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# Cellular and molecular processes leading to embryo formation in sponges: evidences for high conservation of processes throughout animal evolution

Alexander V. Ereskovsky · Emmanuelle Renard · Carole Borchiellini

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**Abstract** The emergence of multicellularity is regarded as one of the major evolutionary events of life. This transition unicellularity/pluricellularity was acquired independently several times (King 2004). The acquisition of multicellularity implies the emergence of cellular cohesion and means of communication, as well as molecular mechanisms enabling the control of morphogenesis and body plan patterning. Some of these molecular tools seem to have predated the acquisition of multicellularity while others are regarded as the acquisition of specific lineages. Morphogenesis consists in the spatial migration of cells or cell layers during embryonic development, metamorphosis, asexual reproduction, growth, and regeneration, resulting in the formation and patterning of a body. In this paper, our aim is to review what is currently known concerning basal metazoans—sponges' morphogenesis from the tissular, cellular, and molecular points of view—and what remains to elucidate. Our review attempts to show that morphogenetic processes found in sponges are as diverse and complex as those found in other animals. In true epithelial sponges (Homoscleromorpha), as well as in others, we find similar cell/layer movements, cellular shape changes involved in major morphogenetic processes such as embryogenesis or larval

metamorphosis. Thus, sponges can provide information enabling us to better understand early animal evolution at the molecular level but also at the cell/cell layer level. Indeed, comparison of molecular tools will only be of value if accompanied by functional data and expression studies during morphogenetic processes.

**Keywords** Porifera · Morphogenesis · Epithelial morphogenesis · Wnt pathway

## General sponge characteristics: four sponge taxa and their relationships

Sponges (Porifera) are aquatic filter-feeding organisms. They have no organs, and thus no separated gut, no nervous and muscular systems, and no gonads. Because of this relatively simple organization, the nature of sponges was the subject of widespread debate until the twentieth century. They were often not fully considered as “true” animals and were grouped with other poorly understood organisms under the fuzzy term of Parazoa. Molecular data at the end of the 1990s closed this debate, showing that they belong to Metazoa and are recognized as one of the most ancient still extant animal lineages. In contrast to the previous classification (Hooper and van Soest 2002), Porifera are now divided into four clades: the Hexactinellida, Demospongiae, Calcarea, and Homoscleromorpha (Fig. 1; Philippe et al. 2009; Gazave et al. 2010, 2012; Pick et al. 2010).

Sponge anatomy, histology, and cellular composition

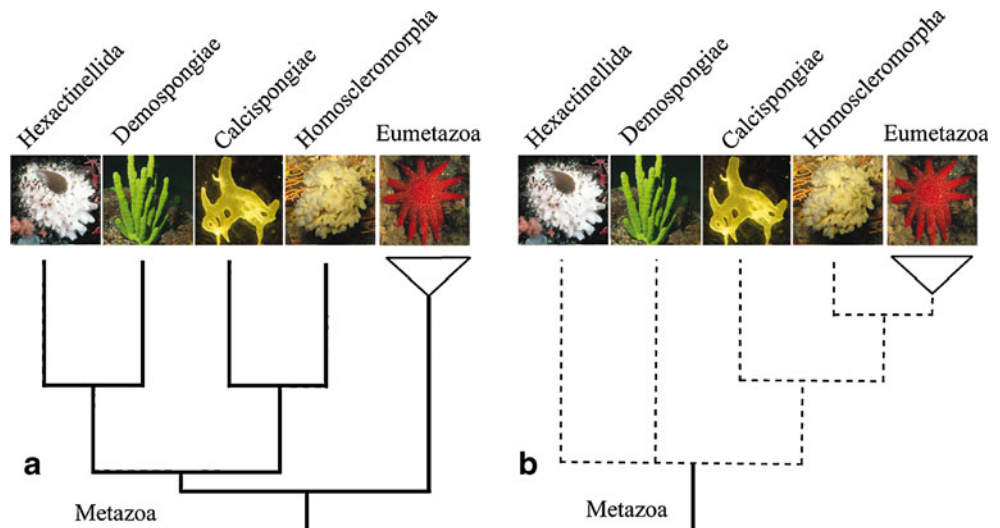
Despite the huge diversity of their body shape, life cycles, and reproductive strategies (Ereskovsky 2000; Meroz-Fine

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**Fig. 1** Phylogenetic relationships between the four lineages of Porifera. **a** Proposed by Philippe et al. (2009). Another recent phylogenomic study (**b**) favors the paraphyly of sponges (Sperling et al. 2009); for this reason, these relationships were indicated with dashed lines



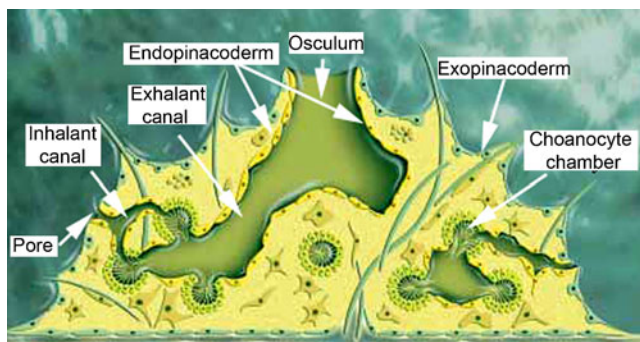
et al. 2005; Mercurio et al. 2006), some common features can be noted. Sponges have two cell layers named pinacoderm and choanoderm (Fig. 2), respectively, formed by pinacocytes and choanocytes. Pinacocytes are flattened cells covering the outer parts of the body (exopinacocytes and basopinacocytes) and lining the canals of the aquiferous system (endopinacocytes; Fig. 2). Choanocytes are flagellated collar cells, lining the filtering cavities of the aquiferous system called choanocyte chambers (Fig. 2). The space between the external pinacocyte layer and the aquiferous system is filled by the mesohyl, a loose layer composed of collagen fibrils, skeletal elements (the nature and form of which depends upon the taxa), and up to ten cell types with different degrees of motility (Bond 1992; Bond and Harris 1988; Ereskovsky 2010; Harrison and De Vos 1991; Simpson 1984). In contrast to this generally shared cellular organization, Hexactinellida harbor syncytial layers and reduced mesohyl (Leys et al. 2007).

In most sponges (Demospongiae, Calcarea, and Hexactinellida), these cell layers do not rely on a basement membrane, and cell junctions remain limited, septate junctions being

present only in a few unusual cases: between the calcite-depositing sclerocytes in calcareous adult sponges (Ledger 1975) and between the spongocytes secreting the gemmule coat in freshwater sponges (De Vos 1977). These observations contrast with molecular data showing the presence of genes orthologous to those involved in eumetazoan adhesion elements. For example, the tetraspanin receptor and the scaffold protein MAGI were identified in *Suberites domuncula* (Demospongiae) (Adell et al. 2004).

In addition, the genome of the demosponge *Amphimedon queenslandica* contains the molecular components needed to form polarized cell layers with adherens junctions, but lacks the tools for occluding junctions or basal lamina. This suggests that cell layers of demosponges may share some but not all of the conserved characteristics of eumetazoan epithelia (Fahey and Degnan 2010).

In contrast, in the fourth sponge clade Homoscleromorpha, cell layers are supported by a basement membrane consisting of collagen IV, tenascin, and laminin (Humbert-David and Garrone 1993; Boute et al. 1996). Furthermore, the cells are connected by specialized cell junctions, both in adult (Ereskovsky and Tokina 2007) and larvae (Boury-Esnault et al. 2003; Ereskovsky et al. 2009): thus, homoscleromorph cell layers can be considered as epithelia, of squamous type in adults and of columnar type in larva (Ereskovsky 2010).



**Fig. 2** Schematic representation of a sponge body plan (sponge with a leuconoid aquiferous system)

### Reproduction and development in sponges

As in other animals, morphogenetic processes occur during ontogenesis in all sponges. For this reason, we review in this section the main developmental features of sponges. Most studied sponge species, whatever their lineage, are capable of both sexual and asexual reproduction, depending on season and environmental conditions (Ereskovsky 2010).

