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Sponge Development and Antiquity of Animal Pattern Formation[1]

BERNARD M. DEGNAN,[2,*] SALLY P. LEYS,† AND CLAIRE LARROUX*

*School of Integrative Biology, University of Queensland, Brisbane Qld 4072, Australia
†Department of Biological Sciences, University of Alberta, Edmonton, Alberta T6G 2E9, Canada

SYNOPSIS. The last common ancestor to all extant animals possessed features shared between the most basal metazoan lineage—Porifera—and the rest of the animal kingdom. To identify ancient and conserved developmental processes, we have been investigating embryogenesis and metamorphosis in the demosponge *Reniera*. Many of the cardinal features of eumetazoan development are displayed during *Reniera* embryogenesis. Specifically, after fertilization there is a period of cell division with little to no cell growth that results in two obvious cell populations distinguished by size as micromeres and macromeres, and by fate: the small cells differentiate into ciliated cells. This is followed by a period of differential cell activities that produces an embryo consisting of two then three layers, where at least 11 populations of differentiated cells are allocated into the different layers and patterned within these layers. This organization yields a swimming larva with the capacity to sense and respond to the surrounding environment, despite a lack of neurons and a coordinating system. During *Reniera* embryogenesis, the clearest example of cell patterning is the formation of a ring of pigment cells at the future posterior pole of the larva. Pigment cell pattern formation has two phases, both of which may require the movement of a large number of cells apparently in response to a morphogen gradient. First, pigmented cells, which initially cover the surface of the embryo, migrate to the future posterior end and form a dark spot. Second, the cells move outwards from the spot and rearrange into a ring. Numerous and diverse transcription factor genes are expressed during *Reniera* embryogenesis, most of which belong to metazoan-specific families and include members of *POU, LIMHD, Pax, Bar, Prox2, NK-2, T-box, MEF-2, Fox, Sox, Ets,* and *nuclear hormone receptor* families. In combination, these observations suggest that the last common ancestor to all extant metazoan lineages already possessed the basic regulatory genetic architecture to direct the specification, patterning and differentiation of multiple cell types. Some of these differentiated cells may have been arranged into localised functional units—*i.e.*, simple tissues.

INTRODUCTION

The evolution of multicellular animals from unicellular protists is one of the key transitions of life on Earth. While we can not directly examine the first metazoans nor the evolutionary steps leading to their origin, we can infer certain details about them through careful comparisons of living animals. Specifically, we can gain insights into the genetic innovations underlying this transition by comparing the genomes of a range of metazoans with representatives in closely related protist lineages, such as the choanoflagellates. We also can infer some features of the last common ancestor (LCA) to all animals by comparing extant metazoan genomes, cells, modes of development and physiologies. Essential to any reconstruction of early animal evolution is the inclusion of representatives from the most basal metazoan lineages—sponges, cnidarians, ctenophores and placozoans.

Morphological and molecular data indicate that metazoans are monophyletic (Zrzavy *et al.*, 1998; Adoutte *et al.*, 2000; Medina *et al.*, 2001). The phylogenetic relationships of basal metazoans remain contentious (*e.g.*, Cavalier-Smith *et al.*, 1996; Nielsen *et al.*, 1996; Collins, 1998; Kim *et al.*, 1999; Collins and Valentine, 2001; Brusca and Brusca, 2003; Rokas *et al.*, 2003), although there is a general consensus that sponges are the most basal metazoan lineage and that cnidarians and ctenophores, along with bilaterians, comprise the so-called Eumetazoa. Combined analysis of nuclear large and small subunit rRNA indicates that siliceous sponges (hexactinellids and demosponges) are the most basal metazoans, although their relationship to calcarean sponges and other basal animals remains unresolved (Medina *et al.*, 2001). Other recent molecular phylogenies also support the basal position of a hexactinellid + demosponge clade (Kruse *et al.*, 1998; Borchiellini *et al.*, 2001). These phylogenies argue for the inclusion of siliceous sponge representatives in studies that seek to reconstruct the LCA of the metazoans, or evolutionary steps leading to this ancestor. The apparent paraphyly of sponges with respect to the eumetazoans (*e.g.* Medina *et al.*, 2001) lends further support for the use of sponges in understanding early metazoan evolution.

