

Epithelia and integration in sponges

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Synopsis An epithelium is important for integrity, homeostasis, communication and co-ordination, and its development must have been a fundamental step in the evolution of modern metazoan body plans. Sponges are metazoans that are often said to lack a true epithelium. We assess the properties of epithelia, and review the history of studies on sponge epithelia, focusing on their homology to bilaterian epithelia, their ultrastructure, and on their ability to seal. Electron micrographs show that adherens-type junctions are present in sponges but they can appear much slighter than equivalent junctions in other metazoans. Fine septae are seen in junctions of all sponge groups, but distinct septate junctions are only known from Calcarea. Similarly, all sponges can have collagenous sheets underlying their epithelia, but only homoscleromorphs are established to have a distinct basal lamina. The presence of most, but not all, gene families known to be involved in epithelial development and function also suggests that sponge epithelia function like, and are homologous to, bilaterian epithelia. However, physiological evidence that sponge epithelia regulate their internal environment is so far lacking. Given that up to six differentiated epithelia can be recognized in sponges, distinct physiological roles are expected. Recognition that sponges have epithelia challenges the perception that sponges are only loose associations of cells, and helps to relate the biology and physiology of the body plan of the adult sponge to the biology of other metazoans.

Introduction and background

It is often said that sponges are simple animals, meaning that along a gradient of increasing complexity in the evolution of body plans in metazoan phyla, sponges are at the lower end. “Simple” implies being constructed of fewer parts with less hierarchical organization. In fact, sponges are usually described as lacking organs, tissues, nerves, muscle, and even epithelia. However, not only is this view not very useful for understanding what characteristics the animals have, there is also mounting evidence that extant sponges in fact possess quite a high level of complexity. We focus here on epithelial tissues because they, especially, determine the extent and manner of integration possible in an organism, since signaling and co-ordination must occur across or via epithelia, and ought to be at least somewhat similar among organisms in different phyla.

It seems remarkable that it is still necessary to examine tissue structure in order to show that a multicellular animal in fact has an epithelium. Nevertheless sponges are the only group of multicellular animals in which the presence of a true epithelium is still questioned by many authors and in many texts. Knowledge of epithelia in other

animals has advanced, while that of epithelia in non-bilaterian phyla has lagged and details have become confused.

All early authors referred to the outer layer of the sponge as an epithelium, but that layer was thought to be syncytial because cell borders were not visible (Bowerbank 1864; Haeckel 1872; Wilson 1910; Parker 1919). It was not until electron microscopic studies confirmed that borders exist between cells that demosponges and Calcarea were understood to have cellular epithelia composed of plate-like cells, pinacocytes. The “parenchyma” referred to by earlier authors is now understood to be a region of extracellular matrix components and motile cells that is enclosed by epithelial tissues. This is in contrast to the situation in glass sponges (Class Hexactinellida), which electron microscopy and live video imaging of moving tissue have shown to be truly syncytial (Mackie and Singla 1983; Leys 1995).

The view reiterated in many reviews and texts, that sponges lack true epithelia, is based on the apparent absence of properties known from other epithelia: apical–basal polarity of the cells, a basal lamina of extracellular matrix fibers, and cell junctions that adhere and, more important seal the

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epithelium, thereby protecting and controlling the internal physiology of the tissue.

It is probably the thinness of the sponge pinacoderm that makes histologists most doubtful of its ability to seal physiologically; although many cell junctions have been found in sponge epithelia, experimental evidence for their ability to seal is extremely limited. With the first sponge genome now on the horizon, there is already some idea of which genes involved in cell junctions are present in sponges, and so we can expect new morphological work testing the presence of junctions containing proteins homologous to those forming junctions in other metazoans.

The “unicellular ancestry” of metazoans

From the comparative study of extant metazoans we can infer the minimal features of the last common metazoan ancestor. Specifically, like modern metazoans, this ancestor likely had differentiated cell types (i.e., division of labor), dimorphic gametes, highly regulated developmental programs, and rudimentary (at least) epithelial tissues. Collectively, these characteristics could have permitted a degree of integration comparable to that of modern sponges.

Current phylogenies robustly support choanoflagellates as the closest unicellular relatives of animals (Lavrov et al. 2005; Rokas et al. 2005; Cavalier-Smith 2006; Steenkamp et al. 2006; Carr et al. 2008). Due to the vast, understudied diversity of modern choanoflagellates, there is less certainty about which of the characteristics of extant lineages were shared by their last common ancestor with metazoans. Minimally, we hypothesize that this ancestor had an obligate unicellular life-history stage and that it was an apically polarized, bacterivorous flagellate. Among modern choanoflagellates, there are numerous examples of transient colony formation but, given differences in their ultrastructure and their phylogenetic distribution within choanoflagellates (Carr et al. 2008), it is not clear that these structures directly relate to metazoan multicellularity. Where known, cell contact in colonies is achieved through direct contact between adjacent membranes at the feeding collar, or through intercellular connections that resemble cytoplasmic bridges (Hibberd 1975; Leadbeater 1983; Karpov and Coupe 1998). Evidence for cellular differentiation within colonies is scant, but colonies and single cells do routinely secrete an extracellular theca that may have elements of homology to the extracellular matrix of metazoans.

What is an epithelium and why is it important?

Epithelia are associations of cells that line a compartment or the outer surface of an organism. Epithelia provide a physiological barrier (controlled or restricted passage) to ions and thereby regulate the internal milieu. They can be formed by single or multiple layers of cells in a variety of shapes—cuboidal, flat or squamous, or columnar. Multiple layers may be stratified (in tiers) or pseudostratified (in which tiers of nuclei are interlayered).

Most importantly, epithelia vary in their capacity to seal, ranging from those that seal absolutely, such as the dermis of terrestrial animals to those that are ‘leaky’, such as mammalian capillary epithelia and some aquatic invertebrate epithelia. Unfortunately, it is difficult to determine epithelial sealing ability. This challenge is illustrated by a situation in which tracers (e.g., Lanthanum or peroxidase or ruthenium red) readily pass through intercellular junctions, as cells are actively pumping ions across, thereby countering leakiness and maintaining osmotic or electrical gradients. Such circumstances indicate that functionally tight epithelia can have leaky intercellular junctions (Skaer and Maddrell 1987). For example, septate junctions of insect Malpighian tubules are physiologically sealed with an electric potential of 100 mV and concentration gradient of 3000:1, yet they allow large molecules like sucrose and charged molecules like Lanthanum to pass through completely or partially (O’Donnell et al. 1984; Skaer et al. 1987); passage by all other routes has been systematically ruled out in these experiments.

