RESEARCH ARTICLE

Epithelia, an Evolutionary Novelty of Metazoans

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ABSTRACT

At the point in animal evolution when cells began to adhere to each other they presumably initially functioned as colonies. The formation of an epithelium that enclosed and controlled an internal milieu would have been the first event to distinguish an individual animal from a colony. To better understand when the first epithelium arose and what its characteristics were, we evaluate the morphological, functional, and molecular characters of epithelia in sponges, considered here the extant representatives of the first metazoans. In particular, we show new claudin-like sequences from sponges align most closely with sequences from *Drosophila* that have a barrier function in septate junctions. We also show that type IV collagen, the main component of the basement membrane (BM), is present in calcareous sponges, and we confirm the presence of type IV-like collagen (spongin short chain collagen) in other sponges. Though in sponges as in other metazoans the epithelium has grades of specialization with varying complexity of junctions and the BM, the main character of a functional epithelium, the ability to seal and control the ionic composition of the internal milieu, is a property of even the simplest sponge epithelium, and therefore the first metazoans likely also had epithelia with these characteristics, which we consider a "true" epithelium. *J. Exp. Zool. (Mol. Dev. Evol.) 314B, 2011.* © 2011 Wiley Periodicals, Inc.

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BIOLOGY OF THE INTEGUMENT

The first animal epithelium was a novel structure that had the distinct (and also novel) function of compartmentalizing regions of the body and controlling the passage of molecules between two environments. The epithelium is, therefore, a good example of an evolutionary novelty, a feature that is "not homologous to a feature in an ancestral taxon" (Hall, 2005). Oddly, it is still not accepted that the first multicellular animals, Porifera, have a true epithelium, and this perspective seems to depend on the level of organization at which homology is required.

Tyler (2003) summarizes a widely held view that epithelia are the "default state of cells in all eumetazoans," which arose in "the stem to the Cnidaria." He says Porifera are not considered to have true epithelia by "established criteria," but admits that Porifera "likely have developed many, if not all, of the mechanisms deemed specific to true epithelium." This statement highlights a long-standing equivocation on the part of comparative morphologists on the question of whether Porifera can be considered epithelial, and therefore whether epithelia are a novelty of Metazoa or only Eumetazoa. Even in the introduction to *The Biology of the Integument*, K. Sylvia-Richards ('84) writes that "Porifera and Cnidaria are both essentially epithelial animals composed of two layers of cells surrounding a central, environment-filled cavity"; yet, Porifera were omitted from that volume primarily based on work that had suggested that sponge epithelia are of "predominantly one sort and are apparently very loosely associated" (Mackie, '84). Cnidarian epithelia in contrast are "histologically more advanced" with both striated and smooth muscle, nerves, and chromatophores, but with the absence of a middle layer (mesoderm) which prevents specialization (Mackie, '84). By this definition, to count as epithelia the tissue ought to contain a diversity of cell types which adhere tightly to

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one another, and therefore presumably also seal and control the ionic constitution of the internal compartment.

Tyler's "established criteria" of epithelia are morphological characteristics (adherens and sealing junctions and a basement membrane (BM)) and the presence of molecular components known from studies in model systems to be involved in the formation of the above junctions and in their physiological adhering, sealing, or signaling functions. Although the ultrastructure of all animal tissues has been known for some time, the accounting of the molecules involved is very much still in progress. What Tyler does not take into account, and about which there is less information, is the physiology of epithelial function, because it is only known for a few model systems (mammalian cell lines, fish, amphibians, *Drosophila melanogaster* and other insects, *Caenorhabditis elegans*, and recently a sponge, *Spongilla lacustris* [see references in Adams et al., 2010]).

Sponges have traditionally been united in one phylum—Porifera—that is characterized by having a porous body containing canals and chambers and a specific cell type—choanocyte—that generates the feeding and respiratory current (Grant, '36). Monophyly of Porifera is supported by one set of molecular analyses (Philippe et al., 2009), but another set finds that sponges are paraphyletic (Sperling et al., 2009). In both instances, the four classes of sponges fall into a clade of siliceous sponges (Silicea) containing Demospongiae and Hexactinellida, and a grouping of calcareous and homoscleromorph sponges. Recent work has shown that the latter group, Homoscleromorpha, has a BM structure containing type IV collagen (Boute et al., '96), which some say is evidence of a "true epithelium" and which has prompted use of the term "Epitheliozoa" (Boury-Esnault et al., 2003; Sperling et al., 2009). But what exactly is a "true" epithelium?