DEVELOPMENT OF THE DEMOSPONGE *RENIERA*

Although phylogenetically crucial, demosponges largely have been overlooked for developmental studies because their adult body plan is simple and appears to have little in common with the rest of the Metazoa (Brusca and Brusca, 2003). There also has been an historical confusion regarding embryogenesis and gastrulation in sponges, in part due to the diversity of external characteristics of different sponge embryos and larvae (Leys and Degnan, 2002; Leys, 2004; Mal-

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[2] E-mail: b.degnan@uq.edu.au

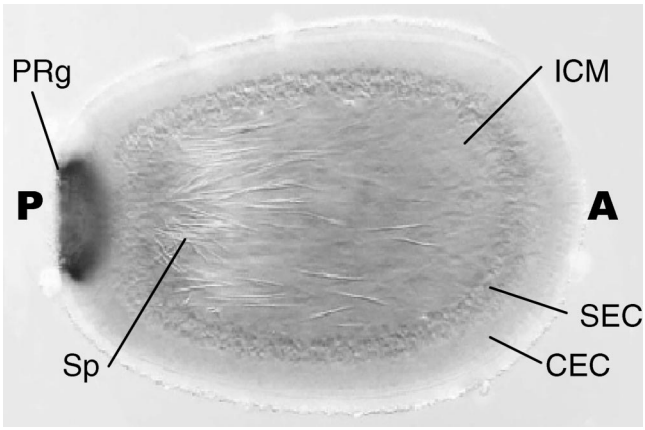

FIG. 1. Cellular organisation of the *Reniera* larva. The whole-mounted larva displayed has been cleared. It consists of three layers. Uniciliated columnar epithelial cells (cec) form the outer layer, except at the anterior (A) and posterior (P) poles. Interspersed throughout this layer are large mucous cells. Flask-shaped ciliated cells are regularly interspersed among the columnar epithelial cells in the anterior third of the larva. Underlying the outer layer of cells is a circumferentially arranged layer cells—subepithelial cells (sec)—embedded in a collagen matrix. This sheet of cells is only interrupted at the posterior end of the larva. The inner cell mass (icm) of the larva is composed of at least 4 cell types that largely are aligned along the AP axis and are surrounded by a thick layer of collagen fibres. Spicule (sp) producing sclerocytes chiefly are localised to the posterior third of the inner cell mass. The anterior end of the larva is not ciliated and is composed of large cuboidal cells. Large cells containing electron dense inclusions protrude from bare posterior pole. The pigmented cells form a ring (prg) at the boundary of the large posterior cells and the columnar epithelial cells. These cells possess a long posterior cilium. See Leys and Degnan (2002) for further details.

donado, 2004). Adult sponges do not have any obvious body axes and appear to lack tissue-level organization, although recent reassessment of these characters suggest they do possess rudimentary axes and tissue organization (Bavestrello *et al.*, 1998; Leys and Degnan, 2001). Though they are described as lacking true tissue organization and certain cell types, including muscle cells and neurons, sponges share a number of cytological and molecular features with the rest of the animal kingdom. Most notably, they possess a collagen-based extracellular matrix, and some groups clearly possess a basement membrane (Boute *et al.*, 1996; Muller, 2001; Boury-Esnault *et al.*, 2003).

Recently, we have developed a demosponge model system–*Reniera* sp.[3]–that is amiable to evolutionary developmental studies and, importantly, has body plan features—A-P polarity, a photoreceptive sensory system, and three apparent cell layers—that suggest direct comparison of the sponge body plan with the rest of the animal kingdom may not be far-fetched (Fig. 1). Using a combination of detailed ultrastructural analyses of *Reniera* embryos and larvae to assign cell types and to track differentiation and morphogenetic events (Leys and Degnan, 2001, 2002; Leys, 2003*a*, 2004), and developmental gene expression data (Larroux *et al.*, unpublished data), we have been reassessing sponge development in light of recent advances in metazoan development.