### Sexual reproduction

In sponges, oviparous, ovoviviparous, and viviparous species can be found as well as hermaphroditic and gonochoristic species, so that the history of reproductive strategies is difficult to trace back. To date, no clear germinal cell lineage has been shown to exist in sponges; as far as we know, gametes differentiate either from archaeocytes (mesohyl cells generally considered as pluripotent) or from choanocytes. Because of the absence of specific gonopores, gametes are conducted outside or inside using the aquiferous system.

After fertilization, in the case of ovoviviparous and viviparous sponges, embryonic development takes place in temporary brood chambers (also referred to as follicles), bordered by flattened cells, that form at the end of vitellogenesis.

In other animals (Eumetazoa), embryogenesis is usually divided into five major stages: cleavage, blastulation, gastrulation, histogenesis, and organogenesis. In sponges, because of the lack of organs, the last step does not occur, and the other steps can hardly be distinguished from one another. As in other Metazoa, the first evident developmental step consists of cleavage. Four main cleavage patterns can be distinguished in sponges: (1) chaotic cleavage in *Homoscleromorpha* and most ovoviviparous Demospongiae; (2) radial-like cleavage in oviparous Demospongiae and Hexactinellida; (3) polyaxial cleavage in *Halisarcida* (Demospongiae) and *Calcinea* (Calcarea); and (4) incurvational cleavage in *Calcaronea* (Calcarea) (Leys and Ereskovsky 2006; Ereskovsky 2010). The spatial position of the blastomeres is usually unstable (except in calcaronean embryos); divisions either become asynchronous very early or are not synchronous at all.

At the end of cleavage, the other classically recognized stages are difficult or even impossible to identify. Firstly, a clear final phase of blastulation is lacking because at the stage commonly referred to as the blastula or the morula, the differentiation of larval cells begins (external ciliated cells and sclerocytes). Secondly, in sponges, the identification of gastrula stage or germ layers is still controversial (Leys 2004; Ereskovsky and Dondua 2006), due to conceptual divergences, difficult correspondence between embryological and adult structures, and high diversity of development types within sponge groups.

Moreover, the constant rearrangement of histological structures adds to the difficulty in determining cell and structural origins. However, aside from these technical and conceptual difficulties in clearly identifying homologous stages, it is evident that almost all types of cell movements characteristic of eumetazoan gastrulation are also found in the poriferan larval morphogenesis: morular delamination (Fig. 3a), cell delamination (Fig. 3b), unipolar and multipolar ingression (Fig. 3c), and invagination (Fig. 3d). In addition to these well-known cellular mechanisms, it is important to

note that some poriferan specific morphogenetic processes can be defined: incurvation in *Calcaronea* (Fig. 3e), formation of blastula (pseudoblastula) by means of ingression of maternal cells into the cleaving embryo *Chondrosia reniformis* (Fig. 3f), polarized delamination (Fig. 3g), unipolar proliferation (Fig. 3h), and multipolar egression in *Homoscleromorpha* (Fig. 3i) (Ereskovsky and Dondua 2006).

All of the sponges studied [except the two demosponge genera *Tetilla* and *Stylocordyla* (Watanabe 1978; Sara et al. 2002)] have indirect, i.e., larval development. Eight larval types have been described: coeloblastula, calciblastula, cinctoblastula, amphiblastula, disphaerula, hoplitomella, parenchymella, and trichimella (Ereskovsky and Korotkova 1999; Maldonado and Bergquist 2002; Ereskovsky 2010). With the exception of disphaerula, which is morphologically intermediate these larvae can either be hollow single-layered larvae (coeloblastula, calciblastula, cinctoblastula, and amphiblastula) or two-layered without cavity (parenchymella, hoplitomella, and trichimella).

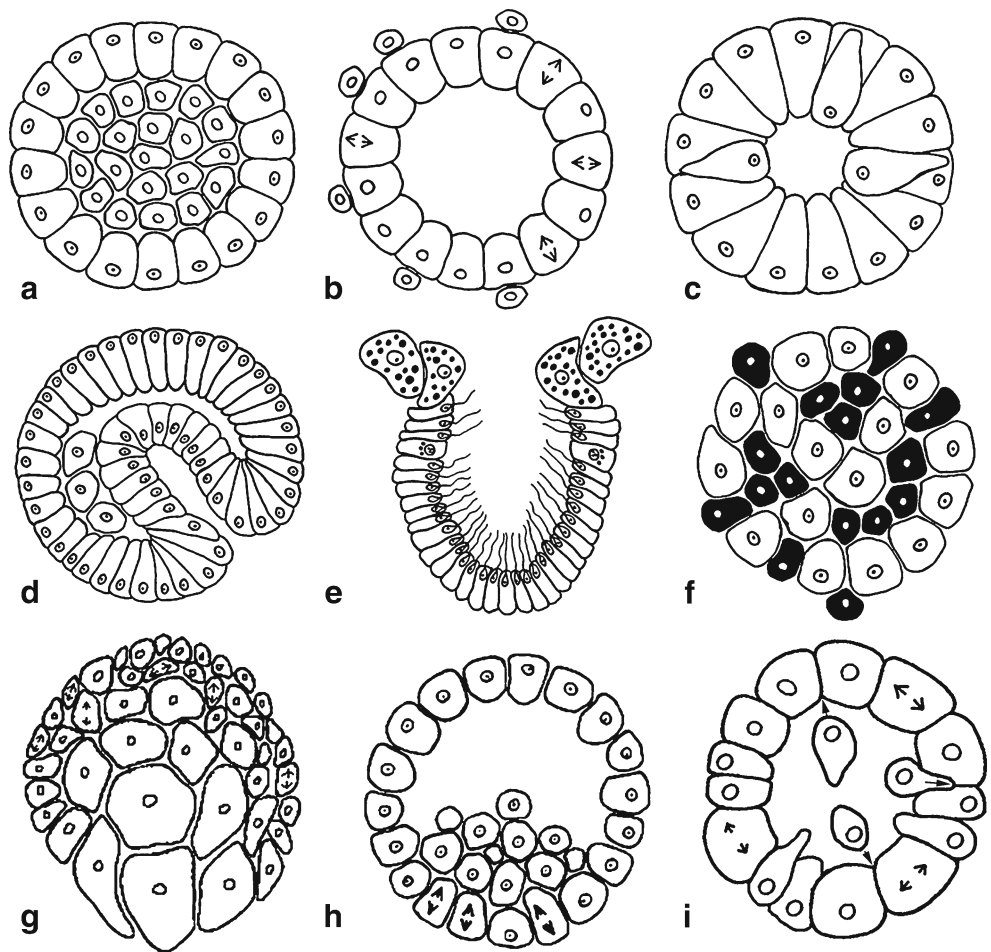
From the larval stage, the attached adult stage is reached by a metamorphosis stage. This is a short stage of postembryonic development during which the larva undergoes radical morphological and physiological changes. Some structures, usually larval or provisional, disappear, while others, that are rudimentary or imaginal, rapidly complete their development. The main feature of metamorphosis is the acquisition of the sponge *Bauplan*, mainly characterized by the aquiferous system. The first adult structure to be formed de novo is the exopinacoderm, which isolates the young sponge from the environment. Subsequent steps include organization of the choanocyte chambers and the water current canals, the opening of the ostia and osculum, and the development of the elements of the adult skeleton. The detailed sequence of these events has only been described in a few species (for review, see Ereskovsky 2010).

### Asexual reproduction

Asexual reproduction occurs in all poriferan clades. It may proceed by fragmentation, gemmulogenesis, and budding (for review, see Simpson 1984; Fell 1993; Ereskovsky 2010).

