# 4 Porifera

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## 4.1 HISTORY OF THE MODEL

Sponges (Porifera) have fascinated scientists for at least 150 years, with two key subjects of investigation remaining vibrant until today and additional areas of research emerging recently. The first of the original subjects is the relationship between sponges, other animals and protists, both in terms of their relative phylogenetic positions and the homology between body plans and cell types. The second stems from the remarkable ability of sponges to regenerate: not only by restoring lost body parts but also by completely rebuilding bodies from dissociated cells. Why can sponges do that and we cannot? While 19th- and early 20th-century biologists were equipped only with microscopes, current scientists have harnessed the power of modern genomics and gene expression analysis to address these fundamentally interesting questions. This section of the chapter sets the stage for sponges as models for biological research by (briefly) reviewing findings and opinions of 19th-century scientists on the position of sponges in the tree of life and the discoveries of sponge regenerative capacity in the early 20th century. The following sections cover modern approaches to both subjects, concluding with discussion of the most recent advances and forecasting future directions of research utilizing sponges as models.

But what are sponges, actually? Perhaps surprisingly, this simple question continues to generate heated arguments, with various answers offered (but never universally agreed on) throughout the past centuries. Are they animals of cellular grade of organization (Parazoa), with a unique body plan and independently evolved cell types? Or are they true animals, with germ layers homologous to our endoderm and ectoderm? Are they living fossils, retaining features of our distant ancestors?

When Robert Grant gave sponges the name "Porifera" (= pore bearing), he referred to the numerus tiny openings (called pores of ostia) which are present on the surface of adult sponges and which lead to (more or less complex, depending on the body plan; see Section 4.5) system of canals and chambers (Grant 1825, 1836) (Figure 4.1). The innermost surface of sponges, an epithelial layer called choanoderm, is composed of choanocytes (collar cells), which are equipped with flagella propelling water through the body. Choanocyte collars capture food particles—often bacteria—and the filtered water is then expelled through a larger opening (or openings) called osculum (plural oscula). All other surfaces of sponges (the outer, the basal and lining of the canals) are composed of flat cells called pinacocytes. In between those two epithelial layers lies the non-epithelial mesohyl layer, containing motile amoeboid cells, cells producing skeletal elements, gametes and—in viviparous sponges—embryos. With these basic building blocks, sponges form a variety of body plans, which are discussed further in Section 4.5 (Figure 4.1). Although Linnaeus listed sponges as "vegetables", Grant considered them animals.

Few decades later, the striking similarity between choanocytes and choanoflagellates, which are single-cell and colonial protists, noticed by James-Clark in 1868 and Saville-Kent in 1880, was interpreted to indicate strong affinity between sponges and protists, in effect relegating sponges from the animal kingdom. Intriguingly, all modern phylogenies place choanoflagellates as the nearest relatives (the sister group) of animals, and the majority of the genome-based phylogenies place sponges as the earliest branching animal lineage (Figure 4.1), consistent with the position of sponges as the link between protists and "true animals" (Eumetazoans).

Ernst Haeckel, considered by many the father of evolutionary developmental biology, noted similarities between body plans of sponges, in particular calcareous sponges, and cnidarians, especially coral polyps. According to his views, the sponge choanoderm was homologous to the coral

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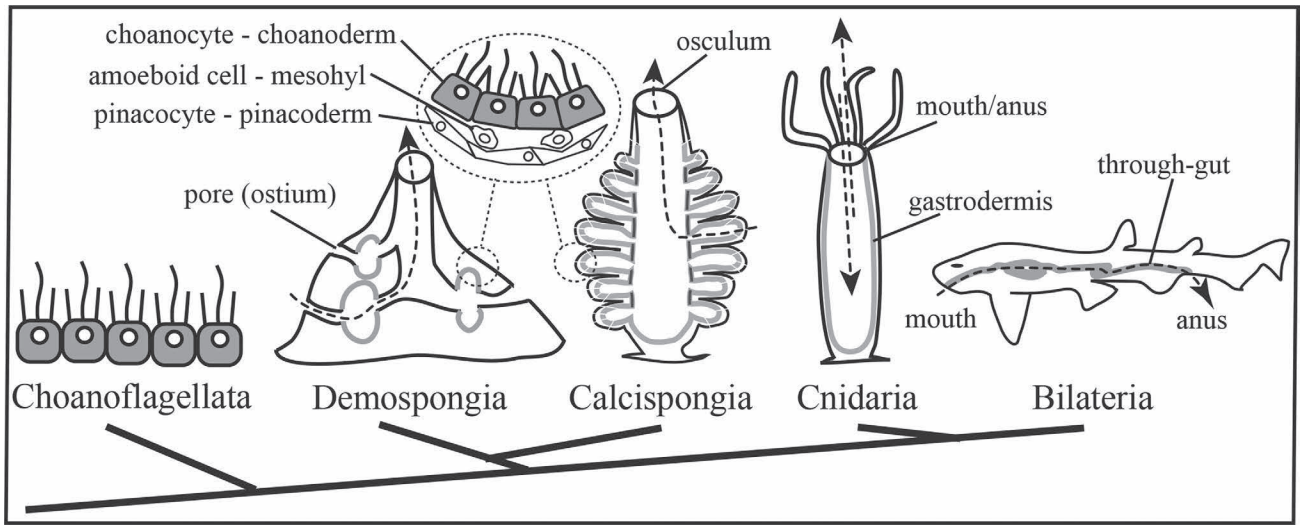