This sponge broods embryos and larvae at all times, allowing year-round access to biological material. The brood chambers contain 50 to 150 embryos that develop asynchronously. Through close inspection of *Reniera* developmental stages and the use of cell-lineage tracers, we have been able to characterise embryogenesis and metamorphosis. We have come to the conclusion that *Reniera* embryogenesis includes many developmental hallmarks usually associated with eumetazoans (Leys and Degnan, 2002). After fertilization there is a period of cell division with little to no cell growth, and cleavage ends with an unequal cell division, each cell giving rise to a small and a large cell, here termed micromeres and macromeres. Cell differentiation is first detected in populations of interspersed micromeres, which quickly differentiate into uniciliated cells. A group of pigmented cells and spicule producing sclerocytes differentiate at the surface (Leys and Degnan, 2002; Leys, 2003*a*; Figs. 2, 3). The pattern of cells within the embryo appears to be individualistic, suggesting that fixed cleavage patterns and cell lineages do not exist in this demosponge.

Cleavage is followed by a period of differential cell movement that appears to occur largely by multipolar delamination (sometimes called morula delamination). At this time, cells lack robust cell junctions but are surrounded by a collagenous extracellular matrix (which is denser centrally than peripherally) and appear to be operating autonomously in a manner similar to mesenchyme cells. These cell activities produce an embryo consisting of two layers in which populations of differentiating cells are allocated into the different layers—macromeres form the inner cell mass and are surrounded by outer micromeres (Fig. 2). The formation of this bi-layered embryo is considered to be gastrulation. At this stage, the differentiating cells are allocated to specific locations.

At or immediately following the formation of the bilayered embryo, we see in both living and fixed embryos the first indications that an anterior-posterior (AP) axis is being established (Leys and Degnan, 2002; Figs. 2, 3). Pigment cells and sclerocytes begin migrating to the future posterior pole in a predictable manner. As the cells of these two populations are interspersed throughout the ectoderm and do not form contacts with other cells of similar type, we infer that they are operating independently. At this stage, the presumptive uniciliated epithelial cells of the outer layer remain loosely associated (Figs. 3, 4), presumably allowing pigment cells, sclerocytes and other localized cells (*e.g.*, flask cells) to migrate to their destinations. Importantly, while intercellular connections at this stage are minimal, most, if not all, of the cell types in this layer appear to be determined and differentiating. The predominant cells in this layer at this stage are the uniciliated columnar epithelial cells, pigment cells

[3] A voucher specimen has been deposited at the Queensland Museum (QM G315611).