The term "true," in this context, seems to come from "good" (Greek = eu) as in "eumetazoa," a term used by Hyman to refer to "animals of the tissue or organ system grade of construction with mouth and digestive tract," including Cnidaria, Ctenophora, and bilaterians (Hyman, '40). Hyman followed Sollas' (1884) who felt sponges were more similar to *infusiora* (protists) than to other metazoans, based on the idea that the sponge ciliated larval epithelial cells were protist characters ("choanoflagellate-cells," p 612) that arose earlier during development than the metazoan characters (gastrulation by in-folding, cellular migration), which happened at settlement. Sollas proposed the term Parazoa for sponges, and Hyman ('40) therefore used the term Eumetazoa for the rest of multicellular animals. [Sollas arrived at this conclusion by studying development in a homoscleromorph sponge (*Halisarca lobularis*, the species now referred to as *Oscarella lobularis*), and ironically it is now homoscleromorphs that are proposed to be closer to other metazoans than other sponges.] Therefore, a "true epithelium" is one that has the characteristics of epithelia in Eumetazoa: adherens and occluding junctions and a BM.

In this article we ask whether it is important to consider the hierarchy of traits in determining "novelty" (that is, whether there are "grades" of epithelia, but ultimately all characteristics are necessary for a "true epithelium") or whether functionality rather than just type of morphological trait is important for "novelty." The challenge is that although formerly an evolutionary novelty was identified only by its morphology, today molecular data is used to account for the ability to produce a structure with a known function and the two do not always agree. For example, although all the genes for muscle and, therefore, mesoderm are in the *Hydra* genome (Chapman et al., 2010), cnidarians are not understood to have a mesoderm, in the sense of a structure that arises as a third layer via embryogenesis (see Siepel and Schmid, 2006, for a discussion of cnidarian mesoderm). Conversely, ctenophores seem to have proper mesoderm that arises as a third layer during embryogenesis, but possess none of the genes for mesoderm (M Q Martindale, Kewalo Marine Laboratory, personal communication). Nevertheless, more often than not gene presence and absence is now used to predict the presence or absence of a structure. In relation to the focus of this article, however, the fact that we lack complete molecular data for more than one species of Porifera remains a major obstacle.

Here we use the definition of novelty as "a feature not homologous to a feature in an ancestral taxon" (Hall, 2005), to assess whether the epithelium was a novel character for all metazoans or only eumetazoans or whether it is a novel structure that evolved within the Porifera. In contrast to the more conceptual articles in this issue (e.g., Hall and Kerney, this issue), we feel that in order to assess whether epithelia are a novelty, important questions need to be settled regarding molecular, functional, and morphological changes. It is these aspects we address.

CELL–CELL JUNCTIONS: MORPHOLOGY AND PHYSIOLOGY

The ultrastructure of adherens junctions, of tight and septate junctions (SJs) (both occluding junctions), and of a BM, is well characterized from both vertebrates and invertebrates (Fig. 1), but not all epithelia show equally good morphology when preserved. Junctions in Porifera are at times clear and at others very indistinct, so much so that, although there are many reports of adherens and SJs in Porifera, some authors still find the ultrastructure "less than convincing" (Fahey and Degnan, 2010). In sponges, adherens junctions seem less electron opaque than in Cnidaria, Platyhelminthes, and in textbook views of vertebrate junctions, although not all images of vertebrate junctions show equally perfect preservation (e.g., Farquhar and Palade, '63). Both zonula and macula adherens junctions, belt and spot adhesion plaques, respectively, contain cadherin-based junctional proteins. Although numerous reports on invertebrate tissues describe the presence of desmosomes (macula adherentes), strictly speaking desmosomes are defined by the presence of desmosomal cadherins and plakoglobins, which are only in vertebrates. Hence, "desmosomes" (properly spot adherens junctions) have been reported in all groups of sponges (Lethias