**FIGURE 4.1** Phylogenetic position, major cell types and body plans of sponges. Dashed lines with arrowheads indicate direction of movement of food particles and waste products; gray color marks cells and tissues involved in food capture and digestion. (Modified from Adamska 2016.)

gastrodermis, the sponge pinacoderm to the ectoderm, and the osculum to the polyp mouth (Figure 4.1). Haeckel credited the development of the gastrea theory (stating that all animals evolved from a gastrula-like pelagic animal), and more broadly recognition of homology of germ layers, to his observations of calcareous sponges and their development (Haeckel 1870, 1874). Following the reasoning of James-Clark and Haeckel, poriferan-grade body organization appears to represent a clear transition stage between the colonial protists and complex animals. However, phylogenetic position of sponges, as well as the nature of the similarity between sponge choanocytes and choanoflagellates on the one side and the gut enterocytes on the other side of the transition (e.g. Peña et al. 2016), remain far from being settled, as discussed again in Section 4.8.

While phylogenetic position and the relationship between sponge cell types and those of other animals might be disputed (Simion et al. 2017; Whelan et al. 2017), the observations of the regenerative abilities of sponges, originally made in the early 20th century, remain as true and fascinating now as they were then. Wilson (1907), working on a marine demosponge, *Microciona prolifera*, discovered that it was capable of forming new, functional bodies after being dissociated into single cells. His experiments were soon reproduced using other sponge species, including freshwater sponges by Muller (1911a, 1911b) and the calcareous sponge *Sycon raphanus* by Huxley (1911, 1921), demonstrating that this remarkable ability is widespread among sponges. Intriguingly, it appears that the cellular mechanisms of sponge regeneration differ significantly across the phylum, and the molecular mechanisms are only beginning to be discovered. We will return to this topic, covering the intriguing recent discoveries and future research avenues, in Sections 4.7 and 4.8.

## 4.2 GEOGRAPHICAL LOCATION

Sponges are found in virtually all marine environments, from cold, deep waters surrounding the poles to shallow tropical environments (van Soest et al. 2012). One lineage of sponges evolved the ability to occupy freshwater environments, with species noted in lakes, rivers and creeks across the globe (Manconi and Pronzato 2002).

Sponges are notoriously difficult in lab cultivation—no sponge species can currently be reliably cultivated throughout its entire lifecycle, and the cell culture methods have only started to be established (Schippers et al. 2012; Conkling et al. 2019). This challenge in combination with interest in sponge biology resulted in proliferation of sponge models, representing all four evolutionary lineages of sponges (Figure 4.2).

From over 9,000 species of marine sponges, laboratories in Europe, North America, Asia and Australia have thus been selecting their model systems focusing attention on species which are easily accessible (abundant in shallow waters or appearing in local aquaria) and relatively robust (permitting transport to laboratories and short-term culture), in addition to possessing unique biological features making them particularly interesting or tractable. This chapter focuses on knowledge obtained using representatives of two lineages: calcareous sponges, especially those from the genus Sycon (the same that inspired Haeckel's theories), and demosponges, especially *Amphimedon queenslandica* (the first sponge to have its genome sequenced). Sponges from the relatively small (but fascinating) lineage of Hexactinellida (glass sponges, a sister group to demosponges) are generally restricted to deep waters, making them difficult to access. However, a few species, such as *Oopsacas minuta*, have been found in relatively shallow cave environments,

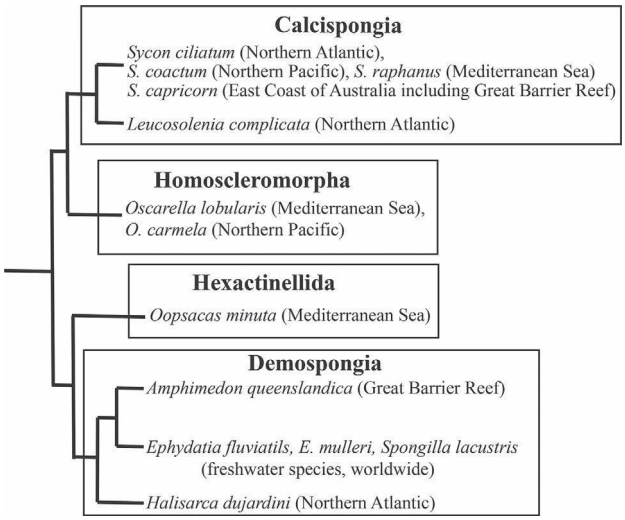


**FIGURE 4.2** Phylogenetic position and geographic location of major sponge model systems.

allowing researchers to study their development leading to formation of syncytial adult body (Boury-Esnault et al. 1999; Leys et al. 2016). The highly derived genomes of Hexactinellids will be mentioned in Section 4.6. Chapter 5 focuses on Homosclermorph sponges, which are the sister group to Calcisponges.

## 4.3 LIFE CYCLE

Like many marine invertebrates, the majority of sponges have a biphasic life cycle, including motile, pelagic larvae and sessile, benthic adults (Figure 4.3). This lifestyle likely reflects the lifestyle of the first animals (Degnan and Degnan 2006) or perhaps even our protistan ancestors (Adamska 2016b). While very few sponge species (such as *Tetilla japonica*) secondarily lost the motile larval stage, becoming direct developers, a spectacular diversity of developmental modes and larval types has been described in sponges (Leys and Ereskovsky 2006; Ereskovsky 2010; Maldonado 2006).