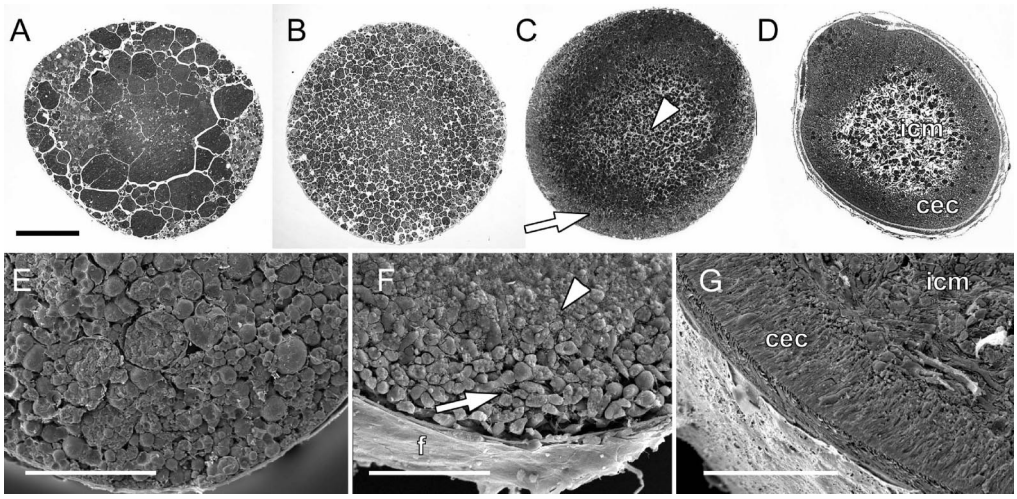

FIG. 2. Epon sections and SEM fractures of embryos. (A) Early blastula with large yolk filled cells. (B, E) Blastulas at the stage when unequal cleavage occurs to form micromeres and macromeres. While micromeres differentiate into ciliated cells at this stage, pigment cells or sclerocytes are not detected. (C, F) Early gastrulas, where ciliated micromeres have migrated to periphery. These embryos have two layers; the outer layer (arrow) is loose collection of migrating cells; cells in the inner layer (arrowhead) are tightly embedded in a dense collagenous matrix. Pigment cells and sclerocytes are present in the outer layer at this stage and are in the process of migrating to the future posterior pole. The embryo is surrounded by a layer of maternal follicle cells (f). (D, G) Late gastrula with two distinct cell layers. A columnar epithelium of ciliated cells (cec) formed around an inner cell mass (icm), the pigment ring is forming (D, upper left), and these embryos are elongating along the A-P axis. Scale bars: A–D, 200 μm; E, 100 μm; F,G, 50 μm. See Leys and Degnan (2002) and Leys (2003a) for methods and detailed descriptions of *Reniera* sp. development.

and sclerocytes, which have begun fabricating siliceous spicules (Leys and Degnan, 2001, 2002; Leys, 2003*a*; Figs. 2–4).

In the later gastrula, the pigment cells, which have coalesced into a spot at the posterior pole, reverse direction and begin migrating anteriorly, so that the spot transforms into an external ring that surrounds the posterior pole (Figs. 1, 3). Later during this migratory phase, the pigment cells appear to be operating in a more cohesive manner, with a defined leading edge of migration evident (Fig. 3D). Around the same time, the sclerocytes located in the outer cell layer, ingress and migrate into the posterior portion of the inner cell mass. They remain localized to this region in the larva (Fig. 1). At this stage the middle cell layer is formed; the origin of this important feature is still unclear. The final result of gastrulation is a fully differentiated larva that hatches and is free-swimming.

Ultrastructural analysis reveals that the *Reniera* larva consists of three layers incorporating at least 11 pluripotent and differentiated cell types arranged in stereotypic patterns along radial and AP axes (see Leys and Degnan, 2001, 2002; Leys, 2003*a*, for details; Fig. 1). The outer layer consists of ciliated columnar cells except at the anterior and posterior poles. At least four other cell types in the outer layer are patterned along the AP axis: (1) the anterior-most unciliated, cuboidal cells; (2) the ciliated flask cells, which are interspersed among the columnar epithelium in the anterior third of the larva; (3) the pigment cells that form a ring around the posterior pole; and (4) the posterior pole cells which protrude from the surface and possess mucus-like inclusions. Underlying the outer layer is a sheet of cells that is interrupted only at the posterior end of the larva. Cells of this middle layer are long and thin with numerous spherulous inclusions. They lie within

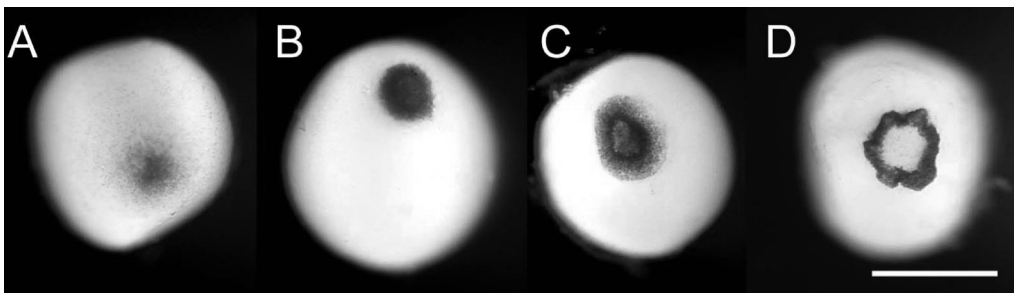

FIG. 3. Migration of pigment cells during *Reniera* embryogenesis. Micrographs of whole-mounted, uncleared embryos. (A) Pre-gastrula with pigment cells present in the outer layer of the embryo. (B) Gastrula, in which pigment cells have coalesced to form a spot at the posterior pole. (C) Late gastrula, in which pigment cells migrate outward from the posterior spot to form a ring. (D) Pre-larva with a forming ring. At this stage, there is a tight leading edge of migrating cells that are typically scalloped shaped. Scale bar, 300 μm.