Budding occasionally occurs in almost all sponges, regardless of taxonomic position or habitat (Simpson 1984; Fell 1993; Ereskovsky and Tokina 2007). A general characteristic of the buds formed in most sponges (except the *Homoscleromorpha* lineage; Ereskovsky and Tokina 2007) is that in the initial stages of development, they are represented by a dense conglomerate of mobile mesohylar cells, mostly totipotent archaeocytes, at the surface of parent sponge. Their development passes by a mesenchymal morphogenesis (see below for details). Thus, a bud lacks choanocyte chambers, canals, and osculum (for review, see Fell 1993). After buds settle on the substrate, the aquiferous

**Fig. 3** Different types of embryonic morphogenesis in sponges, leading to larvae formation. **a** Morula delamination (Demospongiae: orders Dendroceratida, Dictyoceratida, Halichondrida, and Haplosclerida). **b** Cell delamination (Hexactinellida: *Oopsacas minuta*). **c** Multipolar ingression (Demospongiae: order Halisarcida). **d** Invagination (Demospongiae: order Halisarcida). **e** Incurvation (Calcarea: Calcaronea). **f** Pseudoblastula (blastula) formation by ingression of maternal cells (black) inside of the morula (Demospongiae: order Chondrosida, *Chondrosia reniformis*). **g** Polarized delamination (Demospongiae: orders Halichondrida, Poecilosclerida). **h** Unipolar proliferation (Demospongiae: *Vaceletia*). **i** Multipolar egression (Homoscleromorpha; from: Ereskovsky and Dondua 2006, reproduced by permission of Elsevier Ltd.)



system forms and growth begins. In contrast, bud development in homoscleromorph sponges is based on epithelial morphogenesis (see below for details).

In all the sponges studied, gemmules develop from the same cell sources—totipotent archaeocytes conglomeration (Simpson 1984; Weissenfels 1989).

#### Morphogenesis peculiarities: comparison with other metazoans

As previously specified, like other metazoans, sponges need morphogenetic mechanisms to shape their body during development and growth. Like other animals with continuous growth, morphogenetic processes occur throughout the lifespan.

Epithelial tissue organization is generally considered as a major innovation of Eumetazoa (Tyler 2003; Magie and Martindale 2008) because it has drastic consequences in terms of stem cells and the cell type specification system, as well as morphogenetic modes. As a corollary, it is thus usual to cite as the main difference between poriferans and eumetazoan the looseness of layers permitting a higher rate of cell migrations, leading to an almost permanent reorganization of tissue. Nevertheless, this is an oversimplification

because one can expect Homoscleromorpha layers which can be considered as true epithelia to be subject to the same type of constraints than that encountered in eumetazoan. Therefore, a more thorough comparison between the four lineages of sponges and eumetazoans is necessary in order to better understand the evolution of cell layer behavior during morphogenetic processes.

#### Two main groups of morphogenesis in metazoans: epithelial and mesenchymal

Epithelia and mesenchyme are two distinct tissue types existing in every Metazoa.

Epithelia are characterized by obvious apico-basal polarity, and the mechanical integrity of the tissue is ensured by adhesive junctions and the association with specialized extra cellular matrix (ECM), the basal lamina, providing structural support to cells and influencing their behavior (Tyler 2003).

The formation of a continuous polarized cell sheet is one of the most basic forms of multicellular organization, and the epithelial blastoderm is the starting point for morphogenesis during the development of a wide range of Metazoa

(Lecuit and Le Goff 2007). In contrast, the mesenchyme is composed of more motile, loosely associated fibroblast-like cells devoid of cellular junctions. Mesenchymal cells are clearly designed to move. They have a front-to-back polarity that positions leading-edge filopodia that pull cells forward. In relation to these two types of tissue organization, two main groups of morphogenesis are classically defined in metazoans: (1) morphogenetic movements of cell layers are known as epithelial morphogeneses (Keller et al. 2003); and (2) morphogenetic movements of autonomous cells unconstrained by neighbors are referred to as mesenchymal morphogeneses (Bauma et al. 2008).

#### Mesenchymal morphogenesis: epithelial–mesenchymal transition (EMT) and mesenchymal–epithelial transformations (MET)

The interconversion of epithelial and mesenchymal tissues can be found in a certain stage of embryonic development, as well as in malignant transformation of normal tissues (Hay 1995), and involves the profound phenotypic changes summarized below:

Epithelial–mesenchymal transition (EMT) is central to the creation of numerous organs and complex tissues during embryonic development, asexual reproduction, and regeneration and has been well described in Eumetazoa (Keller et al. 2003; Shook and Keller 2003; Hay 2005). For example, it occurs in the derivation of mesenchymal cells in *Drosophila* species from primary epithelia that have undergone an EMT during gastrulation (Tepass et al. 2001). As another example occurs in echinoid species in which the cells of primary skeletogenic mesenchyme migrate into the cavity of the coeloblastula (Hardin 1988).

EMT requires the following profound changes in cell organization (Fig. 4): (1) cells must break the cell–cell cohesion, mediated, in part, by the interaction of cadherin molecules, at specialized cell junction regions called adherens junctions (AJ; reviewed in Kokkino et al. 2010) resulting in the loss of their apico-basal polarity; (2) forces must be generated to drive cell delamination. The contraction of an apical actin-myosin belt (Young et al. 1991) is often implicated, causing a shape change. (3) In order to migrate, delaminated cells must degrade basal lamina by protease activities (Sternlicht et al. 2000; Przybylo and Radisky 2007). (4) As epithelial cells depend on the environmental and cellular context for their survival, growth, and division, changes in gene expression take place preventing apoptotic mechanisms from occurring after delamination (Vega et al. 2004). (5) EMT is accompanied by the expression of new adhesion and signaling molecules (Oda et al. 1998; Moore 2001), which guide

the migrating cells and control the fate of mesenchymal cells (Fig. 4; Bauma et al. 2008). Once free, individual mesenchymal cells usually develop a less well-defined morphology, which can be amoeboid or polarized (Sahai and Marshall 2003), and exhibit an invasive migratory phenotype. During the moving phase, these cells interact weakly with the surrounding ECM and with neighboring cells via transient adhesions (Hay 2005).

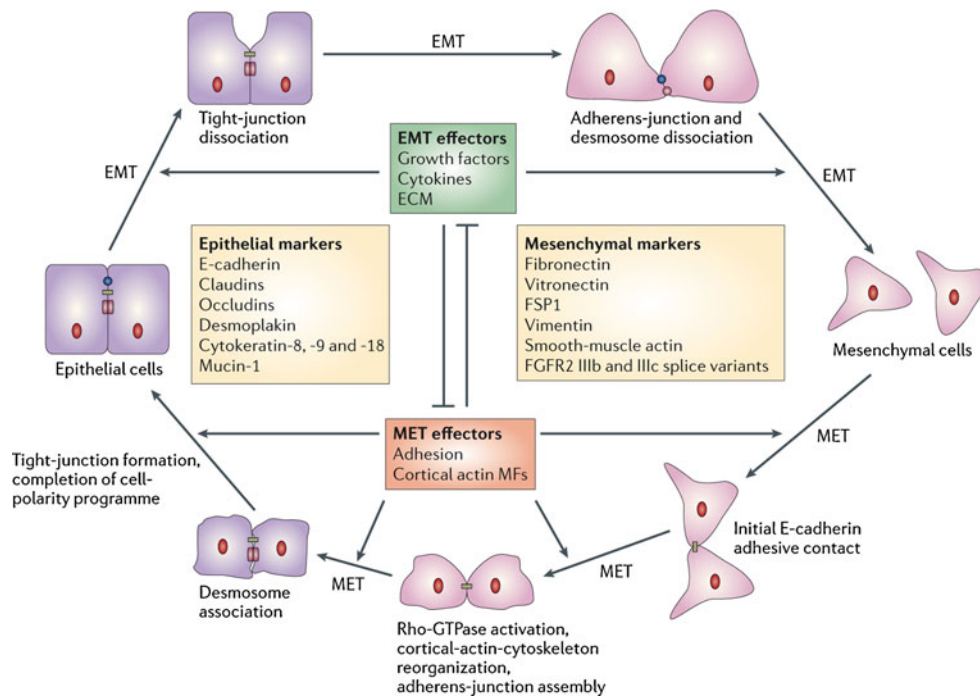
Mesenchymal–epithelial transformation (MET) is the antithesis of EMT involving the participation of mesenchymal cells in the formation of epithelial structures (Hay 2005). The steps described earlier with regard to EMT occur in reverse order in MET (Fig. 4). MET transition plays a major role in certain steps of embryogenesis, metamorphosis, or during regeneration of tissues after injuries. A key role of E-cadherin in MET was demonstrated experimentally by transfection of vertebrate mesenchymal cells with plasmids carrying the E-cadherin gene (Vanderburg and Hay 1996).