Sponges can be either oviparous (that is, releasing gametes to the surrounding water, with the fertilization and subsequence development occurring in the water column) or viviparous, with embryogenesis occurring within the maternal tissues. The majority of sponge species used as models for developmental biology research are viviparous and hermaphroditic. In particular, all homoscleromorph sponges, including *Oscarella lobularis* (see Chapter 5), and all calcisponge species (including *Sycon* sp.) brood their larvae within maternal tissues (Figure 4.3c, d; see also Section 4.4); in both cases, the embryos developing in the mesohyl (the non-epithelial layer sandwiched between pinacoderm and choanoderm) are distributed across the body of the adult. In contrast, in *Amphimedon queenslandica*, the embryos develop in specialized brood chambers, generally found close to the basal region of the sponge (Figure 4.3k). In both scenarios, mature larvae (Figure 4.3e, k) leave the mother sponge through the osculum and, after a period of swimming, settle and metamorphose on suitable substrate.

During metamorphosis, larval cells undergo major rearrangement, differentiation and transdifferentiation; begin production of skeletal elements (spicules, which are built of calcite in the calcisponges, and from silica in all other sponge classes); form the first choanocyte chambers; and finally open ostia and oscula to become feeding juveniles (Figure 4.3f–h, m–o). The juvenile of calcareous sponges from the genus *Sycon* represents one of the simplest body plans found in the animal kingdom: a cup-shaped body composed of two epithelial layers, which are connected by the ostia, with a narrow mesohyl layer containing spicule-producing cells (sclerocytes) and a single apical osculum (Figure 4.3h). This body plan is referred to as the asconoid grade of organization. As development progresses, new radial chambers form to surround the original radial chamber, which becomes the atrium of the emerging syconoid body plan (Figure 4.3i; see schematic representation in Figure 4.1). Despite this substantial change of the body plan, the radial symmetry of the body, with a single osculum, is maintained in many species, including *Sycon ciliatum* (Figure 4.3b). In contrast, the juvenile form of demosponges, such as *Amphimedon queenslandica*, is of leuconoid grade (multiple choanocyte chambers connected by series of canals), with a single apical osculum (Figure 4.3o; see schematic representation in Figure 4.1). As the animal grows, the leuconoid body plan is maintained, but additional oscula are formed, disrupting the original symmetry of the body plan (compare Figure 4.3j).

The life span of sponges also varies significantly across the species. *Sycon ciliatum* can be considered an annual species in the Norwegian fjords. The larvae settling in summer grow through the autumn and resume growth in the spring before they enter the reproductive stage in late spring, with larval release and death of the majority of the post-reproductive specimens in summer (Leininger et al. 2014). In contrast, *Amphimedon queensladica* can live many years based on the apparent growth rate and the size of individuals found in nature (author's personal observations). The most extreme case of sponge longevity on record is a Hexactinellid sponge, *Monorhaphis chuni*, estimated to live 11,000 (yes, eleven thousand!) years (Jochum et al. 2012).

## 4.4 EMBRYOGENESIS

Sponge embryogenesis utilizes a mind-boggling array of cellular mechanisms, including individual and collective movement, differentiation and transdifferentiation, leading to development of very diverse larval types. A significant body of literature has been produced on this topic, including a dedicated book, *The Comparative Embryology of Sponges*, covering all sponge lineages in fine detail (Ereskovsky 2010). Embryonic development of *Amphimedon queenslandica*, the first sponge to have its genome sequenced, received extensive additional attention (recently summarized by Degnan et al.