Epithelia are characterized morphologically by having apical–basal polarity, intercellular junctions, and a basal and apical extracellular matrix that function in adhesion and restrict the passage of molecules and cells into adjacent tissues. Epithelial cell junctions fall into three categories—adhesion (*zonula adhaerens* and *macula adhaerens* also known as intermediate junctions and desmosomes), sealing (*zonula occludens*, also known as tight or septate junctions), and communication (gap junction or aqueous connections)—which were first defined based on their morphological characteristics, but are increasingly understood by their molecular components. Functional differences within these junctional categories in different tissues have physiological consequences, but the mechanisms underlying these differences are not completely understood and do not obviously relate to their

ultrastructure. For example, tight junctions at the blood/brain barrier prevent even select small molecules from entering the brain, whereas tight junctions in nonneural tissues are much leakier (Hirase et al. 1997).

Adhesive junctions, either *adhaerens* junctions or desmosomes, are found between cells that experience tension and may surround the entire cell as a belt, or form punctuate zones of attachment that integrate with the cytoskeleton. Cells and their intercellular junctions in epithelia are considered to be relatively static, but in developing embryos, and in regenerating and growing tissues such as capillary epithelia and mammary glands, epithelial cells are in constant movement and junctions assemble and disassemble (Ewald et al. 2008). Epithelia are also expected to have sealing junctions of some sort. In vertebrates, tight junctions appear as a zipper-like band of protein–protein contacts that bring membranes of opposing cells together and prevent the passage of large ions into the intercellular space. In invertebrates, septate junctions are considered to perform the same function, although most tracer studies show the passage of ions at least part-way between adjacent cells, even in the presence of septae (Leik and Kelly 1970; Hand and Gobel 1972). New work showing that claudins—molecular components of tight junctions—exist in *Drosophila* septate junctions suggests that these sealing junctions have more in common than previously thought (Furuse and Tsukita 2006).

Basement membranes function both as structural barriers and in the adhesion of epithelial cells. Structurally they consist of a sheet of extracellular matrix proteins, including laminins and collagens. In general, Type IV collagen is important for maintaining stability of basement membranes, but during tissue remodeling and development laminins alone are sufficient (Poschl et al. 2004).

In summary, epithelia are suites of cells with versatile sealing and adhesion complexes, with a diverse complement of molecules that enable the physiological control of ionic compartments. The function of molecules, proteins, and cells in sealing is only just beginning to be understood. Epithelia may have some or all of the adhering, sealing, and polarizing molecules and junctions, but the absence of any one of these in many animals does not mean that the tissue does not perform the physiological functions of an epithelium. When so much about tissues still remains to be understood, it does not make sense to have a binding set of criteria of what is to be considered an epithelium.

Evidence that sponges have epithelia

Regardless of morphological evidence for cell junctions in sponge tissues, unless they are found to have the same complement of genes and proteins as in the junctions of other metazoans, there will still be resistance to accepting their homology. Even if the same complement of genes known in other invertebrate junctions is found, unless the morphology appears identical, interpretation of the structure will remain vague in the absence of data detailing the effects of gene function (or loss of function) on tissue function (e.g., the ability to form a physiological seal). For example Sakaraya et al. (2007) have shown that there is “nearly absolute conservation of binding domains and ligands” between sponges and animals with neurons, suggesting potential functional homology of this ‘protosynapse’, but there is no ultrastructural correlate of the postsynaptic density in sponges. So what evidence is there that sponges do have an epithelium?

Reports of the “transient nature” of sponge intercellular interactions (Green and Bergquist 1979) or that epithelial cells “are not joined together by occluding junctions” (Mackie 1990), are based on static sections rather than on physiological evidence. Videos that show sponge cells crawling focus on the highly active cells in the mesenchyme or on pinacocytes at the edge of an explanted fragment of tissue where motility can be ascribed to wounding. Two of us (S.P.L. and E.D.M.A.) have carried out tracer studies on live cells of the surface epithelium of freshwater sponges grown in culture, and find that cells in the undamaged epithelium do not let go of their connections with neighboring cells, any more than cells in other animal epithelia. Cells labeled with the vital lipophilic tracer FM 1-43 maintained their relationship to one another for over 1 h of observation (Fig. 1); for longer periods the entire animal moves, and the epithelium with it as a sheet. Only cells in the mesohyl move in and out of the field of view, but these cells are in the middle collagenous layer of the animal, not in the epithelial sheet.

Behavioral studies show that the entire sponge inflates and contracts to expel water from the canals and chambers (Pavans de Ceccatty 1969; de Vos and Van de Vyver 1981; Weissenfels 1984, 1990; Nickel 2004; Elliott and Leys 2007). As waves of contraction pass through canals, cells in the mesohyl stop crawling and resume motion once the contraction has passed. This observation suggests that a signalling molecule is released into the mesohyl;

