamska *et al.*, 2010, see also Section 4.2). It is also likely that the *Wnt* pathway plays a role in pigment cell migration because the pigmented ring marking the posterior of the *A. queenslandica* larvae becomes established at the same stages at which *Wnt* is expressed at the posterior pole. Functional and/or larval manipulation experiments would help support this hypothesis.

At 'gastrulation' (migration of cells to form the two layers of the larva) of *A. queenslandica*, *TGF-β* is expressed in the outer layer with expression domains at both poles of the larva. Later in development, however, *TGF-β* expression becomes restricted to an anterior pole domain suggesting a role for this pathway in establishing axial polarity (antero-posterior) along with the *Wnts* (Adamska *et al.*, 2007a). *Hedgehog* (*Hh*) family molecules are also found in sponges, however, the *Hh* protein (named *Hedgling* in *A. queenslandica*) lacks important features of true metazoan *Hh* genes (Adamska et al., 2007b). Nonetheless, *Hedgling* is expressed in a sharp narrow band around the developing pigment spot and ring in *A. queenslandica* larvae and the expression of *Wnt*, *TGF-β*, and *Hedgling* molecules form a series of concentric, partially overlapping domains around the forming ring, which might indicate an ancient co-expression cassette (Adamska *et al.*, 2011) along with their probable roles in the establishment of axial polarity.

In *A. queenslandica*, the *Notch* receptor and the *Delta* ligand are co-expressed in the outer layer of the developing larva, and subsequently in cells of the larval subepithelia. Later, *Notch* remains expressed in the subepithelial layer and the *Delta* ligand becomes highly expressed in globular cells that are located beyond the subepithelia (Richards *et al.*, 2008). Richards *et al.* (2008) propose that *Notch-Delta* signalling plays a role in globular cell genesis and that the globular cells of these larvae may be related to eumetazoan neurons. Along with *Notch* and *Delta*, expression of the transcription factor *bHLH* is evident in the outer layer of the gastrulating embryo in subpopulations of cells (before the subepithelial layer forms). At the early pigment ring stage, *bHLH* is restricted to the subepithelial layer along with *Notch* and *Delta* and remains expressed in this layer along with Notch even later in larval development (Richards *et al.*, 2008). Interestingly, *A. queenslandica bHLH* demonstrates proneural activities in both *Xenopus* and *Drosophila* when the sponge gene is over-expressed in these organisms. Like *Xenopus neurogenin*, *A. queenslandica bHLH* can induce ectopic expression of *Xenopus N-tubulin* and *Delta* and can activate *Xenopus Notch*. In flies, *A. queenslandica bHLH* can induce formation of ectopic sensory bristles (Richards *et al.*, 2008). These experiments indicate a conservation of important amino acid residues related to *bHLH* function as found in genes such as

atonal and *neurogenin* in bilaterians. These data support the idea that a functional *Notch-Delta* signalling pathway was also likely to have been present in Urmetazoa (Gazave *et al.*, 2009). However, these authors' notion that expression of *Delta*, *Notch* and *bHLH* during globular cell genesis in *A. queenslandica* is suggestive of bilaterian neurogenesis needs considerable further support.

Two other transcription factors have been evaluated during *A. queenslandica* gastrulation and larval development. *NF-kB* shows post-cleavage expression broadly, but especially in large granulated cells. Later, during cell differentiation and migration, these granular cells migrate to the outer layer and the expression of *NF-kB* persists in these cells in the outer and subepithelial layers. Expression is also observed in the so-called 'flask' cells that are ciliated and interspersed through the columnar epithelia. By late larval stages, expression of *NF-kB* is confined to flask cells of the subepithelial layer along with some expression observed in the inner cell mass (Gauthier and Degnan, 2008). The role that this transcription factor plays in sponge development is unclear, but the expression patterns are dynamic and in some cases cell specific, indicating that this could also serve as a good marker for flask cells during late larval development. It should be mentioned that the repeated expression of genes in 'flask' (globular) cells could reflect a 'stem cell' like property of these cells, but the possibility that those cells are 'sticky' and collect mRNA transcripts indiscriminately must also be considered.

The transcription factor *A. queenslandica Bsh* is expressed during gastrulation in a subset of cells in the outer layer and the inner cell mass. Later, however, during gastrulation, the expression of this gene is restricted to cells of the inner cell mass. Close inspection reveals that at least some of the cells within the inner cell mass that express *Bsh* are the larval sclerocytes (Larroux *et al.*, 2006). *Bar* (*Bar-/Bsh*) expression has also been studied in *Halichondria* where developmental regulation was observed during larval development (Hill *et al.*, 2004), and in this species, *Bar* expression is also restricted to the inner cell mass of larvae (Holstien *et al.*, 2010) and associated with sclerocytes (Hill, pers. obs.). It would be interesting to use functional analysis to determine if sponge *Bar* genes play roles in the specification of both larval and/or adult sclerocytes.