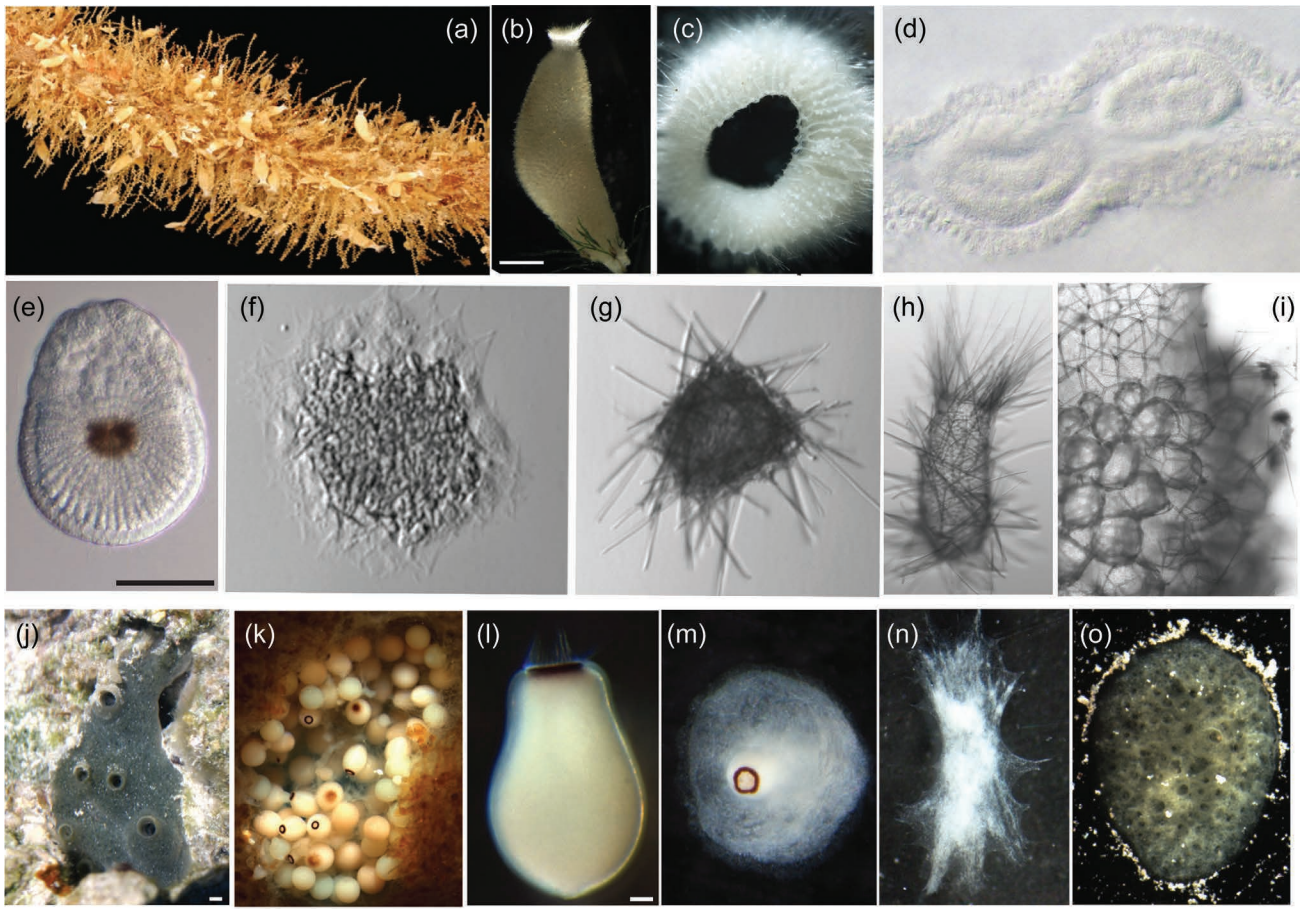


**FIGURE 4.3** **Sponge life cycle**. Adults (a, b, j), embryos within maternal tissue (c, d, k), larvae (e, l), postlarvae (f, g, m, n) and juveniles (h, i) of two sponge model systems: the calcareous sponge *Sycon ciliatum* (a–i) and the demosponge *Amphimedon queenslandica* (j–o). (a) Multiple sponge specimens growing together on *Laminaria sp.*; (j) individual sponge on coral rubble. (d) Fixed slice of tissue with spicules removed to reveal embryos; the remaining samples are live specimens or their fragments. See text for description of embryonic development and metamorphosis. Scale bars: (b, j): 5 mm; (e, l): 50 µm. ([a–i] Reproduced from Leininger et al. 2014, [j–o] from Adamska et al. 2007.)

2015). In this species, embryonic development occurs in a brood chamber, containing a mix of embryos of all stages, from eggs to ready-to-release larvae, with the younger stages close to the edge of the chamber and more mature ones at the center (Figure 4.3k). The embryos are approximately 0.5 mm in diameter and yolky, with a cell division pattern best described as asynchronous and anarchic, leading to formation of a solid, spherical morula composed of cells of different sizes and differing by pigmentation level. Extensive cell movements result in development of a bi-layered, polarized embryo (referred to as gastrula in the original publication describing development of this species; Leys and Degnan 2002, but see Nakanishi et al. 2014 for a different view on the same process). Pigmented cells coalesce at one pole of the embryo to first form a spot and then a ring (Figure 4.3k). This ring, known to be a photosensory steering organ positioned at the posterior pole of the *Amphimedon* larva (Leys and Degnan 2001), is characteristic of parenchymella-type larvae of many other demosponges (Maldonado et al. 2006). There can be an extensive number of cell types present in mature parenchymella type larvae, including sclerocytes (cells producing spicules), archaeocytes (stem cells) and, in some cases, fully differentiated choanocytes and pinacocytes (e.g. Saller 1988).

One of the best studied of the larval types among sponges are the amphiblastula larvae of Calcaronean sponges, the lineage of calcisponges that includes *Sycon ciliatum* and related species (Franzen 1988). The other lineage of calcareous sponges, the Calcineans, has calciblastula larvae very similar to cinctoblastula found in Homoscleromorph sponges (Chapter 5), although it is not clear whether this similarity reflects shared ancestry (as Homoscleromorpha and sister group to the Calcispongiae) or is a result of convergence.

The amphiblastula larva forms through a highly stereotypic series of division followed by differentiation of only three cell types, which further undergo clear differentiation pathways upon metamorphosis. The oocytes are found uniformly distributed across the mesohyl of mature specimens. In the case of *Sycon ciliatum* in the Norwegian fjords, the development is synchronous through the local populations,

with the first round of oocyte growth and fertilization occurring in the late spring (Leininger et al. 2014). Cleavage is complete, with the first two planes of division perpendicular to each other and the plane of the pinacoderm, thus dividing the zygote into four equal blastomeres. The subsequent divisions are oblique, resulting in formation of a cup-shaped embryo, with larger cells (macromeres) closer to the choanocytes and smaller cells (micromeres) facing pinacocytes (Figure 4.1). The embryonic cavity communicates with the lumen of the radial chamber, and through this opening, the embryo inverts itself so that the flagella of the micromeres (which originally form on the inner surface of the embryo) point outward.