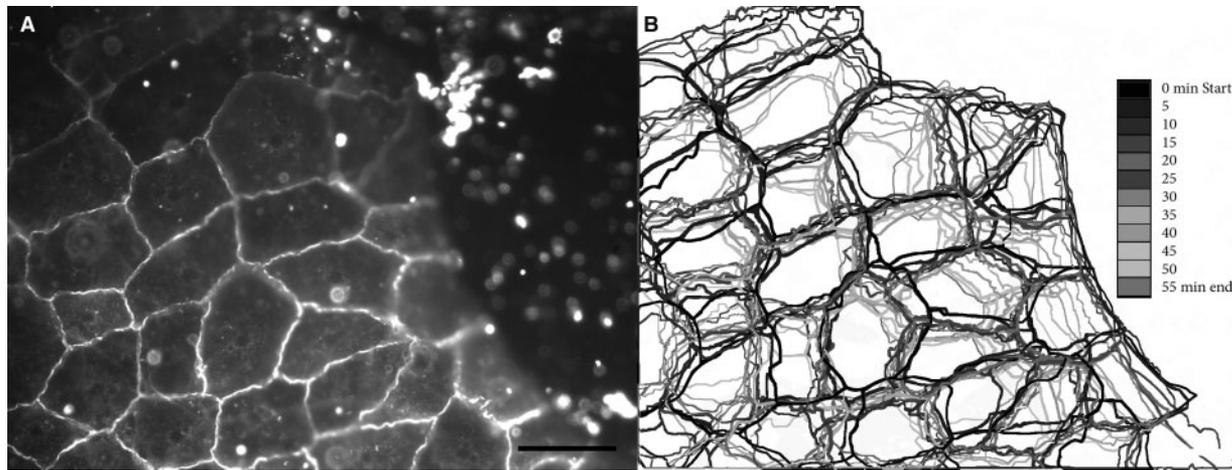


Fig. 1 Exopinacocyte movement in *E. muelleri*. Cell migration was tracked over time using fluorescent microscopy. (A) Apical exopinacocytes in the periphery of a juvenile sponge were highlighted with the lipophilic dye FM 1-43 (Scale = 50 μ m). Images of the external layer of cells were captured every 5 min and cell outlines, (B) were traced using Photoshop CS2. Tracings were overlaid and show epithelial stability over time as evidenced by line overlap particularly in the focal region in the centre. Cells retained their shapes and connections to neighboring cells over time and they acted as a cohesive sheet when the whole sponge underwent a contraction.

if epithelia were not sealed we would expect secreted molecules to be easily lost from the extracellular space, and if the epithelia were not held together well, we would expect contractions to cause the sponge to fall apart. Experiments to determine resistance to passage of ions across the membrane (transepithelial electrical resistance), the ultimate evidence for physiological capacity to seal, have just begun, and preliminary results suggest that sponge epithelia seal at least as well as monocultures of mammalian epithelia (EDMA, unpublished data).

Ultrastructural studies provide morphological evidence for cell–cell junctions in sponge epithelia. The lack of really good ultrastructural evidence for junctions may be due in part to poor fixation of tissues, but presumably it also reflects the absence of some protein complexes. Cells in sponge epithelia typically have a constant spacing of 10–20 nm (Green and Bergquist 1979); along such appositions there are reports of *adhaerens* and desmosome-like junctions (e.g., Pavans de Ceccatty 1981; Lethias et al. 1983) (Fig. 2A–F). In some images, there are regions in which cell membranes appear fused (Fig. 2E); Revel (1966) described these features as “similar or identical to the tight junctions of vertebrates”. He called small areas of contact between cells in *Microciona prolifera* small *macula occludens*, and other wide areas of contact as having “the typical appearance of a tight junction”. The proteins involved in these points of contact remain to be studied.

Many authors report septate junctions between cells but the ultrastructure of these is inconsistent. One image from the demosponge *Hippospongia communis* shows points of adhesion between opposing membranes (Pavans de Ceccatty et al. 1970, Fig. 2G). According to de Vos (1977), interdigitations of cells surrounding the gemmule of *Ephydatia fluviatilis* have septate junctions, but the septae are very difficult to see in the micrographs (Fig. 2H). A septate-like junction has been shown between the apical portions of larval epithelial cells in *Oscarella lobularis* and *Corticium candelabrum* (Boury-Esnault et al. 2003; Maldonado and Riesgo 2008). This junction also appears to have dense regions at the membranes, but variable staining and poor preservation of the membranes makes interpretation difficult (Fig. 2I–K). When cryofixation was used, no evidence of septate junctions was found in larvae of the related species *Oscarella carmela* (SAN). In the glass sponge *Rhabdocalyptus dawsoni* Mackie and Singla (1983, Fig. 2L) described a septate junction between an archaeocyte and the trabecular syncytium. By far the most distinct septate junctions, however, are seen in calcareous sponges. Green and Bergquist (1979) showed septae between some choanocytes in *Clathrina* sp., and Ledger (1975) showed excellent images of septate junctions between all sclerocytes in *Sycon ciliatum* where they are presumed to allow a sealed environment for calcification (Fig. 2M–O).

Basement membranes have not been widely sought in sponges. In homoscleromorph sponges, there is a