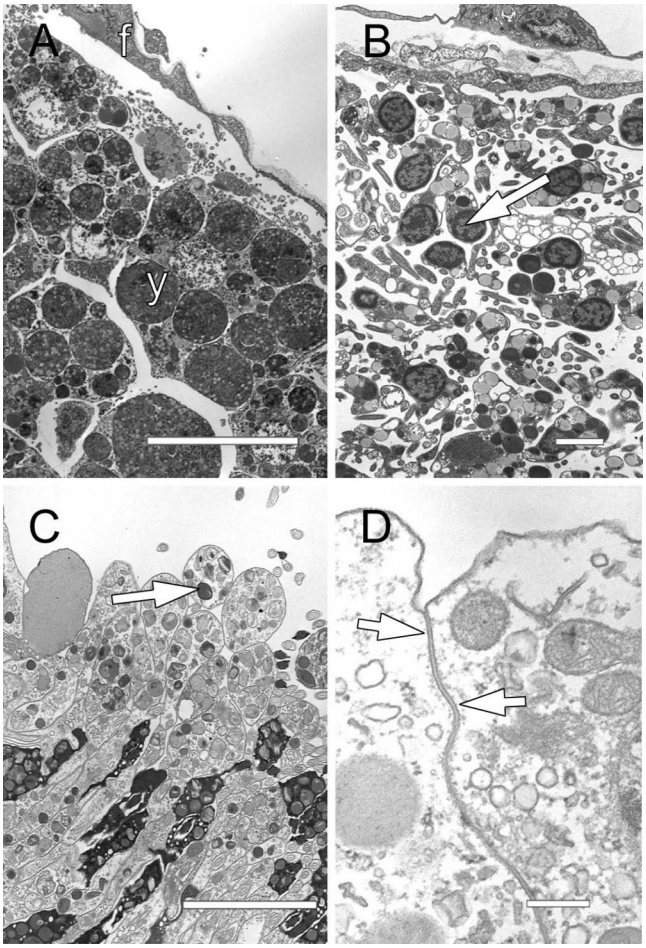

FIG. 4. Thin section transmission electron micrographs (TEM) of the edges of embryos and larvae. (A) Blastula stage (equivalent to Fig. 2B, E). Cells are large and have many yolk granules (y); some cells have begun unequal cleavage at this stage (although no ciliated micromeres are visible in this micrograph). (f, maternal follicle cells). (B) Gastrula stage—the ciliated micromeres (arrow) are migrating to the periphery; the periphery is a loose collection of these cells, whereas the centre of the embryo (seen in Fig. 2C) is a dense collection of macromeres. (C) Pigment granule-containing cells (arrow) at the posterior pole of the fully formed larva. (D) Cell junctions (arrows) between cells at the posterior of the larva are simple; no desmosomes, nor junctional complexes are evident in normal TEM.

a dense collagenous extracellular matrix and are oriented perpendicular to the AP axis of the larva (Fig. 1, sub-epithelial cells). Although a middle layer has been noted in many demosponge larvae (*e.g.*, Woolacott and Hadfield, 1989; Woollacott, 1990, 1993), its significance is not yet clear (see discussion in Leys and Degnan, 2002). The third layer, the inner cell mass, appears to house at least 4 cell types. The sclerocytes are localised within this inner mass, and the location of internally-produced spicules suggests that they are largely restricted to the posterior third of the larva.

Of these localised cell types, only sclerocytes and pigment cells have been assigned functions, namely spicule synthesis and photosensitivity, respectively (Leys and Degnan, 2001; Leys *et al.*, 2002; Leys, 2003*a*). Nonetheless, it certainly appears that the patterning and differentiation of the other cell types confers additional functionality to the *Reniera* larva, which in turn ensures that the larva locates a suitable place to settle and undergo metamorphosis (Jackson *et al.*, 2002).

CELL PATTERNING DURING *RENIERA* EMBRYOGENESIS: MORPHOGENESIS WITH DIFFERENTIATION

Our study of *Reniera* development indicates that demosponge embryos and gastrulae have obvious affinities with the rest of the animal kingdom and thus are an appropriate taxon to compare with other animals to infer ancestral states in the Metazoa. Particularly striking is the role of directional cell migration during embryogenesis in establishing cell layers and in localising cell populations along the AP axis. Currently no other poriferan developmental model exists, but further studies of sponges with external development would be extremely useful (*e.g.*, Borojevic, 1967; Lévi and Lévi, 1976; Watanabe, 1978).