In addition to the flagellated micromeres and larger, non-flagellated macromeres, the larva contains two other cell types: cross cells and maternal cells (Figure 4.1b). The cross cells (four in each larva) are of embryonic origin and differentiate from the outer "corners" of the four original blastomeres, with their final positions forming a cross at the equator of the larvae, conveying tetra-radial symmetry to the larva (Figure 4.1a). The function of these cells remains enigmatic, but they have been proposed to have sensory role and, consistent with this notion, express a number of genes known from other animals to be involved in specification of sensory cells and neurons (Tuzet 1973; Fortunato et al. 2014). Intriguingly, cross cells, along with maternal cells, which migrate inside of the embryo after inversion, degenerate during metamorphosis and do not contribute to formation of the juvenile body (Amano and Hori 1993).

As the larva settles on its anterior pole, the macromeres envelop the micromeres without losing epithelial character and differentiate directly to pinacocytes. The micromeres undergo epithelial-to-mesenchymal transition and become amoeboid cells. After a period of movement (hours to days, depending on species), the micromeres differentiate into choanocytes and other juvenile cell types, including sclerocytes (spicule producing cells) (Figure 4.4b). Finally, the osculum opens at the apical pole and ostia form across the surface, resulting in formation of a functional, juvenile sponge of asconoid grade of organization. The source of porocytes is unclear, but it is likely that they differentiate from pinacocytes.

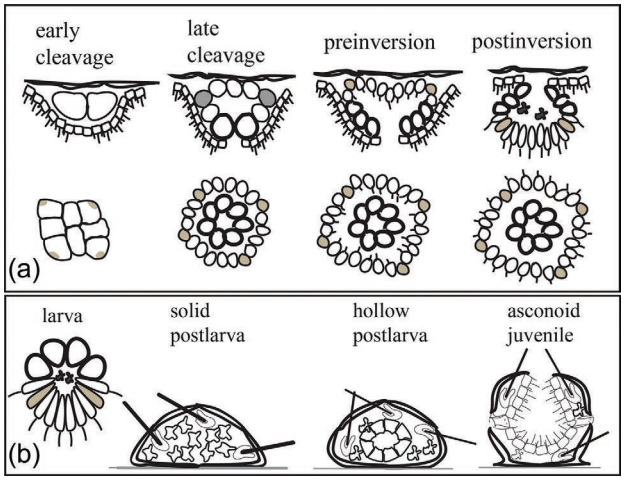


**FIGURE 4.4 Schematic representation of embryonic development (a) and metamorphosis (b) in calcaronean sponges**. In (a), the top row shows cross-sections of embryos surrounded by maternal tissues (pinacoderm and choanoderm); the bottom row is a top view of isolated embryos. Thick lines indicate macromeres and pinacocytes; thin lines indicate micromeres and choanocytes. Embryonic/larval cross cells and the cytoplasm of cleavage stage embryo destined to become cross cell are shaded gray.

## 4.5 ANATOMY

All sponges (with the notable exception of carnivorous sponges, which secondarily lost choanocytes; Vacelet and Boury-Esnault 1995; Riesgo et al. 2007) are built of the same basic building blocks: choanocytes forming choanoderm of the radial chambers, the pinacoderm lining all remaining surfaces, with varying types and numbers of cells inhabiting the mesohyl. The mesohyl can be very cell poor and narrow (for example, in the Homoscleromorph sponges; see Chapter 5) or constitute most of the body of the sponge, as in many Demosponges. Traditionally, the body plans are divided into three major types. The simplest is asconoid, as described for Calcaronean juveniles (Figure 4.3h, 4.4b), with many calcisponge species retaining this body organization, with branching and anastomosing tubes forming as the body enlarges. The second type is syconoid, as in calcisponges from the genus Sycon (Figure 4.1, Figure 4.3b, c, i), with radial choanocyte chambers surrounding endopinacocyte-lined atrial cavity. The most complex, and the most common among sponges (being the typical body plan of Demosponges, the most speciose of the sponge lineages), is the leuconoid body plan composed of choanocyte chambers linked by an intricate network of endopinacyte-lined canals (Figure 4.1 and 4.3j, o). Two lesser-known sponge body plans should also be mentioned. One is the sylleibid body plan found in Homoscleromorph sponges, which can be considered a link between the syconoid and leuconoid body plans, with multiple syconoid-level units connected to the atrium. The most recently described sponge body plan, solenoid, is found in some Calcinean species and can be best described as a complex system of anastomosing tubes of the asconoid grade embedded in a thick mesohyl layer (Cavalcanti and Klautau 2011).

In the majority of sponges, the epithelial and mesenchymal layers are supported by organic and/or inorganic skeletons. The spongin-based organic skeletons of the genus *Spongia* and related species are well known as bath sponges—although, after the natural populations have been virtually exterminated by combination of harvest and pollution of the habitat, natural bath sponges have been all but replaced by artificial ones (Pronzato and Manconi 2008).

The majority of sponges produce inorganic skeletal elements, called spicules, which were traditionally the key to sponge taxonomy, given the paucity of other characters available until the advent of molecular phylogenies (Uriz 2006;

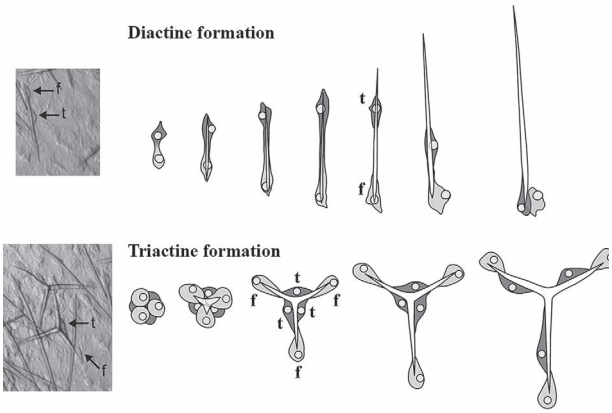


**FIGURE 4.5 Spicule formation in calcareous sponges.** Thickener cells (t) are dark gray, founder cells (f) are light gray. (Modified from Voigt et al. 2017, with the schematic representations re-drawn from Minchin 1908.)