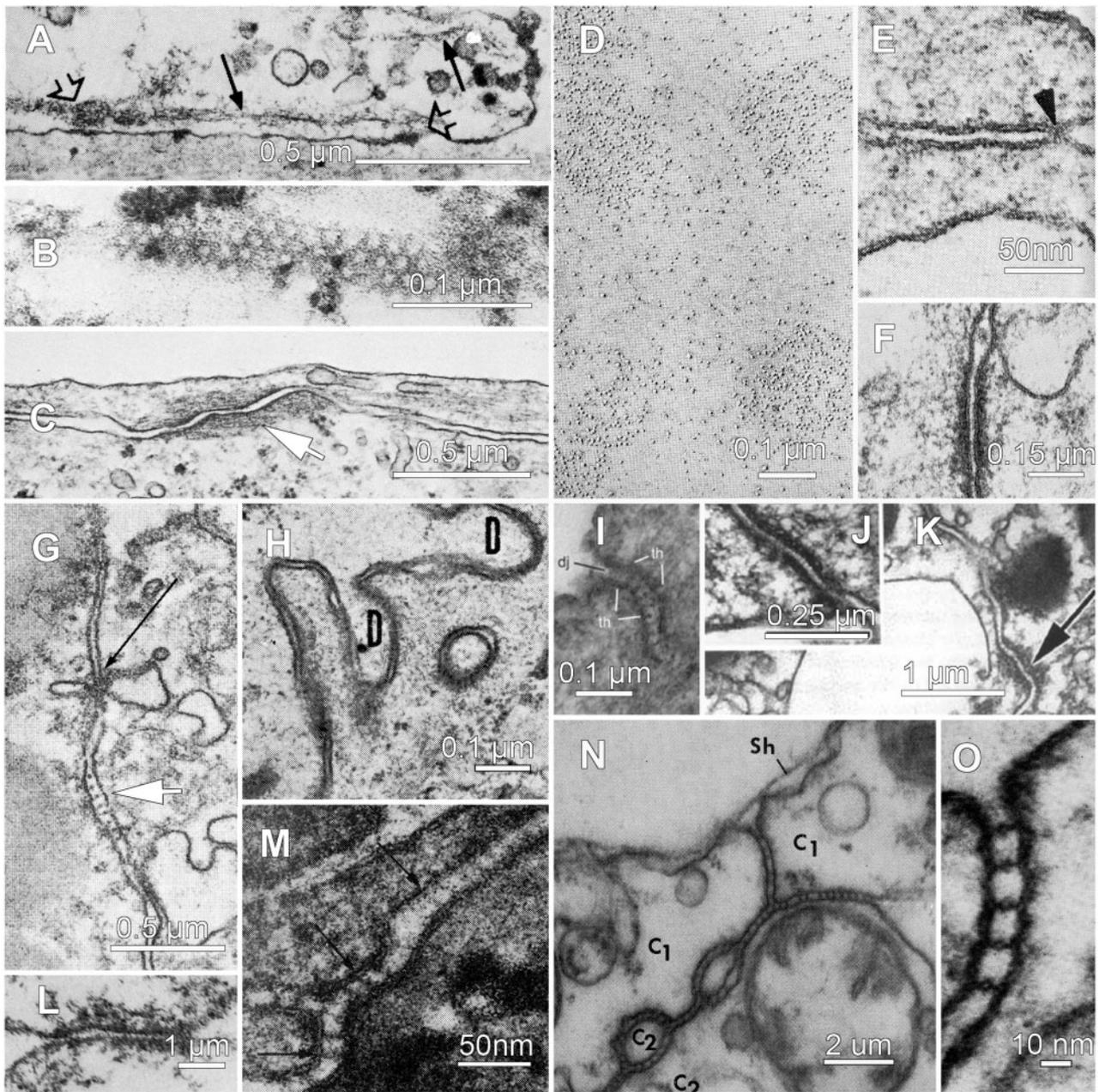


Fig. 2 Ultrastructure of cell junctions in sponges. A suite of potential cell–cell junctions has been shown in different sponges. (A–D) Attachment plaques in *E. muelleri* and *E. fluviatilis*. (A) Filamentous actin (small arrows) in the basal epithelium with possible focal adhesions (open arrows) at the contact with the underlying extracellular matrix. (B) A region of cell contact between two epithelial cells. (C) Desmosome-like junction between two epithelial cells. (D) A freeze-fracture showing groups of particles on the p-face of a pinacocyte. (E) Close apposition of membranes in two epithelial cells in *Verongia cavernicola*. (F) Desmosome-like junction along the membranes of neighboring cells in *H. communis*. (G–O) Septate-like junctions in sponges. (G) Punctate staining (white arrow) and vesicles (black arrow) between two cells in *H. Communis*. (H) Interdigitations (“D”) between cells coating the gemmule in *Ephydatia fluviatilis*. Punctate staining of possible septate junctions between the apical regions of the larval epithelium in (I) *Corticium candelabrum*, and (J and K) *Oscarella lobularis*. (L) Fine punctate staining between membranes in *R. dawsoni*. (M) Septae in the junction between choanocytes in *Clathrina* sp. (N and O) Septate junctions between several cells (C1–C2) in *S. ciliatum*. [Figures reproduced with permission from: A and B, Pavans de Ceccatty (1981); C–F, Lethias et al. (1983); G, Pavans de Ceccatty et al. (1970); H, de Vos (1977); I, Maldonado and Riesgo (2008); J, K, Boury-Esnault et al. (2003); L, Mackie and Singla (1983); M, Green and Bergquist (1979); N, O, Ledger (1975)].

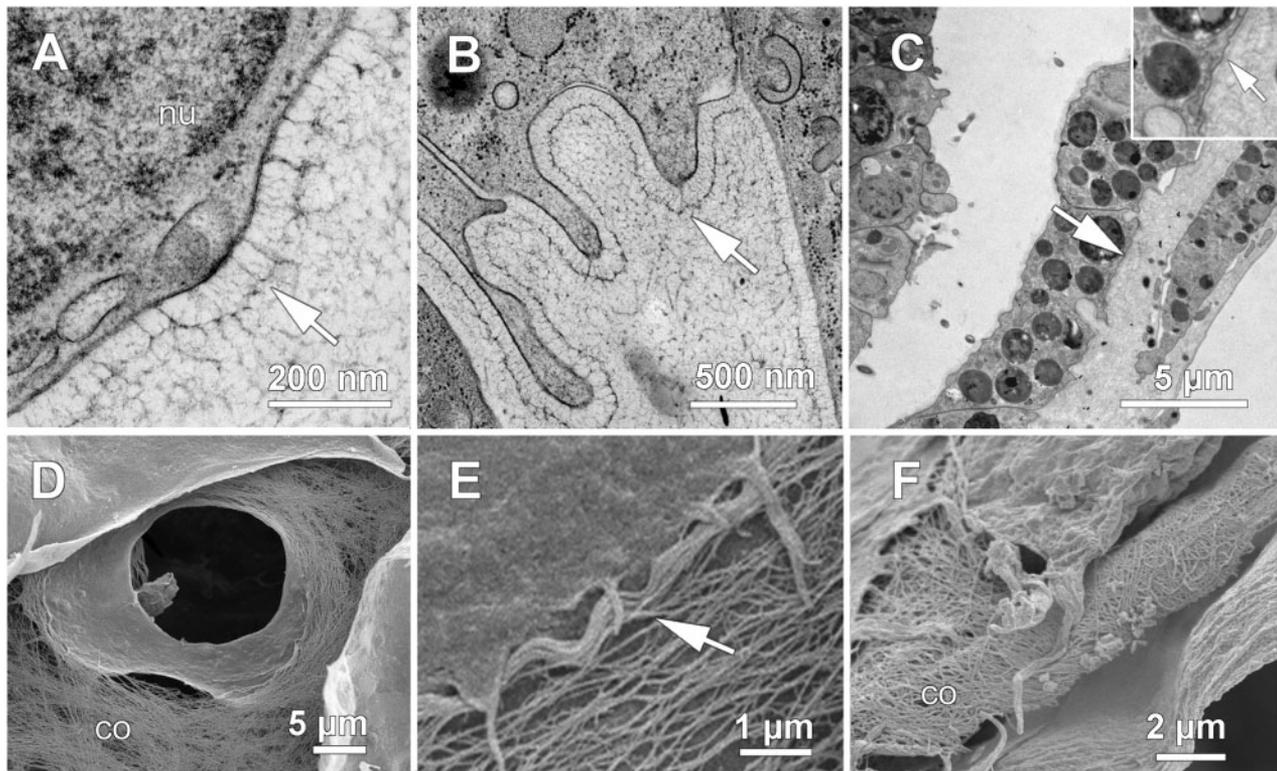


Fig. 3 Basement membrane and collagenous matrix. In the homoscleromorph *Oscarella carmela* thin section transmission electron microscopy shows the basal lamina as a fine extracellular mesh lying 80–100 nm below the choanoderm (A), larval epithelium (B), and follicular envelope (C). A similar feature cannot be found in *Ephydatia* by TEM, but a tightly meshed sheet of collagen underlies the exopinacoderm, (D–F) supporting pinacocytes and porocytes which anchor to the matrix by minute filopodia (A–C, S. A. Nichols unpublished data; D–F, S. Leys unpublished data).