#### Epithelial morphogenesis classification

Epithelial morphogeneses are widely distributed among Eumetazoa, and various types of processes can be distinguished: (1) epithelization of spherical, poorly connected, embryonic cells; (2) inflexion of the epithelial layer (evagination, invagination, and folds formation); and (3) change in the area of the epithelial layer resulting from changing cell shape (spreading and fusion of epithelial sheets, convergent extension, epiboly, flattening, columnarization, elongation, and shortening; Keller et al. 2003).

#### Known conserved molecular tools at the eumetazoan scale

As previously described, MET and EMT transitions that allow reorganization of tissues in Eumetazoa during either embryos, larvae, or adults life are mainly based on modifications of cell–cell or cell–ECM cohesion, cytoskeletal reorganization, and migration events. Many signaling pathways and transcription factors trigger EMT including different members of transforming growth factor- $\beta$  (TGF- $\beta$ ), NF- $\kappa$ B, Wnt, Notch, and others interacting in complex ways not always fully understood (Quintin et al. 2008; Zallen 2007; Thiery et al. 2009; Lap  bie et al. 2011), which converge for the most part at the induction of the E-cadherin repressors. The numerous studies of tumor formations provide a basis for a better description of EMT transitions (Ouyang et al. 2010). Nevertheless, most studies focus on vertebrates therefore generalization at the eumetazoan level is often difficult.



**Fig. 4** Events during which epithelial cells are transformed into mesenchymal cells and vice versa. The different stages during EMT (epithelial–mesenchymal transition) and the reverse process MET (mesenchymal–epithelial transition) are regulated by effectors of EMT and MET, which influence each other. Important events during the progression of EMT and MET, including the regulation of the tight

junctions and the adherens junctions, are indicated. A number of markers have been identified that are characteristic of either epithelial or mesenchymal cells, and these markers are listed in box 1 and box 2. *E-cadherin* epithelial cadherin, *ECM* extracellular matrix, *FGFR2* fibroblast growth factor receptor 2, *FSP1* fibroblast-specific protein-1, *MFs* microfilaments (Thiery et al. 2009)

## Morphogenesis in sponges

The literature provides numerous descriptions of different morphogenesis modes during embryo development, metamorphosis, and asexual reproduction in different sponge lineages. Nevertheless, it is regrettable that, because of current technical limitations, these cellular mechanisms have not been described in sufficient detail (see Ereskovsky 2010).

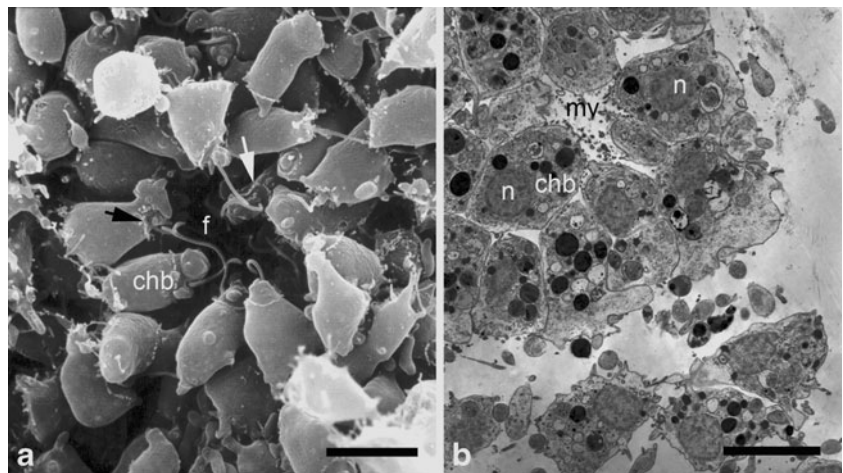
The equivalent of eumetazoan EMT and MET in sponges

Except in the specific case of Homoscleromorpha, no true epithelium exists in sponges because of the absence of basement membrane and limited cell junctions. Nevertheless, choanoderm and pinacoderm somehow behave like epithelia in the sense that cells are polarized and more or less linearly organized and cohesive. In this section, to both facilitate comparison of mechanisms with the previous section and respect the classical nomenclature, we will here use the term “epithelial-like” rather than “epithelial,” consequently EMT will be replaced here by ELMT and MET by MELT. Both ELMT and MELT are widely distributed during all ontogenesis stages in all Porifera taxa.

## EMLT

During the normal ontogeny of sponges, mesenchymal morphogenetic processes are known to occur: Lévi (1956) already described unipolar ingression in blastula of *Halisarca dujardini* (Demospongiae). In the same species, ELMT was also described later at various steps of development: apolar ingression of outer cells into the blastocoel occur as well as cell internalization during the later stages of embryonic development and even in the free-swimming larva (Ereskovsky and Gonobobleva 2000; Gonobobleva and Ereskovsky 2004a). This suggests that the pool of internal cells may be constantly replenished by dedifferentiated external cells of the embryo and the larva (Fig. 5). ELMT can also be observed during the metamorphoses of different larval types in other sponges: in calciblastula (Calcarea and Calcinea), coeloblastula, and parenchymella (Demospongiae; Ereskovsky 2010). Indeed, after settlement of the larva, disorganization of the outer cell layer occurs with desintegration into separate cells. The separated cells acquire amoeboid shape and migrate to the inner part of the larva, forming a dense conglomerate (Fig. 6), in which some cells will retain the ultrastructural characteristics of the larval ciliated cells (Fig. 6) while others remain as dedifferentiated amoeboid cells. This phenomenon has been described in detail in *H. dujardini* (Gonobobleva and

**Fig. 5** Developing choanocyte chambers in the pupa of *Halisarca dujardini* (**a** SEM, **b** TEM). **a** Choanoblasts (*chb*) with flagella (*f*) and microvilli (arrows). **b** Local aggregate of the choanoblasts with the microvilli (*mv*). *f* flagellum, *mv* microvilli, *n* nucleus. (**a** and **b** after: Gonobobleva and Ereskovsky 2004b, reproduced by permission of l'Institut Royal des Sciences Naturelles de Belgique). Scale bars (**a**, **b**), 5  $\mu$ m



Ereskovsky 2004b) and in some Homoscleromorpha species (Ereskovsky et al. 2007, 2009).

Like the primary mesenchymal cells of echinoids, the migrating cells of homoscleromorph *Plakina trilopha* become club-shaped, with rounded proximal parts, and their distal ends sprout long protrusions. During this process, intercellular contacts are destroyed, and pseudopodia are

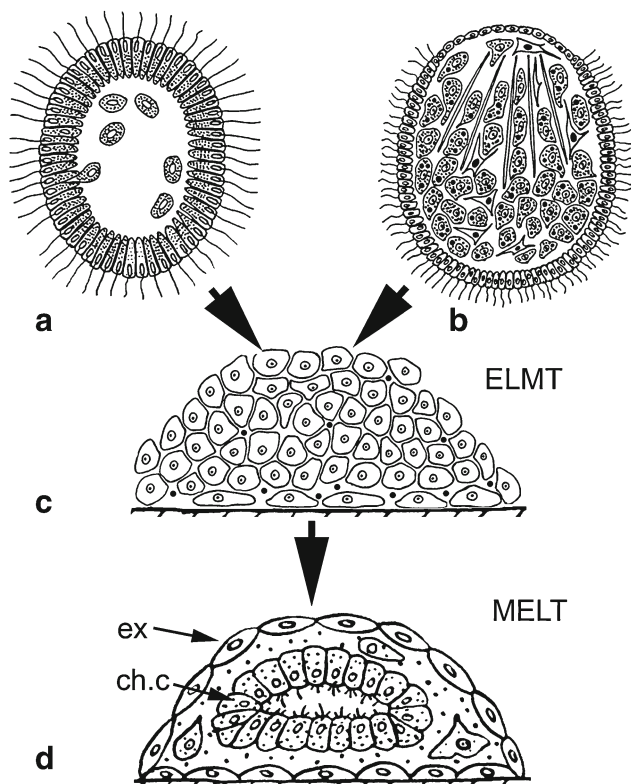
formed in the proximal part of the future mesohylar cell (Ereskovsky et al. 2009).