Sponges, like all animals, consist of populations of differentiated cell types. It is likely that the developmental regulatory processes underlying the formation of specialised differentiated cells are a very ancient and conserved feature of metazoans (Peterson and Davidson, 2000; Davidson, 2001; Larroux *et al.*, unpublished data). Nonetheless, the morphogenetic events leading to the formation of sponge and eumetazoan body plans are commonly assumed to be markedly different (Peterson and Davidson, 2000; Erwin and Davidson, 2002; Brusca and Brusca, 2003). Based on analysis of normal development in *Reniera* (Leys and Degnan, 2001, 2002; Leys, 2003*b*) and other sponges (Leys, 2003*b*, 2004; Maldonado, 2004), we argue instead that cell behaviours and movements in sponge embryos are akin to those occurring during gastrulation and pattern formation in other animals. In some cases, cell patterning leads to the establishment of simple tissues whose organization confers a new functionality beyond the ability of the individual cells that the tissue comprises (*e.g.*, pigment ring; Leys and Degnan, 2001; Fig. 2).

An interesting feature of *Reniera* development is the early determination of embryonic cell types. First, cilia form prior to the separation of macromere and micromere populations (Leys and Degnan, 2002). Second, pigment granules accumulate in pigment cells and biomineralisation proteins are expressed in sclerocytes prior to their migration to the posterior pole. These two observations make it clear that cell differentiation begins prior to morphogenesis in *Reniera*, at least for some cell types. This order of events differs from that observed in many bilaterian systems, where regional specification and pattern formation often precede cell determination and differentiation (Davidson, 2001). There are however examples of differentiation occurring before morphogenesis in bilaterians. For example, differentiation of the ciliated cells that form the pro-

totroch in spiralian trochophores begins prior to or during prototroch morphogenesis (Damen and Dictus, 1994).

A key feature of *Reniera* pattern formation is the heavy reliance on the migration of individual cells. Formation of inner and outer layers appears to occur solely by the movement of individual cells throughout the embryo, by mesenchymal rather than epithelial movements, because junctions do not appear between any cells until the outer columnar epithelial layer has formed (see Leys and Degnan, 2002; Figs. 2, 4); at present, it is unclear how the middle layer forms later in development. The determinants directing multipolar ingression/delamination during early gastrulation in *Reniera* also are unknown, although underlying the different behaviours of macromeres and micromeres is likely to be differential gene expression that yields different populations of receptors and signalling pathway components, cell surface molecules and/or transcription factors. One possibility is that asymmetric cell divisions that give rise to micromere and macromere populations result in the inheritance of determinants that confer different cell behaviours or affinities to these populations. Micromere and macromere populations are mixed throughout the embryo, and sorting of these cell types appears to occur simultaneously across the entire embryo. It is unlikely, therefore, that early cell migration in *Reniera* is the result of a differential response of the two cell populations to a morphogen emanating from a point source. It is possible, however, that the maternal cell layer, a sheet of follicular cells that encapsulates the embryo and which in other sponges is known to provide nutrients to the embryo (Fell, 1969; Figs. 2, 4), may provide signals that direct the differential migration of these cell populations. Alternatively, signals may be produced either by the macromeres and/or micromeres themselves, or by a subset of cells in these populations that promote the aggregation of macromeres internally and of micromeres on the surface.

Formation of embryonic layers in sponges has historically been attributed to a range of gastrulation processes, including epiboly, delamination and ingression, some of which require coordinated movement of sheets of cells (reviewed in Leys, 2004). Problems with the interpretation of gastrulation in the Porifera stem largely from misunderstandings regarding the migration of the outer ciliated cells into the centre of the juvenile to form the choanocytes at metamorphosis. Modern workers are in agreement that these migrations occur after embryogenesis has formed a fully differentiated bi- and even tri-layered larva, and thus are not related to gastrulation (Amano and Hori, 1996; Efremova, 1997; Leys and Degnan, 2002; Maldonado, 2004). Thus the movements that do comprise gastrulation in those embryos studied can be attributed to individual cell migrations, not sheets of cells, during embryogenesis, not metamorphosis.