fine but distinct network of fibrils just under cells in the canal and choanocyte epithelia to which the cells are attached (Fig. 3A). Epithelial cells of the larva (Fig. 3B) and the follicular envelope (Fig. 3C) have the same network, and evidence that this layer contains Type IV collagen (Boute et al. 1996) suggests this is a classical basement membrane. All sponges are known to concentrate collagen fibrils in areas that require support, such as the ectosome (Simpson 1984), but although the collagen there does form a sheet, and may be used by cells as an anchor, in both the adult and the larva (Fig. 3), it rarely resembles the typical basal lamina of homoscleromorphs or other metazoan tissues. Nevertheless, the DNA sequence of nonfibrillar collagen (termed “spongins short chain collagen”) has structural features in common with Type IV collagen and its 3D structure, and hence its function has been estimated by modeling to be the same as Type IV collagen (Exposito et al., 1990; Aoucheria et al., 2006).

In a few months the first sponge genome will be published, but from the publicly available trace files

and other sequences submitted to GeneBank it can be seen that sponges possess genes involved in apical–basal polarity, adhering, and sealing. Whereas molecules known from classical sealing junctions appear to be missing (e.g., *zonula occludens* or ZO1), other genes that encode cell-junction proteins are present in the sponge genome. Four classes of molecules are of particular interest: integrins, cadherins and β -catenin, and proteins of the MAGI family (Fig. 4). Membrane Associated Guanylate Kinases (MAGuK) are scaffolding proteins associated with tight junctions. MAGUK homologs with inverted arrangement, MAGIs, are highly conserved in sponges, cnidarians and other metazoans, and the MAGI gene is expressed particularly in the outer pinacoderm and choanoderm of *Suberites domuncula* (Adell et al. 2004). Integrins and cadherins—which form the functional core of adherens junctions, desmosomes, and focal adhesions—have been demonstrated in a few disparate lineages of sponges and immunolocalization and biochemical data demonstrate that these proteins have conserved localization patterns, binding partners, and regulatory