7.5. Developmental gene expression in larvae

In addition to the molecules discussed above, a few other developmental genes have been evaluated for expression in mature sponge larvae. In *A. queenslandica*, the *BZIP* transcription factor is expressed in two populations of cells of the inner cell mass. One pattern of expression is observed in a subset of cells that is directly adjacent to the boundary of the inner cell mass and subepithelial layer (making a ring pattern around the cell division). The other set of cells that strongly express *BZIP* are in the very centre of the

inner cell mass (Larroux *et al.*, 2006). This finding indicates that there may be some cell specification/differentiation within the inner cell mass of the larvae. The metazoan-specific transcription factor *HNF4* is also expressed in *A. queenslandica* larvae where it is restricted to the outer epithelial layer and localized in small ciliated columnar cells (not in the larger epithelial cells like the mucous or flask cells, and also not around the pigment ring) (Larroux *et al.*, 2006). Thus, the aforementioned transcription factors do not have overlapping expression in mature larvae and at the least could be used along with *Bar* as markers for the inner cell mass and outer epithelial layers.

The *T-box* family of transcription factors has multiple representatives in Porifera (Adell *et al.*, 2003; Manuel *et al.*, 2004; Larroux *et al.*, 2006, 2008). In *Halichondria*, larval expression patterns for five family members have been evaluated (Holstien *et al.*, 2010). For two of the *T-box* transcription factors (*Tbx4/5* and *TbxPor*), expression profiles in free-swimming larvae reveal potential involvement in axial polarity. *TbxPor* is expressed at both the anterior and posterior poles. At the posterior pole, expression is evident and forms a gradient that seems to be strongest in the most posterior cells, whereas in the anterior region, expression is observed in distinct cells along the subepithelial layer and extends around the lateral sides of the larvae (possibly in 'flask' cells of the subepithelium). *Tbx4/5* also shows expression concentrated at both poles of the larvae. Given that some *T-box* transcription factors are known to be downstream targets of *Wnt* signaling it will be interesting to determine whether or not this regulatory connection and function (i.e. establishment/maintenance of axial polarity) is utilized in sponges.

7.6. Gene expression patterns in juvenile and adult sponge tissues and cells

As is the case in other metazoans, many of the genes expressed during early development are also utilized in forming and maintaining the tissues of the adult sponge. Many of the transcription factors discussed above are also expressed during various stages of metamorphosing, juvenile and adult sponges (e.g. Seimiya *et al.*, 1997; Coutinho *et al.*, 2003; Adell and Müller, 2004; Hill *et al.*, 2004, 2010; Larroux *et al.*, 2006). However, little is known about their tissue-specific expression patterns or the roles they play in shaping adult sponge body plans.

In the demosponge *S. domuncula*, the homeobox transcription factor *Iroquois* is expressed in cells adjacent to canals (probably endopinacocytes) that form the epithelium of the canal structure. This gene is also turned on during the formation of canals in developing sponge aggregates (primorphs) (Perović *et al.*, 2003). In *Chalinula loosanoffi*, the transcription factors *PaxB* and *Six1/2* are also transcribed in pinacodermal cells (i.e. pinacodermal lining of the atrial opening of the oscular chimney) as well as in the immediately adjacent choanosome (Hill *et al.*, 2010). In *E. muelleri*, *PaxB* and *Six1/2* are both

expressed in multiple cell types, and most notably in endopinacocytes of the canals. *PaxB* and *Six1/2* are also both expressed in cells or small groups of cells that are at the junction between choanocyte chambers. Given the placement, shape and occurrence of these cells, it is likely that they are archeocyte cells that are specified to develop into choanocytes. Very low levels of *PaxB* and *Six1/2* are found in fully formed choanocyte cells (April Hill, personal observation). Recent data indicate that when *PaxB* or *Six1/2* expression is knocked-down by RNAi, the resulting sponges have poorly formed canals with missing pinacocytes (Hill, personal observation). Thus, it seems likely that one role for *Iroquois*, *PaxB* and *Six1/2* may be in the development of the endopinacocytes of adult sponges.

In the homoscleromorph sponge, *Oscarella lobularis*, an *NK* homeobox transcription factor is expressed strictly in the choanocyte cells of the chambers in both reproductive and non-reproductive adult tissue (Gazave *et al.*, 2008). In the freshwater sponge *E. fluviatilis*, two additional genes are also known to be expressed in choanocytes. While these genes are not formally transcription factors or signal transduction pathway genes, they merit mention here. The *annexin* gene is expressed in choanocyte-committed archeocytes and chamber-forming choanocytes (Funayama et al., 2005a; see also Section 3.5). Additionally, the stem cell marker *Piwi* is expressed in archeocytes and choanocytes of *Ephydatia* and studies from this group suggest that choanocytes might represent ancestral-type stem cells (Funayama *et al.*, 2010; see also Section 5.4).