van Soest et al. 2012). The spicules are of two types—built of calcite in the calcisponges and of silica in the remaining three lineages. Not only the material but also the cellular mechanism of spicule synthesis and subsequent positioning differs. The demosponge spicules are produced intracellularly, within vacuoles, and are subsequently moved to their final position by a concerted action of carrier cells (Mohri et al. 2008; Nakayama et al. 2015). In contrast, calcareous spicules are produced by groups of cells, the numbers of which depend on the type of the spicule and tend to remain in situ, without subsequent movement. For example, single-rayed spicules (diactines) are secreted by two cells, one known as the founder cell and the other as the thickener cell. On the other hand, the tri-radial triactines are produce by sextets of cells, with three founder cells and three thickener cells working together to produce one spicule (Minchin 1908; Voigt et al. 2017) (Figure 4.5). Different types of spicules form supporting structures along the body, with the long, slender diactines often found forming a crown or a collar around the osculum (Figure 4.3b).

## 4.6 GENOMIC DATA

The first insight into gene content of sponges was provided by transcriptome rather than genome analyses. Most significantly, the analysis of developmental regulatory genes in the transcriptome of the homoscleromorph sponge *Oscarella carmela* revealed that sponges possess multiple components of developmental signaling pathways used by animals to regulate their development (Nichols et al. 2006). However, the complete developmental regulatory gene repertoire of a sponge could only be fully appreciated by whole genome sequencing. The first sponge for which this was achieved was the demosponge *Amphimedon queenslandica*, a species inhabiting reefs fringing the Heron Island of the Great Barrier Reef (Srivastava et al. 2010). This was not only the first but also likely the last sponge genome sequenced using the traditional Sanger method. *Amphimedon* genome analysis revealed that for the overwhelming majority of developmental regulatory gene families, whether signaling molecules or transcription factors, *Amphimedon* possesses fewer family members than the more complex animals (Cnidarians and Bilaterians). This pattern, perhaps expected, was consistent with the notion that a simple animal would have a simpler regulatory gene repertoire.

It was therefore surprising when analysis of the second sponge species to be sequenced—the calcareous sponge *Sycon ciliatum*—revealed developmental gene family sizes on a par with those found in bilaterians. For example, while humans have 19 Wnt ligands and *Amphimedon* has 3 (Adamska et al. 2007), Sycon has 21 (Leininger et al. 2014). Even more strikingly—and controversially—the *Sycon* genome appears to possess a ParaHox gene, Cdx, which is clearly absent from the *Amphimedon* genome (Larroux et al. 2007; Fortunato et al. 2014). A systematic comparison of transcription factors present in *Amphimedon* and *Sycon* demonstrated that genomes of calcisponges and demosponges underwent independent events of gene loss and family expansions (Fortunato et al. 2015).

Gene content analysis of two Hexactinellids (glass sponges) revealed a different kind of surprise—it appears that neither *Oopsacas minuta* nor *Aphrocallistes vastus* possesses key components of the Wnt signaling pathway (Schenkelaars et al. 2017). As this pathway is used across the animal kingdom (including other sponges; See section 4.7) to pattern the major body axis, this finding is another key indication that insights from one lineage of sponges cannot be assumed to reflect the genome composition of all sponges—and of the last common ancestor of all animals. Instead, it thus appears that, since the divergence approximately 600 million years ago, sponge gene repertoires underwent dramatic changes, in contrast to the body plans which remained apparently stable throughout this time.

But sponge genomes can provide insight into more than just gene content: a gateway to understand evolution of genome function in animals. One of the mechanisms known to regulate gene expression in vertebrates (but not in the majority of invertebrates) is DNA methylation. However, the evolutionary history of this mechanism is not well understood. A recent study revealed that—in parallel to the differences found in gene content—sponge genomes are methylated to very different levels. While the *Amphimedon* genome is highly methylated (in striking similarly to vertebrate genomes), methylation in *Sycon* is more moderate, consistent with independent acquisition of genome methylation in sponges (de Mendoza et al. 2019).

Gaiti and colleagues (2017) used the *Amphimedon* genome find out whether two other regulatory features of animal genomes are found in sponges: the posttranslational modifications of histone H3 (linked to precise regulation of gene expression in animals) and micro-systenic units harboring distal enhancers of developmental regulatory genes.

Perhaps surprisingly, both features were found, demonstrating that they predate (and were perhaps the key to) divergence of animal lineages (Gaiti et al. 2017).

The very recent advances in genome sequencing technologies, allowing relatively cheap generation of (almost) chromosomal-level assemblies, opened the way to comparing large-scale synteny (gene order) analysis in addition to micro-synteny studied before. The first sponge genome to be assembled to this contiguity level, that of *Ephydatia mulleri*, demonstrated strong synteny conservation between this freshwater demosponge and other animals but not with choanoflagellates (Kenny et al. 2020). Time (and ongoing sequencing efforts) will tell if genomes of sponges representing other lineages also maintained this conservation or whether they hold further surprises.