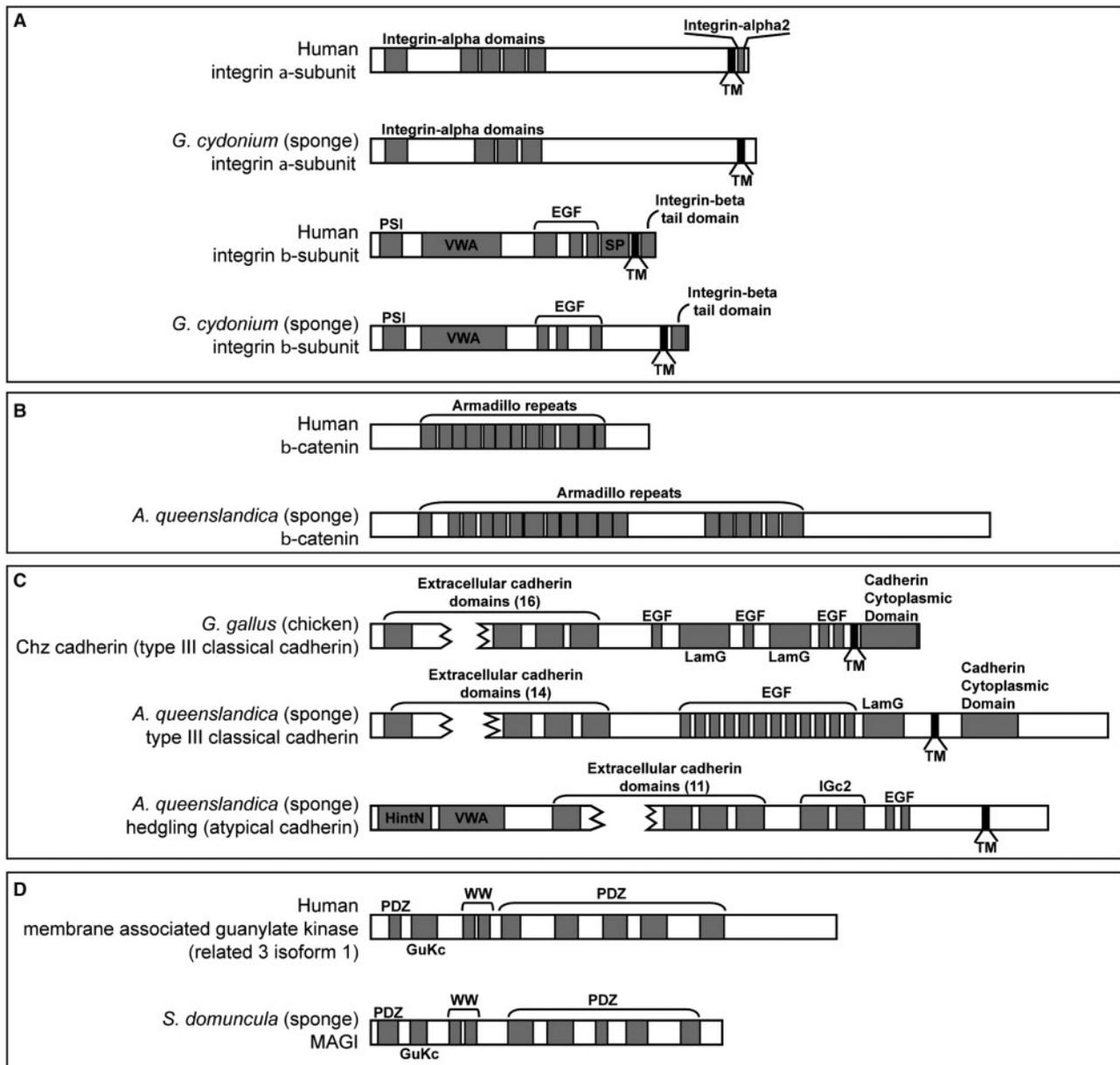


Fig. 4 Conservation of select components of cell junctions in sponges. The conservation of cell-junction proteins in sponges raises the possibility that epithelial cell interactions are regulated by mechanisms akin to those that operate in bilaterians. Specifically, integrins (A) localize to basal-cell membranes where they interact with components of the extracellular matrix at focal adhesions, whereas catenins (B) and cadherins (C) localize to adherens junctions and desmosomes at points of cell–cell contact. The molecular evidence for barrier-forming junctions in sponges is presently restricted to the tight-junction scaffolding protein MAGI, (D) [MAGI sequences from Human MAGI3 isoform1: NM_001142782.1; (Wu et al. 2000), Suberites MAGI: CAE45014; (Adell et al. 2004); Geodia integrin α : CAA65943; Geodia integrin β : CAA77071; Amphimedon hedgling: ABX90059.1 (Sakaraya et al. 2007)]. SP, signal peptide; PSI, plexin/semaphoryin/integrin domain; Arm, armadillo domain; LamG, laminin G domain; CCD, cadherin cytoplasmic domain; INB, Integrin β subunits; HintN, Hint (Hedgehog/Intein) domain N-terminal region; Beta_TD, beta tail domain; VWA, von Willebrand factor A domain; Int_ α 2, Integrin α β -propellor repeats; EGF related, epidermal growth factor domain related; Int_ α , integrin α cytoplasmic tail domain; TM, transmembrane domain; Igc2, immunoglobulin C-2 type domain; EC, extracellular cadherin domain; PDZ, Post-synaptic density protein 95; Drosophila discs large suppressor and Zonula occludens-1; WW, conserved tryptophans domains; GuKc, P-loop containing nucleoside triphosphate hydrolases.

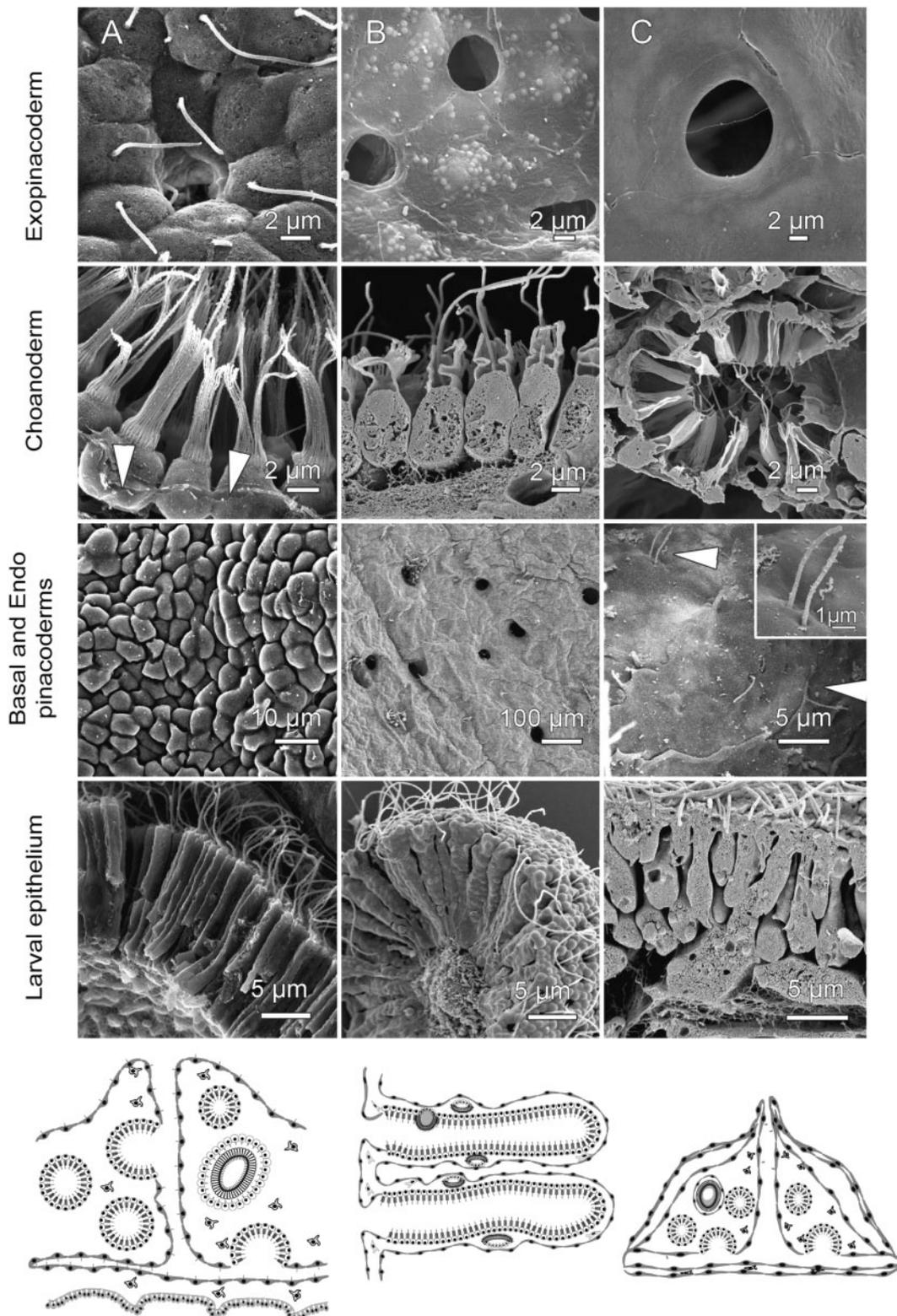


Fig. 5 Differentiated epithelia. (A) The homoscleromorph, *Oscarella carmela*, (B) calcareous sponge, *Sycon coactum*, and (C) demosponge, *Ephydatia muelleri*. The exopinacoderm in *O. carmela* is quite unusual in that cells are all ciliated. Cells are also more cuboidal than in either *S. coactum* or *E. muelleri*. Choanocytes form a feeding epithelium, the choanoderm, in all sponges. In *Oscarella* the fine network of basal lamina can be seen at the base of the choanocytes (arrowheads). Other regions of the adult sponges show differentiated epithelia. The basal pinacoderm of *Oscarella* lacks cilia, as do endopinacocytes of the atrial cavity in *Sycon*. However, endopinacocytes lining the osculum of *Ephydatia* and other freshwater and marine demosponges possess cilia. The function of cilia in each of these epithelia remains unknown. The larval epithelium of all three sponges is composed of columnar ciliated cells; the larval epithelium of *Eunapius fragilis* is shown in C. Diagrams illustrate the regionalization of epithelia in each sponge.

mechanisms in sponge epithelial tissues (Sabella et al. 2004; Sakaraya et al. 2007; Abedin and King 2008; S. A. Nichols manuscript in preparation).