*MELT*

In contrast to ELMT, mesenchymal–epithelial-like transition (MELT) is rarer during sponge embryogenesis. It has been observed during multipolar egression in Homoscleromorpha (Ereskovsky and Boury-Esnault 2002; Maldonado and Riesgo 2008), where after the formation of an apolar morula, internal blastomers migrate to the periphery of the embryo resulting in the formation of a hollow single-layered pre-larva (Fig. 3i).

Interestingly, this mechanism observed in Homoscleromorpha is similar to that of schizocoely, a process widespread among the Eumetazoa, for example, during formation of the endodermal lining in cnidarian medusae through buds (Boero et al. 1998), and by the formation of coelomic cavities in annelids (Anderson 1973).

In other sponge lineages, MELT is involved in the development of the aquiferous system during metamorphosis. During metamorphosis of calciblastula, coeloblastula, and parenchymella, the choanoderm and endopinacoderm are formed by migration of separate cells from internal cell conglomerate, and their association is followed by differentiation (Fig. 7; Ereskovsky 2010). These mesenchymal movements to form epithelial-like structures have been well described for example, in *H. dujardini* and *A. queenslandica* (Leys and Degnan 2002; Gonobobleva and Ereskovsky 2004b).

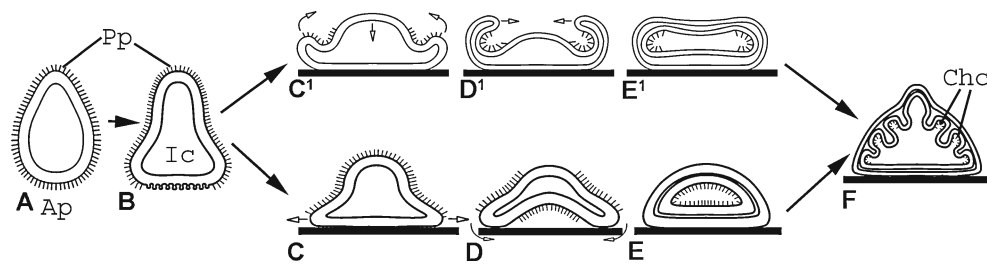


**Fig. 6** Epithelial-like–mesenchymal transition (ELMT) and mesenchymal-like–epithelial transformation (MELT) during metamorphosis of sponge larvae. **a** Calciblastula larva. **b** Parenchymella larva. **c** Settler with entire destruction of the covering epithelium. **d** Pre-rhagon. *Ch.c* choanocytes chamber, *ELMT* epithelial-like–mesenchymal transition, *ex* exopinacoderm, *MELT* mesenchymal-like–epithelial transformation

Epithelial or epithelial-like morphogenesis in sponges

*With or without true epithelia, the processes are similar to those of eumetazoans*

The formation of the continuous epithelial (Homoscleromorpha) or epithelial-like (other cellular sponges) sheets



**Fig. 7** Diagram of the different paths of formation of the rhagon during metamorphosis of *Plakina trilopha*. **B** First stage of metamorphosis. **C, C', D, D'** Second stage of metamorphosis. **E, E'** Third stage of metamorphosis. **A** Cinctoblastula. **B** Attachment to substrate. **C–E**

Basal invagination. **C<sup>1</sup>–E<sup>1</sup>** Extension and folding up of lateral sides of settler. **F** Rhagon. Scale bar, 150  $\mu$ m. **Ap** anterior pole, **Chc** choanocyte chambers, **Ic** internal cavity of larva, **Pp** posterior pole

from spherical, poorly connected, non-polarized cells has been observed in all investigated larval types of Demospongiae, Calcarea, and Homoscleromorpha during external cell layer development via the morula stage. The external sheet allows isolation of the inner compartment of the organism from the external environment while maintaining the tissue integrity and polarity. The cellular mechanisms of epithelization involved are very similar to those described in other metazoans, for example, in Cnidaria (Kraus 2006).

The coeloblastula of the calcareous sponges *Guancha arnesenae* (Ereskovsky and Willenz 2008) and of the demosponges *Polymastia robusta* (Borojevic 1967) and *H. dujardini* (Gonobobleva and Ereskovsky 2004a) consist of a columnar layer of polarized cells closely adjoined, but without specialized intercellular junctions at this stage.

In contrast, in homoscleromorphs (Ereskovsky 2010), the pre-larva shows a surface cell layer looking like palisade epithelium: highly polarized (cilium in the apical part and collagen synthesis in the basal part), tightened by specialized intercellular contacts in the apical zone (zonula adherens), and lined by a basement membrane (Boury-Esnault et al. 2003).

#### *Inflexion of the layers: invagination and evagination*

Epithelial folding is a basic morphogenetic process reiterated throughout all types of development in Eumetazoa (Wolpert 1998). During this processes, interactions between epithelial cells and the extracellular matrix play a fundamental role. Two main types of movements are commonly defined: invagination and evagination. Invagination is the inpocketing or furrow formation at a specific region of an epithelium, with the apical surface being the concave side. In contrast, evagination is the outpocketing or ridge formation within an epithelium such that the apical surface is the convex side of the pocket.

Both processes are rare during embryonic development of sponges, except in Homoscleromorpha where they occur quite often. In this sponge lineage invagination occurs during folder formation in pre-larva, both invagination and evagination occur during aquiferous system development in metamorphosing

larva, the exopinacoderm invagination occurs during ostia formation, and evagination occurs during the first steps of budding during asexual reproduction and lobe formation phase of sponge growth (see Ereskovsky 2010). Even if functional evidence is still lacking, the presence of a basement membrane may be expected to be partly responsible for this observation.

*Focus on invaginations* Invagination of cell layers in sponges can be performed by two distinct phenomena linked with two different cell shape modifications: (1) invagination involving bottle-shaped cells. The function of so-called wedge-shaped or bottle cells is critical to the proper initiation of invagination in metazoans, for example, in echinoids (Kimberly and Hardin 1998), and in anthozoans (Kraus and Technau 2006); and (2) invagination resulting from the increase in the surface area, a mechanism that also occurs in Eumetazoa, for example, during the neurulation in cephalochordates (Whitaker 1997).

The first mechanism is a key morphogenetic event occurring during metamorphosis of all investigated homoscleromorphs cinctoblastulae (Fig. 7; fully detailed in Ereskovsky et al. 2007, 2009). This morphogenesis often proceeds as a dome-shaped invagination of the settler's basal layer of cells that have retained the cilium. The bottle-shaped cells are formed in the zone of fold or invagination formation. During their migration, bottle cells do not lose junctions with neighboring cells. The basal (expanded) part of protruded cells extends and forms lamellopodia and filopodia, while the apical part plays an anchorage role in the epithelial layer because of zonula adherens. The result of these cell movements is that the marginal flattened parts of the settler tuck up or down, merging either above the apical pole or below the basal pole. Thus, the rhagon acquires a second internal cell layer that develops into the aquiferous system. Canals of aquiferous system and endopinacoderm are derived from the proximal parts of the internal cell layer (Fig. 7, label D). The choanocyte chambers develop from distal parts of the internal folds (Fig. 7, label E).