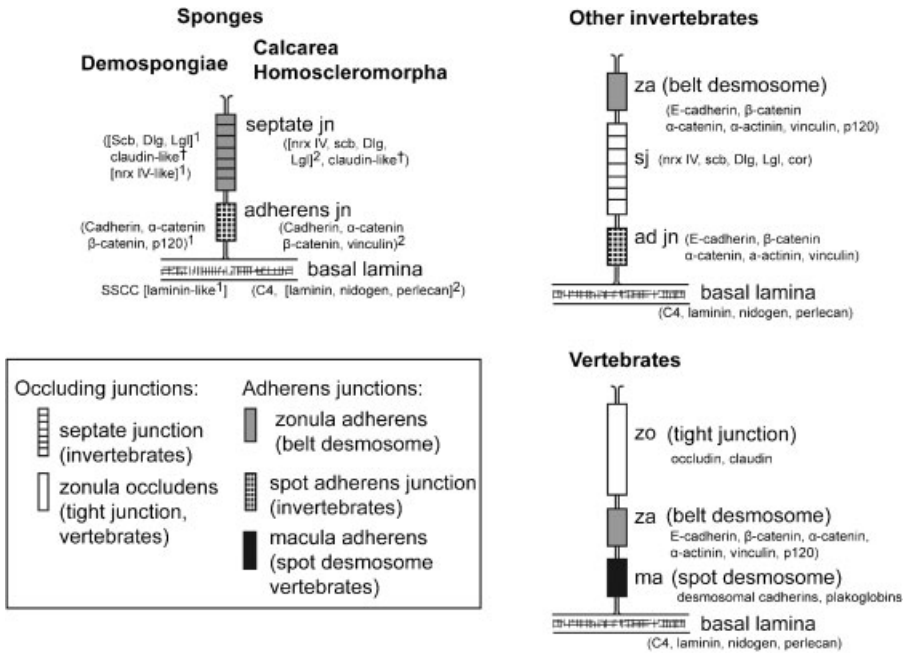

Figure 1. Cartoons depicting cell junction and basement membrane structures in sponges, other invertebrates, and vertebrates. A selection of the genes known to be present in each is shown in parentheses. Sequence data from [1]Fahey and Degnan, 2010; [2]Nichols et al. 2006; [†]this article.

et al., '83; reviewed in Leys et al., 2009), but in none of the groups (hexactinellids, demosponges, calcarea, homoscleromorphs) does the ultrastructure appear as it does in bilaterians. In fact, equally "convincing" adherens junctions can be seen by TEM in the slime mold *Dictyostelium* (Grimson et al., 2000; Dickinson et al., 2011), where they link cells in the inner region of the fruiting body. Those junctions have even been shown to label with actin and the *Dictyostelium* protein homolog of β-catenin, Aardvark. No data are yet available on the proteins used in sponge adherens junctions, but thanks largely to the presence in the sponge genome of the catalog of molecular components known to be required for making adherens junctions (including several cadherins), the presence of adherens junctions in sponges is not questioned (Fahey and Degnan, 2010).

As to occluding junctions, the ladder-like structure of SJs is particularly faint in demosponge and homoscleromorph tissues and yet can be very distinct in calcareous sponges. This is attributed to the presumed need to have a sealed compartment to secrete calcium carbonate in calcareous sponges, but not in siliceous sponges (including homoscleromorphs). Nevertheless, based on these ultrastructural data, all sponges have been considered to have SJs (see Leys et al., 2009, and references therein), and new physiological data now show that demosponge epithelia do seal and control the ionic composition of their internal milieu (Adams et al., 2010).

The physiological ability of an epithelium to seal is determined either by measuring transepithelial resistance across the cell sheet (Shaw, '58) or block of tracers, e.g., 10 kd dextrans (Asano et al., 2003; Nelson et al., 2010) or 0.85 kd ruthenium red (Hori, '87). The extent of sealing depends on the specialization of the tissue, but in all instances there is at least a resistance of $>10\,\Omega\,cm^2$ or block of 10 kd molecules (see Supplementary Table 1 in Adams et al., 2010). Sponge tissues tested with the same techniques had a resistance of $500–1500\,\Omega\,cm^2$ and blocked entry of ruthenium red, just as it is blocked by SJs in the epithelium of the planarian *Dugesia* (Fig. 2A–C). Yet, it is still not understood how the block occurs in sponges not only because the septae appear so "faint" in the transmission electron microscope (TEM) images (for siliceous sponges and homoscleromorphs), but also because the molecular components for SJs known in other invertebrates are not so readily found in the sponge genome. An intriguing possibility is that sponge cell junctions may combine molecules used in septate and tight junctions (TJs) to generate the seal. TJs are the sealing junctions of chordates, but cell biologists have also considered sponges to have TJs based on TEM images (Revel, '66), and similar "kissing points" (Tsukita and Furuse, '99) can be found in sponge and mammalian epithelia (compare Figs. 14, 15, and 23 in Farquhar and Palade, '63; with Figs. 3 and 4C, D in Adams, 2010). If sponge epithelia seal and the SJs have an unusual ultrastructure, perhaps the junctions used in sealing in