Occurring concomitantly with early cell differentiation and cell layer formation in *Reniera* is the establishment of a larval AP axis. This is inferred from the movement of pigment cells and sclerocytes to the future posterior end of the embryo prior to the completion of micromere/macromere sorting (Leys and Degnan, 2002; Figs. 1–3). Pigment cells and sclerocytes initially cover the surface of the embryo and are not localized to any territory. Posterior migration begins before the columnar epithelial layer forms, when all micromeres of the outer layer are still loosely associated (Figs. 3, 4).

The initial directional movement of these two populations of differentiating cells is very similar, which suggests that they are responding to the same signal emanating from one or both poles of the embryo. We speculate that this signal may be produced either from embryonic or maternal cells positioned at the pole or from the pigment cells and/or sclerocytes themselves. Since both cell types behave in a similar manner and are tracking together, the source of the signal may be independent of these cell types. An embryonic organizer may be established by a localized cytoplasmic determinant or by inductive events during cleavage or early gastrulation. In the latter scenario, pigment cells and sclerocytes could act as organizers where all cells release an equal amount of morphogen and stochastic unequal distribution of cells creates a subtle concentration gradient that becomes reinforced as cells migrate directionally towards regions of higher morphogen concentration. Chemotaxis is a feature of metazoan development, tissue maintenance and innate immunity and requires cell motility, polarity and directional sensing (reviewed in Iijima *et al.*, 2002). Regardless of the source of the morphogen, the directional migration of pigment cells and sclerocytes in *Reniera* indicates that they are uniquely competent to respond to a signal gradient that exists on the surface of the *Reniera* gastrula. At the posterior pole, pigment cells initially coalesce to form a tight pigment spot and then transform into a ring configuration (Fig. 3). Pigment ring formation requires a second set of signalling events to occur. In this second phase, the pigment cells migrate in an anterior direction to a fixed distance from the posterior pole, suggesting that they are responding to a signal emanating from the posterior pole.

The fully formed pigment ring is symmetrically localized around the posterior pole and consists of ciliated pigment cells. This configuration, along with the stereotypic response of these cells to light, which includes orientating their cilia in relation to the direction of the light, directs the larva to a dark location presumably where it will settle and undergo metamorphosis (Leys and Degnan, 2001; Jackson *et al.*, 2002; Leys *et al.*, 2002). This pigment ring has the hallmarks of being a metazoan tissue in that it consists of one cell type whose organisation creates a unit whose function (i) is the result of organization of the cells and (ii) doesn't exist at the level of the individual cell.

EVOLUTION OF METAZOAN PATTERN FORMATION

Early metazoan evolution, prior to the emergence of the clade that gave rise to crown taxa, involved a num-

ber of innovations. These include multicellularity through sexual reproduction and embryogenesis, and an extracellular matrix that allows cell support, movement and localized differentiation. In these organisms, there existed multiple differentiated cell types that may have initially operated independently, both within and between cell types. These cells are likely to have had the capacity to communicate using extant metazoan signalling pathway components such as receptor tyrosine kinases, and to inhabit a self-generated extracellular matrix using cadherins and C-type lectins, as these systems are encoded in choanoflagellate protist genomes (King and Carroll, 2001; King *et al.*, 2003). Indeed, the first metazoan cell differentiation events may have been contingent upon this and other signalling systems (*e.g.*, TGF-beta and Wnt pathways; Suga *et al.*, 1999; Adell *et al.*, 2003) and may initially have been the result of responding to endogenous and exogenous (environmental) signals (Wolpert, 1994) and gradients formed within the organisms. A threshold response to a morphogen gradient, regardless of the source of the signal, would yield localized populations of differentiated cells within the organism. Specification of multiple cell types also would require the evolution of a sophisticated regulatory architecture, which would be manifested in the genome of the metazoan LCA. Evidence of multiple metazoan-specific transcription factor families being expressed during sponge embryogenesis, including members of *POU, LIM-HD, Pax, Bar, Prox2, NK-2, T-box, MEF-2, Fox, Sox, Ets* and *nuclear hormone receptor* gene families (Larroux *et al.*, unpublished data), substantiates this supposition. One of the key innovations in early metazoan evolution could well have been the linking of existing signalling pathways to the control of transcription factor function. This would allow localised cell populations to respond to a gradient of extracellular molecules in a stereotypic manner. Another key step would be the emergence of an inherited self-sustaining system to create multiple cell types (*i.e.*, embryogenesis), in which the encoding of successful multicellular configurations could be passed on to the next generation. Regional specification of cells within the embryo would evolve to be chiefly contingent upon the predictable deployment of local and long-distance signals. These metazoan innovations likely created the platform for localized populations of cells to come under the control of a common regulatory program, which in turn could be used to integrate these cells into a common functional unit (*i.e.*, a tissue) via autoregulation and/or local interactions. Selection of individuals with particular tissue configurations allows for developmental innovation in the way these units form, and further scope to evolve. Existing and new signalling systems could be co-opted and used to further pattern these simple tissues.