The second mechanism of invagination has been well described during embryonic development of *H. dujardini* (Fig. 3d; Ereskovsky and Gonobobleva 2000; Gonobobleva and

Ereskovsky 2004a). In this case, the larval outer cells proliferate, actively yielding to numerous folds and protrusions of the surface layer. After the end of proliferation and the acquisition of disphaerula cell characteristics, the invagination of the ciliated layer proceeds perpendicularly to the anterior–posterior axis of the larva and results in a single-layered closed structure formed by ciliated cells, with their cilia directed inwards.

**Focus on evagination** The mechanism of evagination (synonyms: incurvation and inversion) is well known in Eumetazoa, for example, during an early development (in blastulae) of *Nematostella vectensis* (Fritzenwanker et al. 2007), during asexual bud formation and tentacle development in *Hydra* (Philipp et al. 2009), or in imaginal disc development of *Drosophila melanogaster* (Fristrom and Fristrom 1975). In sponges, it is characteristic for Calcarenea (class Calcarea) embryos (Fig. 8) (Dubosq and Tuzet 1937). Recent light and electron microscopic observations on *Sycon ciliata* (Franzen 1988) and *Sycon calcaravis* (Yamasaki and Watanabe 1991) show that inversion in syconoid calcareans results from coordinated movements of the layer of parent choanocytes and the granular cells of the stomoblastula. At the beginning of inversion in *S. ciliata*, the choanocytes lose the collar and the flagellum, and their basal parts produce long flattened offshoots spreading along the embryo (Fig. 8a, b). Such transformed choanocytes establish contacts with granular stomoblastula macromeres, which, in turn, spread filopodia towards the former. As a result of this interaction, the mass of granular cells is drawn into the choanocyte chamber. Transformed flattened choanocytes migrate along the external surface of the stomoblastula, pulling the granular cells along with them and making the stomoblastula turn inside out. At the end of inversion, the larva lies within the choanocyte chamber (Fig. 8d).

#### Changes of cell shapes involved

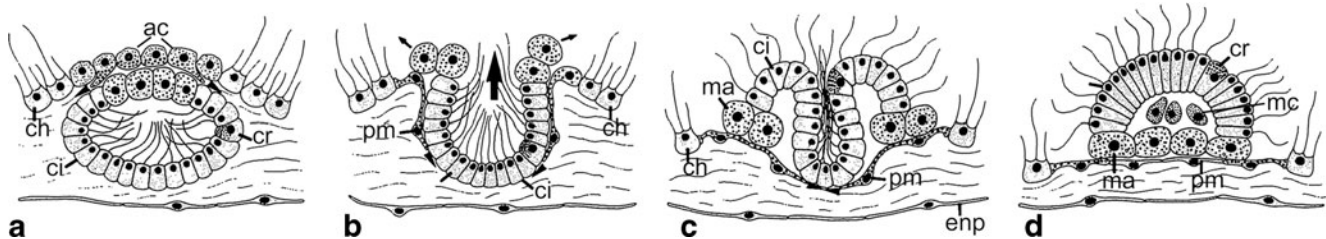
As mentioned previously, changes of cell shape often occur during morphogenetic processes and often play central

roles. Spreading and fusion of cell layers and convergent extension are often accompanied by the flattening, columnarization, elongation, and shortening of cells. Even if all these events are rarely observed in the main three lineages of sponges, the majority of these morphogenetic mechanisms are more widely distributed in homoscleromorphs as well as in Eumetazoa.

**Flattening versus columnarization** A common cell shape change during epithelial morphogenesis is the shortening of the lateral sides of a cell. This event results in the flattening of the epithelial sheet such that the epithelium increases its apical and basal surfaces (Schöck and Perrimon 2002). Well-known examples are the flattening in the epiboly of frog embryos and flattening that occurs during the formation of the wing blades in insects (Fristrom 1988).

Clear examples of flattening in sponges, and especially in Homoscleromorpha, are found in metamorphosis of larvae, bud formation, and in reparative regeneration of the exopinacoderm (described in *Oscarella lobularis*). In contrast, the reverse process, columnarization, is more unusual.

Three main mechanisms of flattening have been described so far: (1) the columnar larval epithelium of homoscleromorph cinctoblastula, flatten during the first step of metamorphosis accompanying cell dedifferentiation. Cells first change from columnar to cubical then from cubical to flat. This results in the extension of the surface area of the settler. The cell junctions and basement membrane remain intact throughout the flattening process (Ereskovsky et al. 2007, 2009); (2) during the metamorphosis of *P. trilopha*, a “cytoplasmic shedding” was described (Ereskovsky et al. 2009). Here, the cell shape changes from prismatic to almost cubical due not only to the flattening of cells but also due to the cytoplasmic shedding and the decrease of cell volume; and (3) during the metamorphosis of the demosponge *H. dujardini*, an “oblique” type of contact cell polarization is involved. Here, it is interesting to note that the ciliated cells in the posterior larval hemisphere retain specialized intercellular contacts of the *zonula adherens* type (fully detailed in Gonobobleva and Ereskovsky 2004b).



**Fig. 8** Scheme of *Sycon* stomoblastula inversion. **a** Stomoblastula, beginning of placental membrane development from the accessory cells complex (arrows). **b** Early and middle (**c**) stages of inversion, accompanying by placental membrane growth (arrows). **d** End of

inversion. *ac* accessory cells, *ci* ciliated cells, *ch* choanocytes, *cr* cross cells, *enp* endopinacocyte, *ma* macromeres, *mc* maternal cells, *pm* placental membrane (from: Franzen 1988, reproduced by permission of Springer)

Whereas the two latter flattening mechanisms seem rare in other sponges, they are very common in eumetazoan morphogenesis (Mergner 1971; Belousov 1998).

**Spreading and fusion** The movement and fusion of epithelial sheets can be subdivided into three distinguishable and related steps: first, leading cells are specified and brought into position; second, cells execute a coordinated forward movement by changing shape and/or migrating over a substratum; and third, epithelial cells fuse. Many of these processes have been well described in Metazoa, such as epiboly during neural tube and palate closure in vertebrates (Harrington et al. 2009), or during ventral enclosure in *Caenorhabditis elegans* (Raich et al. 1999). Extensive and directed movements of two epithelial faces are then involved. One of the characteristics of this phenomenon is the formation of filopodia, originating from cells that are located at the leading edge of a cell sheet. When the epithelial sheets meet, nascent junctions form by rapid contacts. This “filopodial priming” leads to a rapid creation of adherens junctions. Spreading and fusion processes have been described in Homoscleromorpha. They occur, for example, in the follicle formation of eggs. Follicles form during the later stages of egg vitellogenesis when endopinacocytes surround mature eggs and embryos. During follicle formation, leading edge cells of the endopinacoderm appear at the border between two layers of endopinacocytes from adjoining exhalant canals (Ereskovsky and Boury-Esnault 2002).

### Have molecular tools been conserved at the metazoan level?

Review of the main molecular tools used in eumetazoans and in early sponge Evo-Devo

The confirmation by molecular data of the phylogenetic position of sponges within the metazoan clade has made it possible to consider that the molecular bases of pluricellularity, development, and morphogenesis were probably acquired in the common ancestor of all the animals including Porifera. As far as eumetazoan morphogenesis is concerned, at the cellular level, these complex phenomena can be dissected in terms of cellular differentiation, migration, adhesion, and coordination. These cellular mechanisms have been shown to be regulated by two main types of molecular tools: transcription factors and signaling pathways.

Although sponge embryology has been studied since the nineteenth century, developmental genetics began in the 1980s, and finally, the first study of developmental genes in sponges dates back only to 1993. The investigations first focused on the homeobox genes (Degnan et al. 1993). Even though the degenerate PCR approach proved to be long and

difficult with sponges and did not allow the characterization of this gene family, this was the essential starting point to become aware, little by little, of the presence of the unexpected molecular richness regarding the relative morpho-anatomical simplicity of sponges. The first description of a homeobox gene was made in a fresh water demosponge *Ephydiatia fluviatilis* (Seimiya et al. 1994) and on marine sponge *Geodia cydonium* (Kruse et al. 1994).