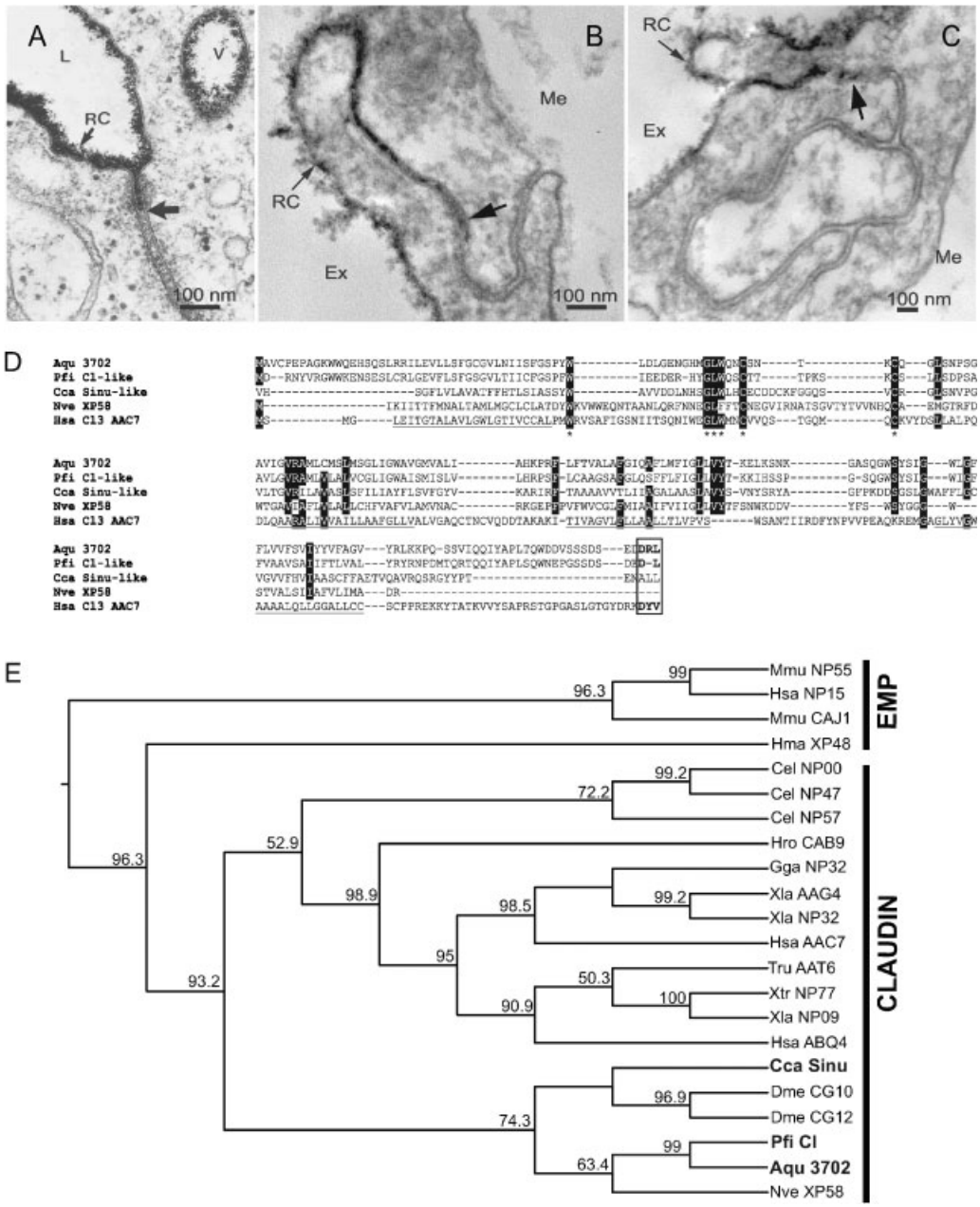

Figure 2. Sealing junctions. Block of ruthenium red by septate junctions in **A**, the planarian *Dugesia* and **in B** and **C**, *Spongilla lacustris*. RC, ruthenium coat; L, lumen; V, vesicle; Ex, external medium; Me, mesohyl. Black arrows mark the point at which ruthenium is blocked from penetrating the junction (A, from Hori, '87, with permission; and B and C from Adams, 2010, with permission). C, Representative multiple alignment of claudin-like sequences from sponges (AquClaudinSF, *A. queenslandica*: Fahey and Degnan, 2010; Pfi *Petrosia ficiformis*; Cca *Corticium candelabrum*), anemone (Nve *N. vectensis*), and human claudin 3. Alignments were generated with MAAFT version 6 (http://mafft.cbrc.jp/alignment/server/) and viewed in BioEdit 7.0.5.3. Dashes indicate gaps. The W-GLW and two conserved cysteines are marked with asterisks. The PDZ domain is marked with a box. Black shading is used to show >75% similarity in the residues. **D**, Phylogenetic analysis by maximum likelihood of epithelial membrane proteins (EMP) and claudin protein sequences. The phylogenetic tree was generated using an amino acid alignment of EMP and claudin proteins from 22 metazoans with PhyML (Guindon and Gascuel, 2003; http://atgc.lirmm.fr/phyml/). The JTT substitution model was optimized with ProtTest (Abascal et al., 2005; http://darwin.uvigo.es/software/prottest_server.html) with a γ-shaped parameter of 5.186. Statistics for the ML tree: log-likelihood = −8447.09, unconstrained likelihood = −1347.81. The cladogram was generated with FigTree 1.3.1 and rooted using EMPs. Bootstrap values are shown only when they are greater than 50%.

the first epithelia used a mix of proteins to seal, and these later diverged to take on the specific functions of those forming tight and SJs in bilaterians.