Analysis of sponge development and body plans reveals that the metazoan LCA is likely to have possessed a regulatory genetic architecture that directed the formation of more complex and integrated multicellular structures (*i.e.*, simple tissues), such as the larval pigment ring and the adult choanocyte chamber (Fig. 5).

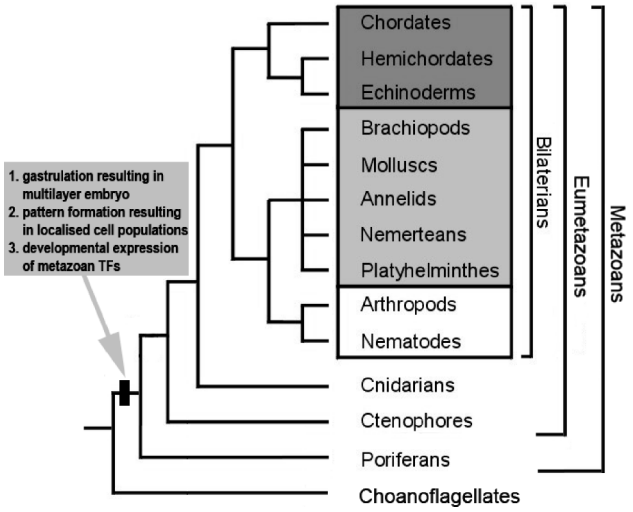

FIG. 5. Developmental features present in the last common ancestor to all metazoans. These are mapped onto a phylogenetic tree of the Metazoa and choanoflagellates that displays only some major phyla (based on Adoutte *et al.*, 2000; Medina *et al.*, 2001). Major bilaterian superphyletic clades are boxed: dark grey, deuterostomes; light grey, lophotrochozoans; white, ecdysozoans. The possible paraphyly of sponges is not displayed in this figure; basal ''poriferans'' may consist only of demosponges and hexactinellids. Based on comparisons of demosponge development with that occurring in the eumetazoans, we propose that the LCA to all metazoans possessed: (1) the capacity to form a multilayered embryo following cleavage largely through the directional migration of populations of cells to specific regions of the embryo (*i.e.*, the ability to gastrulate); (2) the capacity to establish stereotypic body axes with populations of specified and differentiated cells patterned along these axes to form rudimentary tissues; (3) a genome that encoded a suite transcription factors and signalling pathway components, many of which appear to be metazoan-specific, that were expressed during embryogenesis and presumably regulated this process.

Observation of *Reniera* development reveals that the morphogenetic programs underlying the formation of these structures are likely to be similar to those found throughout the Metazoa. The formation of such structures requires asymmetric cell division, organizers, morphogen gradients and populations of specified cells competent to respond to a subset of the developmental signals within the embryo. These capacities are relatively sophisticated, and suggest that the LCA to all metazoans had evolved the inherited ability to localise populations of differentiated cell types to specific territories within the body plan and to configure them into simple functional tissues. The formation of cell layers after a period of rapid cell division (*i.e.*, gastrulation) appears to be a hallmark of metazoan development and may represent the first entrainment of a patterning process in the metazoan genome.

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