The first EST libraries and the beginning of the genome sequencing of the demosponge *A. queenslandica* in 2005 (Srivastava et al. 2010a) significantly increased the accumulation of data. These different studies revealed that sponges possess genes homologous to those acting in the eumetazoan developmental program. In this section, we propose to offer a short survey of the state of the art of knowledge of sponge transcription factors and signaling pathways, but it is clear that we are still far from understanding the roles of each actor and far from being able to depict a clear scenario. In particular, it should be emphasized that all the following data and interpretations are based on currently available genomic data obtained from a limited sampling of eukaryotic lineages, EST data for a few sponge species (thus non-exhaustive) and on a single sponge genome (one species not being representative of a whole group). Concerning the last point, one cannot rule out the possibility that the absence of a gene may be the result of a secondary loss specific to *A. queenslandica* or to the demosponges, as has already been evidenced for different genes (Peterson and Sperling 2007; Gazave et al. 2008; Larroux et al. 2008; Schierwater et al. 2009; Adamska et al. 2011). Moreover, the proposed scenarios must be viewed with caution because interpretation of the data relies on a phylogenetic context (Schierwater et al. 2009), and unfortunately, the relationships between the four extant lower metazoan phyla (Placozoa, Porifera, Cnidaria, and Ctenophora) still remain controversial (Dunn et al. 2008; Hejnol et al. 2009; Philippe et al. 2009, 2011; Schierwater et al. 2009; Sperling et al. 2009; Mallatt et al. 2010; Pick et al. 2010). Another pitfall to be taken into account is the often encountered difficulty of assigning genes to known eumetazoan orthology groups.

Early or late origin of transcription factors?

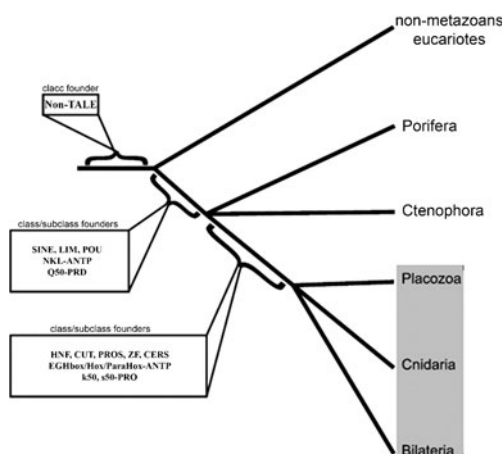
### Homeobox genes

The homeobox gene superfamily encodes transcription factors that, in eumetazoans, act as master regulators of development through their ability to activate or repress a diverse range of downstream target genes. Classes are defined on the basis of conservation of the homeodomains (HD, a 60 amino acid DNA-binding proteic domain) they encode, as well as additional motifs that contribute to DNA binding and interactions with other proteins. Not only in metazoans but also in all multicellular Eukaryotes, these transcription

factors are extensively used in fundamental development processes and are highly diversified. They are supposed to have played a key role in the acquisition of multicellularity in Eukaryota (Derelle et al. 2007; King 2004).

A recent extensive phylogenetical study of HD at the eukaryotic scale showed that HDs can be grouped in two clades: TALE (three extra residues between helix 1 and helix 2 of the homeodomain) and non-TALE. Moreover, these results show that the last common ancestor of Eukaryotes (LECA) already possessed these two types of HDs, non-TALE genes being lost secondarily in *Monosiga* (Derelle et al. 2007; King et al. 2008; Larroux et al. 2008, Sebé-Pedrós et al. 2011). Later, duplications occurred independently during diversification of eukaryotic lineages. As a result of this very wide diversification (by duplication and divergence, or via domain shuffling), animal homeobox genes are at present divided into 11 classes and more than 125 gene families, non-TALE genes being more diversified than TALE ones (Holland et al. 2007; Mukherjee and Bürglin 2007; Ryan et al. 2010).

**Non-TALE genes** Major diversification events of non-TALE genes occurred before metazoan radiation. Indeed, data on sponges revealed that many of non-TALE classes were already present in the metazoan ancestor, such as ANTP, PRD, POU, LIM, and SIX, and that others may have appeared later, after the Porifera–Eumetazoa divergence because, to date, no HNF, CUT, PROS, or CERS classes have been discovered in demosponges (Larroux et al. 2006, 2007, 2008; Ryan and Baxevis 2007; Ryan et al. 2010; Degnan et al. 2009; Srivastava et al. 2010a, 2010b). Figure 9 gives a more detailed view of Non-TALE gene history.



**Fig. 9** Origins of non-TALE class and subclass founders on phylogenetic hypothesis according Ryan et al. (2010). All the results obtained in sponges reveal that first diversification events of non-TALE gene have occurred before metazoan radiation and a second after the Porifera–Eumetazoa divergence

**TALE genes** The TALE category includes IRO, MEIS, PBX, and TGIF classes (Holland et al. 2007; Mukherjee and Bürglin 2007). In demosponges, several TALE genes have been characterized (Perovic et al. 2003; Larroux et al. 2006, 2008). All but one seem to be related to the IRO class. Larroux et al. (2008) proposed that the common ancestor of Metazoa may have possessed at least two genes, resulting from an ancestral MEIS-like gene (King et al. 2008). However, the data recently obtained on *Capsaspora*, although poorly supported, suggest the presence of a PBX class in the Holozoan ancestor and a secondary loss in *Amphimedon* and *Monosiga* (Sebé-Pedrós et al. 2011). The interpretation is thus, once again, pending the acquisition of other genomic data.

#### Other transcription factors

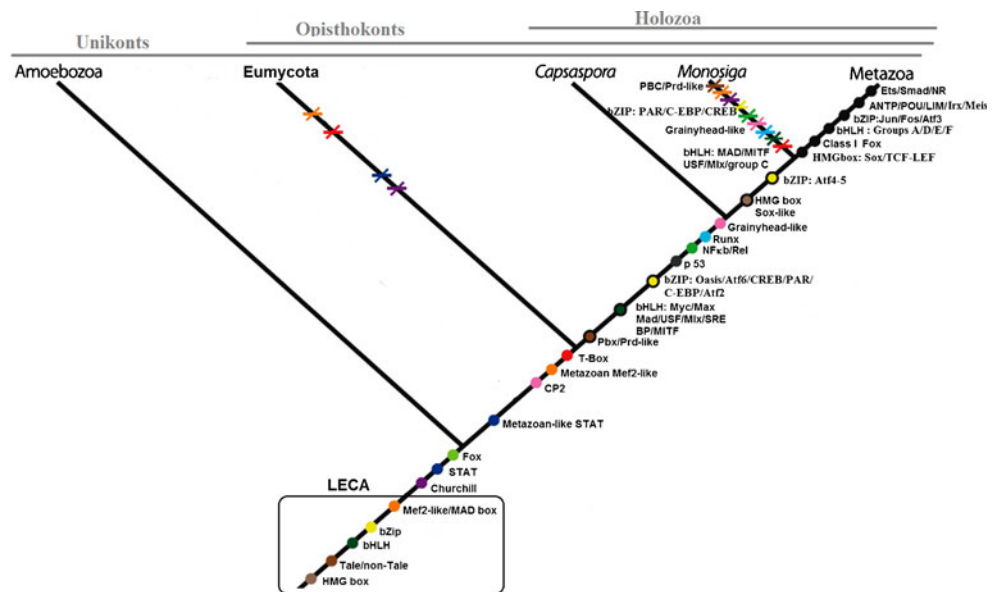
Sponges also possess other TFs (Fig. 10), and evolutionary scenarios have been proposed for each of them. Generally, although many classes of transcription factors are older in origin (Derelle et al. 2007; King et al. 2008; Larroux et al. 2008; Shalchian-Tabrizi et al. 2008; Degnan et al. 2009; Mikhailov et al. 2009; Sebé-Pedrós et al. 2011), many other classes and families of transcription factors seem to be metazoan innovations. These animal genetic novelties are thought to have played an important role in animal evolution by providing new tools that increased morphogenetic possibilities. Nevertheless, as already mentioned, this impression of sudden emergence may only be the artificial result of insufficient sampling at the eukaryotic scale. In the same way, the lack of certain genes in the *Amphimedon* genome may not reflect the ancestral condition in sponges but rather specific gene losses (Peterson and Sperling 2007; Adamska et al. 2011). To resolve this question, the acquisition of genomic data from other sponge lineages is needed. The most recent study on the subject, using the newly available genome of the sporozoan *Capsaspora*, led Sebé-Pedrós et al. (2011) to propose a new very comprehensive evolutionary scenario (Fig. 10).