Genomic accounting of the epithelium has been carried out in general assessments of the genomes of *Hydra, Nematostella, Trichoplax, Amphimedon*, and *Monosiga* (Putnam et al., 2007; King et al., 2008; Srivastava et al., 2008, 2010; Chapman et al., 2010), but more specifically in comparative analyses of the domain structure of genes known to be involved in forming the epithelia and junctions (Sakaraya et al., 2007; Alié and Manuel, 2010; de Mendoza et al., 2010; Fahey and Degnan, 2010); some of these are shown in Figure 3. Among the animals at the base of the metazoan tree, however, drift in sequence is often so great that, as noted by Aouacheria et al. (2006), domains, such as collagen IV, are not recovered by automatic domain detection software (e.g., Pro-Dom, PFAM, SCOP, PROSITE, and Interpro). Sequence similarity may also be so low between species of distant phyla that phylogenetic analysis does not offer much insight, but the proteins of these phyla are still estimated to form nearly identical 3D structures. Until the ligands are also known, the actual interactions of these proteins can only be speculated upon. With more data from additional poriferan species, however, it becomes apparent that even though the sequences are divergent, they do seem to be present in a wider selection of Porifera. We focus here on specific examples of genes involved in forming sealing junctions (septate and tight) and the basal lamina.

SEALING JUNCTIONS: GENE ACCOUNTING

Because sponge epithelia do seal, either SJs or TJs are expected to be present (it is less likely that sponges have a unique way of forming seals). Given the ultrastructure of faint septae in junctions seen in demosponges and homoscleromorphs and of strong septae in Calcarea (Fig. 2 in Leys et al., 2009), we would expect some of the proteins and genes involved in SJs to be present in sponges. SJs are found in the apical side of junction complexes in epithelia of invertebrates, whereas in vertebrates they are only found at the paranodal junctions of axons and glia (Hortsch and Margolis, 2003).

Proteins involved in forming SJs are best known from *Drosophila*, but exactly which proteins are involved in each junction and what their functions are is still being determined. In *Drosophila*, there are two types of SJ: smooth SJ (sSJ) which occur in the midgut and malpighian tubules, and pleated sheet SJ (pSJ) which occur in the epidermis, salivary gland, and photoreceptors (so-called primary epithelia). The molecular components of pSJ are best characterized and consist of the adhesion proteins neuroglian, contactin, and neurexin IV (a contactin associated protein or Caspr). All three have been shown to be necessary for barrier function at pSJ in the fly, but of these only neurexin IV is needed to retain the ultrastructure of septae that characterize this junction (Baumgartner et al., '96; Genova and Fehon, 2003; Faivre-Sarrailh et al., 2004). Neurexin IV is not found at sSJ, which also lack strong ladder-like septae in electron micrographs (Baumgartner et al., '96; Baumann, 2001). sSJ are characterized by fasciclin III, ankyrin, and α,β-spectrin. Neuroglian is not localized to sSJ, but rather is found around muscle and regenerative cells in the midgut (Baumann, 2001). So, the proteins that one might be looking for in order to assess whether SJ are in sponges or arose as a novelty of cnidarians, depend on what kind of SJ we expect sponges to have.

A neurexin IV homolog, found in the homoscleromorph sponge *Oscarella carmela* (Nichols et al., 2006; Nichols, UC Berkeley, personal communication), aligns well with two fragments found in the genome of the demosponge *Amphimedon queenslandica* (Fahey and Degnan, 2010). The sponge sequences are short, so it is not known if they are able to function in the same way as other neurexin IVs. Neither neuroglian nor contactin were found in *Amphimedon*, but other genes associated with pSJ in *Drosophila* do have homologs in this sponge, such as those encoding for the linker proteins Scribble and Discs-large, although these may also be associated with other aspects of cell polarity (Fahey and Degnan, 2010). Genes encoding for proteins of the 4.1 m family, which has been shown to be essential for barrier function in *Drosophila* (Lamb et al., '98), were not assessed in the study by Fahey and Degnan (2010), nor was fasciclin III, but blast searches identify potential fragments in the *A. queenslandica* sponge genome. The ultrastructure of smooth SJ in *Drosophila* is very similar to that of SJ seen in sponges, and given that the sponge epithelium functions more like the tubules of a gut (for absorption and secretion and slow peristaltic contractions, rather than for maintaining strength or robustness), it would not be at all surprising if a similar combination of proteins to those in sSJ make up the sponge junctions. On the other hand, we might expect a neurexin-based complex in calcareous sponges where the ultrastructure shows firm septae between choanocytes and between spicule secreting sclerocytes (Ledger, '75; Green and Bergquist, '79). In sum, although there are many proteins involved in SJ for which homologs of genes have not yet been sought in sponges, with those already identified the sponge might operate a SJ and use this to seal. However, immunocytochemistry is needed to determine the location of the proteins and RNAi or equivalent tests are needed to confirm function. It would not be surprising to find some of these proteins localized to SJs, but sponges may also use other novel proteins in barrier function, and one possibility is claudins.