8. The Immune System

8.1. Molecules with a potential role in the immune response in sponges

A host of genes that are involved in the immune system function in bilaterians are present in sponge genomes. Importantly, these include genes that play roles in cell adhesion and possibly allorecognition (i.e. aggregation factors; see Müller, 2003), as well as a host of metazoan specific factors such as molecules in the *Toll-like receptor* (*TLR*) and *interleukin1 receptor* (*IL-1R*) pathways, *macrophage expressed gene 1* (*MPEG1*), *interferon regulatory factor-like proteins* and *MDA-5-like* RNA helicases regulated upon exposure to LPS in primorphs (Schröder *et al.*, 2003). Thus, it may be that these lectin proteins are involved in sponge bacterial defense.

One of the most important and best-characterized sets of pathways involved in innate immune defense are the *TLR* and *interleukin 1* (*IL-1R*) receptor pathways. First isolated and reported for sponges in *S. domuncula*, *TLR* transcripts are expressed constitutively in epithelial layers of the sponge surface (Wiens *et al.*, 2007). In an earlier study, Wiens *et al.* (2005) found

that *myeloid differentiation factor 88* (*MyD88*), a protein containing a Toll/interleukin-1 receptor domain and a death domain, is up-regulated when sponges are exposed to LPS. Another gene of the macrophage-expressed protein class is also expressed highly at the surface in the sponges after LPS treatment. These data indicate that sponges may have a system in place to recognize Gram-negative bacteria (Wiens *et al.*, 2005). Additional work showed that *caspase* gene product is increased in *S. domuncula* during treatment with LPS, and that when recombinant *TLR* was added, it could prevent the induced expression of *caspase* (Wiens *et al.*, 2007). These data support the hypothesis that an active *TLR* pathway is utilized in sponges. Recent sequencing of the *A. queenslandica* genome revealed that although a conventional *TLR* or *IL-1R* receptor is not present, there are two highly-related receptors that contain *IL-1R-like* immunoglobulins (*Ig*) extracellular domains and an intracellular *TLR-like* (*Toll/interleukin 1*) receptor/resistance domain (called *IgTlRs*) (Gauthier *et al.*, 2010). Most of the other members of the conserved pathway (e.g. *Tollip*, *MyD88*, *Pellino*) are also present in this demosponge (Gauthier *et al.*, 2010). The authors of the latter study correctly point out that the *Toll* pathway is also known to play important roles in animal development and their study clearly demonstrates expression of *IgTlR*, *MyD88*, *Tollip* and *Pellino* orthologs during larval morphogenesis. Interestingly, *IgTlR*, *Tollip* and *Pellino* are expressed in the globular cells of the larvae (Nehyba *et al.*, 2009; Gauthier *et al.*, 2010; Srivastava *et al.*, 2010).

One possible marker in sponges of cells involved in innate immunity is the *lectin* gene. In *E. fluviatilis*, this gene is not expressed early in sponge development, but is expressed at late stages in large, differentiated cells that are distributed throughout the sponge body and localized beneath epithelial cells (Funayama et al., 2005b). The *E. fluviatilis lectin* gene is most similar to a *lectin* gene isolated in *S. domuncula* called *GBP* (*galactose-binding protein*). The *S. domuncula GBP* gene is able to bind to lipopolysaccharides (like the bacterial endotoxin LPS) and can inhibit bacterial growth (Schröder *et al.*, 2003). Indeed, *S. domuncula GBP* is up-regulated upon exposure to LPS in primorphs (Schröder *et al.*, 2003). Thus it may be that these *lectin* proteins are involved in sponge bacterial defense. Studies determining whether or not the *TLR* and *interleukin 1* receptor pathways play roles in immunity as well as development in *A. queenslandica* will help clarify the function of these pathways in innate immunity.

9. Conclusions

Whether or not sponges represent the oldest extant phylogenetic lineage, they are clearly a crucial group of ancient non-bilaterian animals whose structure and physiology will shed light on the evolution, development and

molecular genetics of all animals. Here, we try to revisit the comparative morphology and physiology of sponges in light of a growing body of data that reveals the molecular machinery underlying cell and tissue function in these animals. Important advances have been made in understanding the sponge epithelium, cell adhesion and junctions, differentiation of the aquiferous system, coordination and contraction, as well as possible aspects of a sensory system. A host of signaling pathway components and other genetic factors have also been uncovered leading to a more complete assessment of the molecules that play roles in sponge development and function. However, it is clear that there is much more work to be done on every level (molecular, cellular, physiological) before we can understand more fully the origin of multicellularity, the evolution of tissues, signal pathways, body polarity and coordination systems in the sponges and, indeed, across the animals.

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