Types of epithelia in sponges

The emerging picture is then, that diverse types of sponges have a number of ways of forming epithelia with grades of adhesion junctions and basement laminae, although the subtleties of the functioning of sponge tissue remain to be explored. In terms of predicting the functional complexity of sponge tissues, however, up to six different types of epithelia can be distinguished, which has not previously been highlighted. Here we give examples from only three groups of sponges, but many others remain to be analyzed.

First, *Oscarella carmela*, a homoscleromorph, has at least five morphologically distinct epithelial tissues (Fig. 5A) that include the outer epithelium (the exopinacoderm) which lines the exterior of the organism and internal water canals, the feeding epithelium (the choanoderm) comprised of collar cells, the attachment epithelium (the basal pinacoderm), the follicular epithelium that surrounds developing embryos, and the larval epithelium. The great ultrastructural differences between these epithelia suggest that they serve distinct structural or physiological functions.

Second, the calcareous sponge *Sycon coactum* appears to have four distinct morphological epithelial phenotypes (Fig. 5B): a plate-like outer (exo) pinacoderm, the choanoderm with 100 μm^2 sheets of choanocytes that can be peeled off intact from the live animal, the endopinacocytes lining the atrial cavity, and the larval epithelium.

Third, in freshwater sponges (Demospongiae, Spongillidae) at least three morphologically distinct epithelia can be recognized (Fig. 5C). These are the external surface (exopinacoderm), the basal pinacoderm which adheres the sponge to the substrate, and the larval epithelium. It is unclear whether the follicular envelope or lining of the sac in which embryos develop possesses a collagenous underpinning. However, in *Ephydatia muelleri* and in many marine demosponges, regions of the endopinacoderm are further specialized, as seen by the presence of cilia on all cells lining the osculum, and on certain cells of the canal epithelium. Cells forming these epithelia tend also to be elongate and contractile, unlike those of the exopinacoderm.

Thus, there are morphologically distinct epithelia in discrete regions of the sponge body in a diverse range of sponges. It is expected that the differences

between these tissues are functionally significant and that the developmental patterning of these tissues has relevance for relating the body plan of sponges to those of other animals.

Conclusions

Many animals, at some point in their development or in some region of their body, have surface tissues that lack one or more of the criteria commonly considered to define an epithelium, yet we still call these tissues epithelia. Sponges are no different. Taking together what we know about the morphology, molecular biology and physiology of sponge tissues, there is no good case for denying that sponges, too, have differentiated epithelia. Our questions should now focus on how sponge epithelia differentiate, and from which cell population during development, as well as on the levels of differentiation of different epithelia. We know a little about how choanocyte epithelia differentiate during growth, but we do not know how each epithelium differentiates during development, or whether cells in each epithelium are fully differentiated or are able to re-differentiate.

The significance of recognizing that sponges have differentiated epithelial tissues is two-fold: First, it challenges the all-too-common textbook view of sponges as “loose associations of transiently differentiated cells” and helps relate the biology and physiology of the adult sponge to the biology and physiology of other metazoans. Second, it provides a context for interpreting the emerging evidence that the last common metazoan ancestor had a gene set much like that of modern animals. Many cellular, developmental, and physiological processes known from bilaterians are likely to operate in sponges, but with less dramatic morphological manifestations. At the very least, with the understanding that there are epithelia in sponges, we can now ask how they function in sponges, and how their functions changed in the lineages leading to other animals.