It is a fairly recent discovery that claudins are involved in SJ barrier function in both *Drosophila* and *C. elegans* (Asano et al., 2003; Nelson et al., 2010). Claudins are members of the PMP-22/EMP/MP-20/Claudin superfamily, which together with occludins and junctional adhesion molecules are involved in integrity and control of paracellular transport in vertebrate TJs (Heiskala et al., 2001). This complex interacts with MAGUK homologs (ZO 1–3) and other proteins to bind to the actin cytoskeleton of the cell, and variation in TJ permeability occurs by variability in claudin

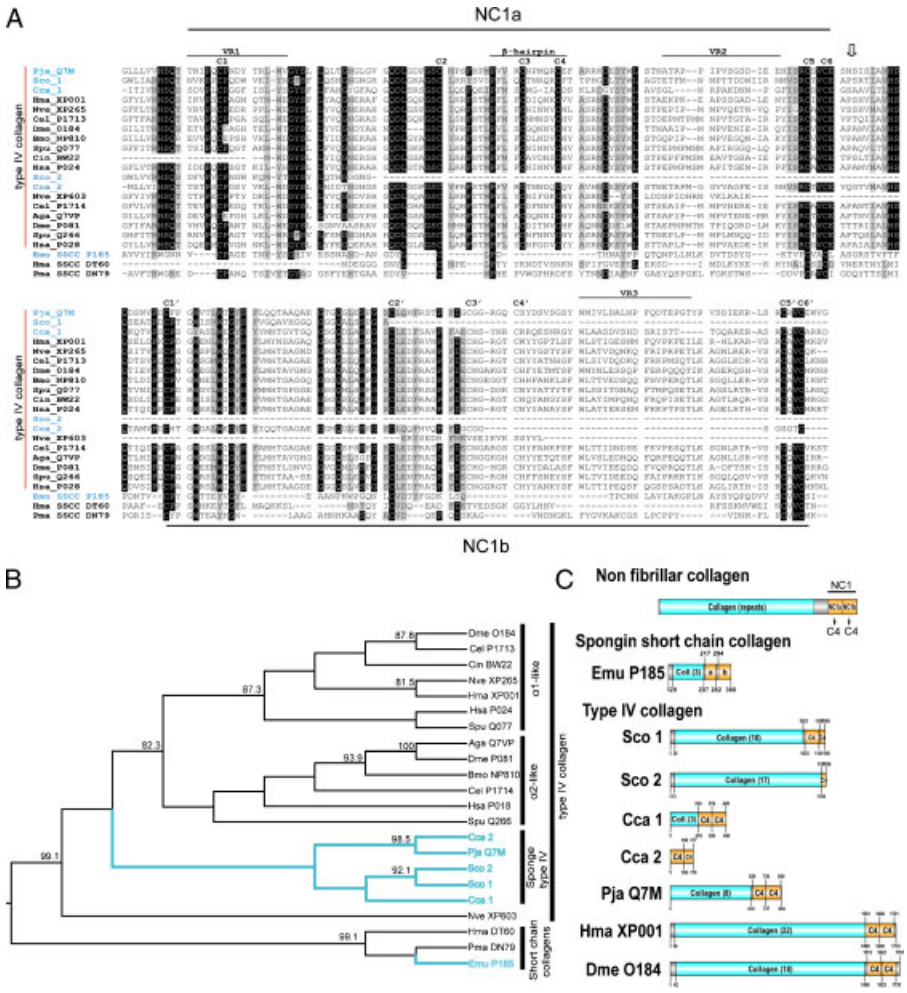

Figure 3. Conserved protein structure of type IV collagen and spongin short chain collagen-related NC1 domains. **A**. Representative multiple alignment of type IV collagen and spongin short chain collagen-related NC1 domains. The alignments were generated with Muscle and viewed with SeaView (http://pbil.univ-lyon1.fr/software/seaview.html). Dashes indicate gaps. The abbreviations used are presented in Supplementary Table 1. The NC1 domains (NC1a and NC1b), position of variable regions (VR), and of the ß-hairpin are indicated. Conserved cysteines are numbered consecutively. Limit between subdomains is indicated with an arrow. Black shading is used to show >75% similarity in the residues. **B**. Phylogenetic analysis by maximum likelihood of spongin short chain collagen-related and type IV collagen subdomain sequences. Phylogenetic tree was generated using an amino acid alignment of NC1 subdomains of spongin short chain collagen-related and type IV collagen from 22 metazoans with PhyML (Guindon and Gascuel 2003; http://atgc.lirmm.fr/phyml/). The JTT substitution model was optimized with ProtTest (Abascal et al. 2005; http://darwin.uvigo.es/software/prottest_server.html) with a γ-shaped parameter of 0.811. Statistics for the ML tree: log likelihood = −6872.24, unconstrained likelihood = −1239.64. The cladogram was generated with FigTree 1.3.1 and rooted with short chain collagen proteins. Bootstrap values are shown only when they are greater than 50%. **C**. Protein structure of type IV and SSCC visualized using DOG (http://dog.biocuckoo.org/software.php). Collagen domain repeats (comprised of approximately 60 residues each) are colored in blue and the number of repeats is indicated between parentheses; NC1a and NC2b subdomains (called C4 in Type IV collagen) are colored in orange.

expression. The working components seem to be the W-GLW-C-C motif on the large extracellular loop, and a *C*-terminal PDZ domain that is involved in localizing claudin to the tight/SJ. A claudin candidate was found in the demosponge *Amphimedon* genome (AmqClaudinSF; Fahey and Degnan, 2010). We searched EST data from two other sponges (*Petrosia ficiformis*: demosponge and *Corticium candelabrum*: homoscleromorph) and found two more sequences. An alignment of the sponge, cnidarian and