## 4.7 FUNCTIONAL APPROACHES: TOOLS FOR MOLECULAR AND CELLULAR ANALYSES

Evolutionary genomics and developmental biology strive to go beyond cataloguing genes, attempting to reveal the links between gene expression and function. Decades of research revealed that across the animal kingdom, key developmental events, such as establishment of germ layers and polarity of embryos, as well as cell fate specification, are governed by a conserved set of regulatory genes. As soon as homologues of these genes were uncovered in sponge transcriptomes and genomes, in situ hybridization methods were developed, allowing interrogation of expression patterns of the candidate genes (Larroux et al. 2008).

One of the key examples of pan-metazoan functional conservation is the role of the Wnt pathway in specification of the primary body axis, with Wnt ligands expressed in the posterior poles of cnidarian and bilaterian embryos, as well as the apical region of cnidarian polyps. In several sponge species, Wnt ligands are expressed in the posterior pole of sponge larvae and around the osculum of sponge adults (Figure 4.6), suggesting that this role is conserved in sponges and therefore predates animal divergence (Adamska et al. 2007; Leininger et al. 2014; Borisenko et al. 2016). Similarly, genes involved in specification of animal sensory cells, such as components of the Notch pathway and the transcription factor bHLH1 (related to atonal and neurogenin in bilaterians), are expressed in the sensory cells of *Amphimedon* larvae (Richards et al. 2008).

However, gene expression patterns, while certainly suggestive, still do not demonstrate gene function. Disappointingly, functional gene expression analysis—through interference with gene function by morpholino or RNAi, or generation of transgenic animals to understand effects of gene overexpression—is still not a routine methodology in sponges. This is despite multiple efforts, some giving tantalizing results, such as successful generation of transgenic sponge cells, although with a success rate in the range of 1 in 10,000 cells (Revilla-I-Domingo et al. 2018), or downregulation genes targeted by RNAi, although with change level that required qPCR to demonstrate it (Rivera et al. 2011). Despite this limited success so far, efforts to establish robust functional genomics strategies continue in many sponge laboratories across the world. In the meantime, biologists utilize a range of other methodologies to gain functional insights into sponge development. For example, taking a drug interference approach, Windsor Reid and Leys (2010) demonstrated that the Wnt pathway is involved in specification of the main body axis of the demosponge *Ephydatia mulleri*.

## 4.8 CHALLENGING QUESTIONS BOTH IN ACADEMIC AND APPLIED RESEARCH

Perhaps surprisingly, the two major topics that attracted biologists to sponges in the 19th century, namely origin of the animal body plan and regeneration, continue to provide background for vibrant research programs in many laboratories—and ongoing debates in the research field. Until very recently, the relationship between sponge cell/tissue types and body plan organization was interrogated using the candidate gene approach. As discussed in Section 4.7, results of these analyses are consistent with homology of the major body axis (specified by the Wnt pathway) in sponges and cnidarians, therefore suggesting that the first animals also used the Wnt pathway to pattern their bodies (reviewed by Holstein 2012). Moreover, subsequent gene expression analyses focusing on genes involved in specification of animal endomesoderm, revealing that these genes are expressed in sponge choanocytes, are also consistent with Haeckel's idea that the sponge choanoderm is homologous to the cnidarian gastrodermis (Leininger et al. 2014; Adamska 2016a, 2016b). However, the fact that sponge cell fate specification is unusually fluid, allowing choanocytes to transdifferentiate into pinacocytes (thus apparently changing germ layer identity), makes some researchers unwilling to accept that notion (Nakanishi et al. 2014). While the question of cell type homology between sponges and other animals remains open for now, a novel approach based on expression of genes with conserved microsynteny yielded results consistent with the proposed homology of choanocytes and cells involved in cnidarian digestion (Zimmermann et al. 2019; Adamska 2019).

On the other side of the evolutionary transition leading from protists to complex animals, the similarity between choanocytes and choanoflagellates, understood to indicate homology of the collar apparatus throughout the 20th century, has become controversial again (Mah et al. 2014). Some authors take evidence of morphology, function and molecular composition of collars and flagella in choanocytes and collar cells as strong support for the proposed homology (Peña et al. 2016; Brunet and King 2017). Yet others used comparison of *Amphimedon* cell-type gene expression with cell-state gene expression data from choanoflagellates and a range of other protists to suggest that choanocyte