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References

- Abedin M, King N. 2008. The premetazoan ancestry of cadherins. *Science* 319:946–8.
- Adell T, Gamulin V, Perovic-Ottstadt S, Wiens M, Korzhhev M, Muller IM, Muller WEG. 2004. Evolution of metazoan cell junction proteins: the scaffold protein MAGI and the transmembrane receptor tetraspanin in the demosponge *Suberites domuncula*. *J Mol Evol* 59:41–50.
- Aouacheria A, Geourjon C, Aghajari N, Navratil V, Deléage G, Lethias C, Exposito J-Y. 2006. Insights into early extracellular matrix evolution: spongin short chain collagen-related proteins are homologous to basement membrane Type IV collagens and form a novel family widely distributed in invertebrates. *Mol Biol Evol* 23:2288–302.
- Boury-Esnault N, Ereskovsky A, Bezac C, Tokina D. 2003. Larval development in the Homoscleromorpha (Porifera, Demospongiae). *Invertebr Biol* 122:187–202.
- Boute N, Exposito JY, Boury-Esnault N, Vacelet J, Nor N, Miyazaki K, Yoshizato K, Garrone R. 1996. Type IV collagen in sponges, the missing link in basement membrane ubiquity. *Biol Cell* 88:37–44.
- Bowerbank JS. 1864. A monograph of the British Spongiadae, Vol. 1. London: Ray Society.
- Carr M, Leadbeater B, Hassan R, Nelson M, Baldauf S. 2008. Molecular phylogeny of choanoflagellates, the sister group to Metazoa. *PNAS* 105:16641–6.
- Cavalier-Smith T. 2006. Cell evolution and earth history: stasis and revolution. *Phil Trans R Soc B* 361:969–1006.
- de Vos L. 1971. Etude ultrastructurale de la gemmulogues chez *Ephydatia fluviatilis*. *J Microsc* 20:15–20.
- de Vos L, Van de Vyver G. 1981. Etude de la contraction spontanée chez l'éponge d'eau douce *Ephydatia fluviatilis* cultivée en vitro. *Ann Soc R Zool Belg* 111:21–31.
- Elliott G, Leys SP. 2007. Coordinated contractions effectively expel water from the aquiferous system of a fresh water sponge. *J Exp Biol* 210:3736–48.
- Ewald AJ, Brenot A, Duong M, Chan BS, Werb Z. 2008. Collective epithelial migration and cell rearrangements drive mammary branching morphogenesis. *Dev Cell* 14:570–81.
- Exposito J-Y, Garrone R. 1990. Characterization of a Fibrillar Collagen Gene in Sponges Reveals the Early Evolutionary Appearance of Two Collagen Gene Families. *Proc Nat Acad Sci* 87:6669–73.
- Furuse M, Tsukita S. 2006. Claudins in occluding junctions of humans and flies. *Trends Cell Biol* 16:181–8.
- Green CR, Bergquist PR. 1979. Cell membrane specialisations in the Porifera. *Colloq Int Cent Natl Rech Sci. Biologie des Spongiaires* 291:153–8.
- Haeckel E. 1872. Die Kalkschwämme, Eine Monographie. Berlin: Verlag von Georg Reimer.
- Hand AR, Gobel S. 1972. The structural organization of the septate and gap junctions of Hydra. *J Cell Biol* 52:397–408.
- Hibberd DJ. 1975. Observations on the ultrastructure of the choanoflagellate *Codosiga botrytis* (Ehr.) Saville-Kent with special reference to the flagellar apparatus. *J Cell Sci* 17:191–219.
- Hirase T, Staddon J, Saitou M, Ando-Akatsuka Y, Itoh M, Furuse M, Fujimoto K, Tsukita S, Rubin L. 1997. Occludin as a possible determinant of tight junction permeability in endothelial cells. *J Cell Sci* 110:1603–13.
- Karpov S, Coupe S. 1998. A revision of choanoflagellates genera *Kentrosiga*, Schiller, 1953 and *Desmarella*, Kent, 1880. *Acta Protozool* 37:23–7.
- Lavrov DV, Forget L, Kelly M, Lang BF. 2005. Mitochondrial genomes of two demosponges provide insights into an early stage of animal evolution. *Mol Biol Evol* 22:1231–9.
- Leadbeater BSC. 1983. Life-history and ultrastructure of a new marine species of *Proterospongia* (Choanoflagellida). *J Mar Biol Ass UK* 63:135–60.
- Ledger PW. 1975. Septate junctions in the calcareous sponge *Sycon ciliatum*. *Tiss Cell* 7:13–8.
- Leik J, Kelly D. 1970. Septate junctions in the gastrodermal epithelium of *Phialidium*: a fine structural study utilizing ruthenium red. *Tiss Cell* 2:435–541.
- Lethias C, Garrone R, Mazzorana M. 1983. Fine structure of sponge cell membranes: comparative study with freeze-fracture and conventional thin section methods. *Tiss Cell* 15:523–35.
- Leys SP. 1995. Cytoskeletal architecture and organelle transport in giant syncytia formed by fusion of hexactinellid sponge tissues. *Biol Bull (Woods Hole)* 188:241–54.
- Mackie GO. 1990. The elementary nervous system revisited. *Am Zool* 30:907–20.
- Mackie GO, Singla CL. 1983. Studies on hexactinellid sponges. I Histology of *Rhabdocalypptus dawsoni* (Lambe, 1873). *Philos Trans R Soc Lond B* 301:365–400.
- Maldonado M, Riesgo A. 2008. Reproductive output in a Mediterranean population of the homosclerophorid *Corticium candelabrum* (Porifera, Demospongiae), with notes on the ultrastructure and behavior of the larva. *Mar Ecol* 29:298–316.
- Nickel M. 2004. Kinetics and rhythm of body contractions in the sponge *Tethya wilhelma* (Porifera: Demospongiae). *J Exp Biol* 207:4515–24.
- O'Donnell MJ, Maddrell SH, Gardiner BO. 1984. Passage of solutes through walls of Malpighian tubules of *Rhodnius* by paracellular and transcellular routes. *Am J Physiol* 246:R759–69.
- Parker G. 1919. The elementary nervous system. Philadelphia, PA: JB Lippincott Company.
- Pavans de Ceccatty M. 1969. Les systemes des activités motrices, spontanées et provoquées des éponges. *C R Acad Sci Paris* 269:596–99.
- Pavans de Ceccatty M. 1981. Demonstration of actin filaments in sponge cells. *Cell Biol Int Rep* 5:945–52.
- Pavans de Ceccatty M, Thiney Y, Garrone R. 1970. Les bases ultrastructurales des communications intercellulaires dans

- les oscules de quelques éponges. Symp Zool Soc Lond 25:449–66.
- Poschl E, Schlotzer-Schrehardt U, Brachvogel B, Saito K, Ninomiya Y, Mayer U. 2004. Collagen IV is essential for basement membrane stability but dispensable for initiation of its assembly during early development. *Development* 131:1619–28.
- Revel J-P. 1966. Fine structure of intercellular contacts in the sponge *Microciona prolifera*. *Biol Bull* 131:402.
- Rokas A, Kruger D, Carroll S. 2005. Animal evolution and the molecular signature of radiations compressed in time. *Science* 310:1933–8.
- Sabella CA, Faszewski EE, Kaltenbach JC, Kuhns WJ, Burger MM, Fernandez-Busquets X. 2004. Immunocytochemical detection of integrin alpha 3 and beta 1 in allografts of the marine sponge, *Microciona prolifera*. *Biol Bull* 207:162.
- Sakaraya O, Armstrong KA, Adamska M, Adamski M, Wang I, Tidor B, Degnan BM, Oakley TH, Kosik KS. 2007. A post-synaptic scaffold at the origin of the animal kingdom. *PLoS ONE* 2:e506.
- Simpson TL. 1984. *The cell biology of sponges*. New York: Springer Verlag.
- Skaer HB, Maddrell SH, Harrison JB. 1987. The permeability properties of septate junctions in Malpighian tubules of *Rhodnius*. *J Cell Sci* 88:251–65.
- Skaer HLB, Maddrell SHP. 1987. How are invertebrate epithelia made tight? *J Cell Sci* 88:139–41.
- Steenkamp ET, Wright J, Baldauf SL. 2006. The protistan origins of animals and fungi. *Mol Biol Evol* 23:93–106.
- Weissenfels N. 1984. Bau und funktion des Süßwasserschwamms *Ephydatia fluviatilis* (Porifera) XI. Nachweis einer endogenen Kontraktionsrhythmik durch Infrarot-Reflexion. *Zoomorphology* 104:292–7.
- Weissenfels N. 1990. Condensation rhythm of fresh-water sponges (Spongillidae, Porifera). *Eur J Cell Biol* 53:373–83.
- Wilson HV. 1910. A study of some epithelioid membranes in monaxonid sponges. *J Exp Zool* 9:537–77.
- Wu Y, Dowbenko D, Spencer S, Laura R, Lee J, Gu Q, Lasky LA. 2000. Interaction of the tumor suppressor PTEN/MMAC with a PDZ domain of MAGI3, a novel membrane-associated guanylate kinase. *J Biol Chem* 275:21477–85.