chordate claudin sequences shows close agreement of amino acids in the key motif and also a functional PDZ *C*-terminal domain (Fig. 2D). Phylogenetic analysis groups the sponge sequences with one cnidarian and two *Drosophila* claudins (Sinu and Kune) that have been shown to have barrier function (Wu et al., 2004; Nelson et al., 2010) (Fig. 2E). The similarity to vertebrate sequences is low outside the main motifs and transmembrane domains, but the *Drosophila* and *C. elegans* sequences (Asano et al., 2003; Nelson et al., 2010) also share low sequence similarity with vertebrate sequences, so it is quite possible that claudins function in sealing the epithelium in sponge junctions too. Although no occludin gene was found in the *Amphimedon* sponge genome, *A. queenslandica* has a MAGUK protein homolog (*Amphimedon* CARD; de Mendoza et al., 2010) that is ancestral to ZO 1–3 genes. Expression of a MAGUK protein in the epithelium of another demosponge (Adell et al., 2004) suggests it would be well worth investigating protein localization of claudin by immunogold and testing function in sponges.

All in all, the available molecular and ultrastructural data suggest SJs were already well-established in all Porifera and are not a novelty of either just one group of sponges or of cnidarians. Specializations of these junctions by acquisition of new proteins by combining new domains with ancestral domains and restructuring ancestral domains (described as type II and III novelties, respectively; Putnam et al., 2007; Fahey and Degnan, 2010) seems to have been a subtle process of modification of the business components of the junction, not the kind of radical structural change that would elevate it to the level of a novel structure: there are homologs of all the components in all sponges.

BASEMENT MEMBRANE: A NEW STRUCTURE?

A third component of all metazoan epithelia is the BM. Morphologically, the BM consists of a bilayered basal lamina (formed by the *lamina densa* and *lamina lucida*) and a single reticular lamina (*lamina reticularis*). The basal lamina is the structure most often referred to when speaking of a BM. It is a 50–100 nm thick layer that lies just below the cells of the epithelium; in fact, its path follows every undulation of the cells which secrete it. The *lamina reticularis* is usually considered a more rigid sheet that does not follow every contour of the cells in the epithelium above, but which nevertheless provides support and information to the cells in the epithelium. The main components of the basal lamina are type IV collagen, laminin, nidogen/entactin, and perlecan, but each BM has a suite of other molecules specific to the type of epithelium (Hynes and Zhao, 2000; LeBleu et al., 2007). The BM functions in support, as a semi-permeable selective barrier (e.g., in the kidney), and in signaling it guides differentiation, cell proliferation, and migration; its evolution seems, therefore, intimately tied to the evolution of differentiated epithelia (LeBleu et al., 2007).