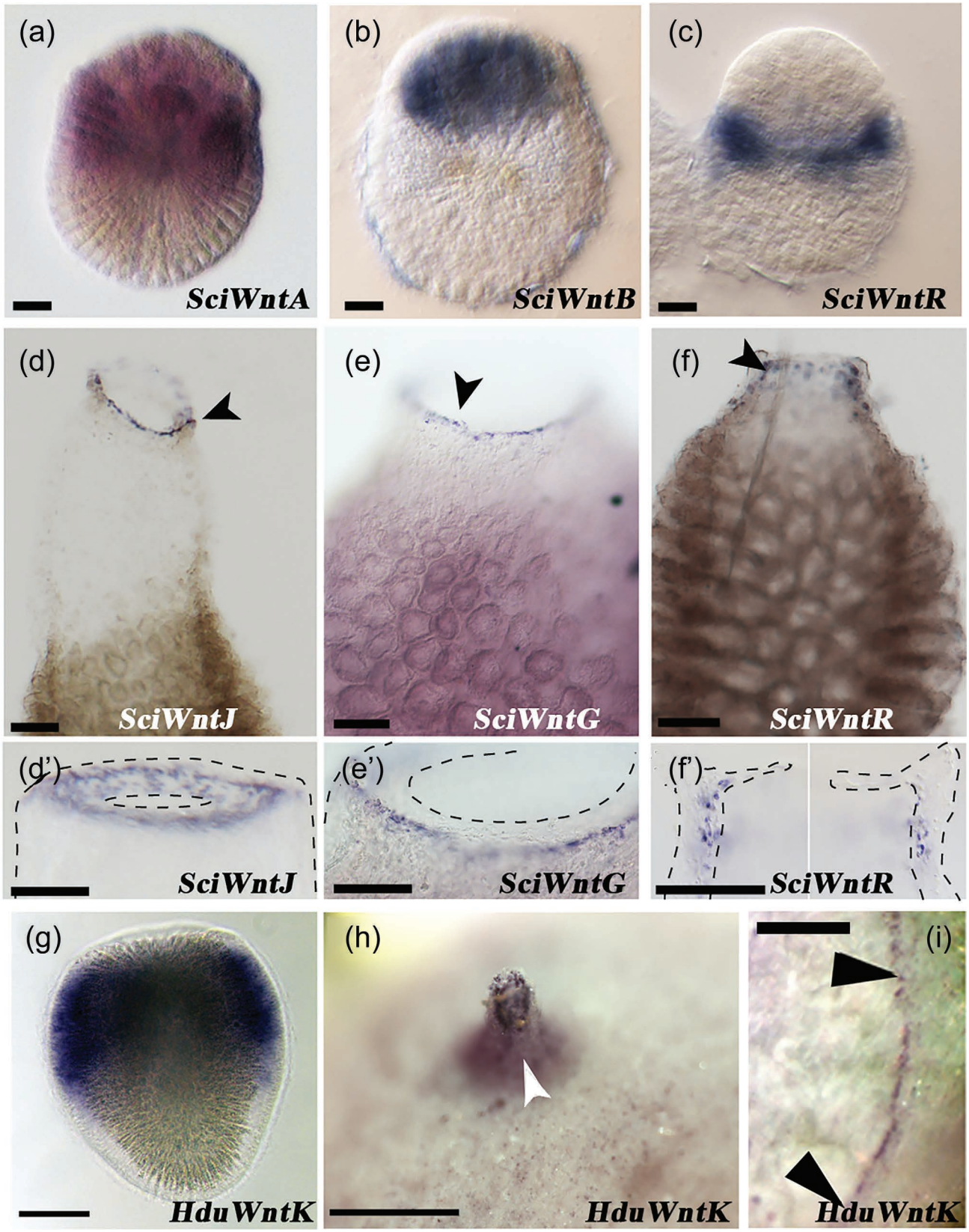


**FIGURE 4.6** **Expression of Wnt ligands in sponges**. (a–c) Larvae of the calcareous sponge *Sycon ciliatum*. (d–f) Oscular regions of *S. ciliatum* (' indicates higher magnification; dashed lines delineate transparent tissues). (g, h, i) The demosponge, *Halisarca dujardini:* larva, the osculum and regenerating epithelium, respectively. Larval posterior and osculum are at the top of each image. Scale bars: (a–c): 10 μm, (d–f'): 100 μm, (g): 50 μm, (h–i): 3 mm. ([a–f] Reproduced from Leininger et al. 2014, [g–i] from Borisenko et al. 2016.)

morphology evolved independently from choanoflagellates (Sogabe et al. 2019). That these seemingly academic questions are also exciting to the general audience is evidenced by popular science magazines covering this debate (Cepelewicz 2019).

Less "academic", as understanding of sponge regeneration capacity might potentially be applicable to human regenerative medicine, is the question of how sponges regulate their spectacular regenerative capacities. Recent research reveals that some of the regeneration mechanisms might indeed be shared between sponges and other animals, as many of the developmental signaling pathways known to be involved in mammalian regenerations are also activated during regeneration of sponges, including re-building of bodies from dissociated cells (Soubigou et al. 2020). The most exciting aspect of sponge regeneration appears to be the capacity of sponge cells to directly transdifferentiate upon injury (Ereskovsky et al. 2015; Ereskovsky et al. 2017; reviewed by Adamska 2018). Would it be possible to utilize mechanisms involved in transdifferentiation of sponge cells to reprogram mammalian cells for therapeutic purposes?

The pharmaceutical industry has been investigating sponges as potential sources of bioactive compounds, with great success, for over 50 years. In 1969, the first sponge-derived anti-cancer drug, cytarabine (also known as Ara-C, Cytosar-U or Depocyst), originally extracted from the Caribbean demosponge *Tectitethya crypta*, was approved by the Food and Drug Administration (FDA). In 1976, the FDA also approved vidarabine (Ara-A, Vira-A) as an antiviral drug derived from the same sponge species (reviewed by Brinkmann et al. 2017). More recently, eribulin mesylate (E389, Halaven), an analog of halichondrin B isolated from Japanese demosponge *Halichondria okadai*, was approved as treatment for metastatic breast cancer (reviewed by Gerwick and Fenner 2013).

In addition to being useful, the secondary metabolites found in sponges are all the more fascinating as they are in fact produced by microbes living in close symbiosis with their poriferan hosts. The study of sponge microbiomes revealed essential roles in nutrient cycling and production of vitamins in addition to the secondary metabolites likely responsible for protection of sponges from potential predators and fouling organisms (see Reiswig 1981; Maldonado et al. 2012). It appears that the complex, species-specific assemblages of bacteria can be transmitted both horizontally (from the surrounding water) and vertically (from mother to larvae) (e.g. Schmitt et al. 2008; Webster et al. 2010). However, the molecular mechanisms involved in establishment and maintenance of these symbioses are not understood and remain an area of open and exciting investigations.

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