It is already clear that this feature did not arise de novo in so-called eumetazoans, because there is nice ultrastructural preservation of a basal lamina in homoscleromorph sponges. Also, the homoscleromorph sponge, *Pseudocorticium jarrei*, has been shown to have type IV collagen and antibodies to the protein localize to the basal side of epithelial cells (Boute et al., '96), but it is still unknown if that sponge has the full complement of basal lamina proteins. The ultrastructure of the BM is definitely different in homoscleromorph sponges than in other sponges. Yet, it is difficult to pinpoint the precise difference between this structure in homoscleromorphs and the collagen mat that is found beneath epithelia of demosponges, which contains spongin short chain collagen (SSCC) (Exposito et al., '91), a collagen considered ancestral to type IV collagen (Aouacheria et al., 2006). Like type IV collagen, SSCC also has the NC1 domains that produce the globular heads particular to type IV collagen and which are required for assembly of the unique scaffold of the BM (Aouacheria et al., 2006; LeBleu et al., 2007). Threading (using mGenThreader and FUGUE) of the SSCC NC1 domain onto the human type IV collagen α1/α2 domains showed such similar 3D structure that Aouacheria et al. (2006) predicted that SSCC and type IV collagens very likely have similar functions, suggesting that just like type IV collagens, SSCCs can form a basal lamina under the epithelium. Still, no electron micrographs show a proper basal lamina in sponges other than homoscleromophs. Perhaps other components are missing which prevents its proper preservation. To form a BM, type IV collagen needs to interact with laminins. Although a suite of laminin-related proteins were found in the *Amphimedon* genome, none were considered similar enough to vertebrate laminins in domain architecture (Fahey and Degnan, 2010). Nichols et al. (2006) report both laminin α and β, as well as perlecan and nidogen in the homoscleromorph *O. carmela*; perlecan and nidogen were not found in *A. queenslandica*. But, differing interpretations of domain structures lead to different conclusions on the presence/absence of genes, so more complete data from other sponges are needed in order to say whether laminins exist in homoscleromorph sponges and not in others. Although we have not yet carried out that survey, we have found type IV collagens in other sponges.

In new transcriptome data from a calcareous sponge (*Sycon coactum*) and another homoscleromorph sponge (*C. candelabrum*), we found two new type IV collagen genes in each (Fig. 3). This provides the first evidence for type IV collagen domains in sponges other than homoscleromorphs and suggests that α1 and α2 domains may have already diverged in the Porifera (Fig. 3B and C). The finding of type IV collagen in Calcarea is fascinating, because nowhere in this group has a BM-like structure been noted; the only mat-like collagenous feature in Calcarea we are aware of is a sheath that surrounds spicules. Perhaps, it is not so much the type IV collagen that differs between homoscleromorph and demosponge BMs, but rather the links provided by laminin, perlecan, and nidogen between the collagen mat (SSCC or type IV collagen) and the epithelium, and it may be these molecules that

allow signals to be transduced to the epithelium for cellular differentiation and proliferation. These are hypotheses that need exploring by detailed searches of other sponge genomes.

EPITHELIAL ROOTS PREDATE SPONGES

Roots of genes involved in epithelial structure are not only well-entrenched in the Porifera, but those involved in epithelial polarity and forming adherens junctions are now known to predate metazoans (Abedin and King, 2008; Dickinson et al., 2011). If we go by the ultrastructure and complement of gene families known to be involved in adherens and sealing junctions, and in polarity and BM features, the distinction between the epithelium of the different cellular sponge groups (demosponges, Calcarea, and homoscleromorphs) is not clear cut. All sponges have sealing epithelia—this has been shown for demosponges, and based on the similarity in junctions across sponges, it may be assumed that the epithelia of homoscleromorphs and certainly of calcareaous sponges also seal. Collagen IV domains are not novel to homoscleromorphs (they are also in Calcarea) and these are very similar to the SSCC NC1 domains in demosponges (Aouacheria et al., 2006). Whether the linker proteins laminins, nidogens, and perlecans are innovations of homoscleromorphs awaits further analysis of more sponge genomes. *If* these linker structures are specific to homoscleromorphs alone or to homoscleromorphs and Calcarea only and are not present in any demosponge, then those two groups might be usefully termed, along with other animals, the "Laminazoa," and this would also lend support to the hypothesis that sponges are indeed paraphyletic. However, further data on the structural components of BMs and their function in sponges is needed before they can be considered substantially different across Porifera or substantially different between Porifera and other Metazoa; especially considering that even within vertebrates different epithelia can have different levels of structural and functional complexity, and not all epithelia possess all of the same components, and not all function in the same way. Therefore, we suggest that the functional character of an epithelium—one which seals (or at least controls the passage of ions)—is more important to the definition of a "true epithelium" than the structure alone. All sponges have pinacoderms with barrier function, and therefore all sponges have true epithelia. But it would be interesting to determine whether in sponges there are different types of epithelia with different levels of integrity and barrier function as in other animals.

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