In other animals, ten TFs have been identified to be involved in EMT regulation during embryogenesis and carcinogenesis. Among them, some are direct repressors of the gene coding for E-cadherin (such as Snail, Twist, Fox, Gsc, and KLF). Interestingly, these TF are regulated by well-known signaling pathways Wnt, Notch, TGF- $\beta$ , and Hh (Takebe et al. 2011).

#### Signaling pathways or how to make “new” from “old”

In multicellular organisms, signaling pathways are an essential intercellular means of communication enabling the realization and the coordination of the embryonic development and more broadly of morphogenetic processes. Cell

**Fig. 10** Cladogram showing TF origin and evolution on phylogenetic hypothesis according to Sébé-Pedrós et al. (2011). Each color represents domain class. Origin of domain is indicated by a colored dot. Specific protein family appearance is indicated by a black-circled dot in our taxon sampling. Loss of the domain or specific protein family is indicated by a cross. Metazoan apomorphies are shown as black dots



responses to intercellular signals include cell proliferation, secretion, and motility via the regulation of the expression regulation of target genes using signal-dependent transcription factors (Barolo and Posakony 2002). In eumetazoans, fewer than 20 different signal transduction pathways are needed to generate the wide variety of cell types, patterns, and tissues observed (Pires-daSilva and Sommer 2003).

Among these 20 pathways, only seven participate in the control of development processes: Wnt, TGF- $\beta$ , Hedgehog (Hh), receptor tyrosine kinase (RTK), Jak/STAT, nuclear hormone receptor, and Notch (Barolo and Posakony 2002; Gerhart 1998). Complete descriptions of these pathways are the subject of several reviews (Adamska et al. 2007a, b, 2010, 2011; Sakarya et al. 2007; Richard et al. 2008; Gazave et al. 2009; Richard and Degnan 2009; Srivastava et al. 2010a).

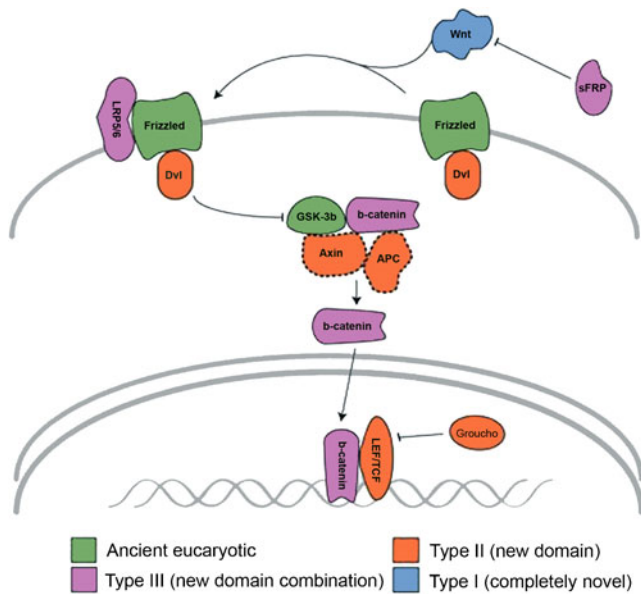
#### Focus on the Wnt pathway

Among these major signaling molecules, the WNTs are metazoan-specific ligands that control diverse processes such as cell proliferation, cell-fate determination, cell migration, and differentiation during multiple steps of embryonic development. This key pathway is active in axis specification as well as in morphogenesis movements such as gastrulation processes in all the eumetazoan models studied and in the MET/EMT regulation (Kusserow et al. 2005; Guder et al. 2006; Lee et al. 2006; Adamska et al. 2007a, 2010, 2011; Momose and Houlston 2007; Ryan and Baxeavanis 2007; Momose et al. 2008; Manuel 2009; Takebe et al. 2011).

In the *Amphimedon* genome, the majority of the components of Wnt canonical or non-canonical signaling have been described (Adamska et al. 2007a, 2010, 2011; Richards and Degnan 2009; Srivastava et al. 2010a). Nevertheless, a major diversification of some gene families

is thought to have occurred after the demosponges–eumetazoan split. Indeed, *Amphimedon* only possesses three Wnt gene families (assignment poorly corroborated) and two frizzled receptors (compared to the 12 Wnt and four frizzled receptors thought to be present in Ureumetazoa; Kusserow et al. 2005). According to these first data (pending complementary data on other sponge and non-metazoan lineages), we can assume the presence of one to three Wnt genes in the last common metazoan ancestor. This assertion will have to be checked because *Amphimedon* has already been shown to have lost several key elements of both Wnt pathways, for example, dickkopf absent in *Amphimedon* but present in *Oscarella carmela* (Nichols et al. 2006; Adamska et al. 2010). Whereas Wnt genes seem to be a metazoan synapomorphy, most of the other Wnt pathway components antedate the emergence of the metazoan lineage (Putnam et al. 2007; Fig. 11).

Despite the lack of specific protein–protein binding motifs required for interaction of ACP and Axin with each other and with  $\beta$ -catenin, it is thought likely that Wnt signaling is functional in sponges (Richards and Degnan 2009; Adamska et al. 2010, 2011). It may be involved in axial patterning in demosponge embryos and in epithelium morphogenesis and patterning in *Oscarella* adults (Adamska et al. 2007a, 2010, 2011; Lapébie et al. 2009; Windsor and Leys 2010). During the first phase of cell movements in *Amphimedon* blastula, the cells organize into two layers. The first cells to become distributed asymmetrically at the future posterior pole are those with strong expression of *AmqWntA*. The existence of a Wnt expression gradient in relation with embryonic polarization has been proposed in *Amphimedon* (Adamska et al. 2007a, 2010, 2011). Functional studies on *Ephydatia muelleri* demonstrate the involvement of the Wnt pathway in the axial patterning of a gemmule-hatched juvenile (Windsor and Leys



**Fig. 11** Origin and evolution of Wnt signaling pathway according to the scenario proposed by Adamska et al. (2010). Each color represents evolutionary origin of each component. Dashed lines indicated absence of specific protein–protein binding motif

2010). In juveniles treated with molecules simulating Wnt pathway activation (GSK3 inhibitors LiCl and alsterpaullone), the authors observed multiplication of oscula, which can be interpreted as multiplication of body axes. Since over-activation of the Wnt pathway in eumetazoan model systems commonly leads to the multiplication of the body axis (Broun et al. 2005; Müller et al. 2007; Philipp et al. 2009), the involvement of the Wnt pathway in axial establishment appears to be ancient in metazoan history. Pharmacological inhibition experiments have also been used to test the role of Wnt signaling in *O. lobularis* adults (Lapébie et al. 2009). The activation of the Wnt/ $\beta$ -catenin pathway using GSK3b inhibitors resulted in the formation of ectopic ostia, as in eumetazoan epithelial appendages (Philipp et al. 2009). These results suggest that deployment of Wnt signaling in epithelial patterning and morphogenesis has been conserved after the divergence of Homoscleromorpha and Eumetazoa.

#### What is known concerning the others?

Similarly to Wnt signaling, the richness of sponges regarding TGF- $\beta$  and Notch components is compatible with the functionality of these two pathways in sponges (Gazave et al. 2009; Richards et al. 2008; Richards and Degnan 2009; Adamska et al. 2010, 2011; Srivastava et al. 2010a). In contrast, too few components of Hedgehog and growth factors/receptors tyrosine kinase pathways have been found in *Amphimedon* (Fig. 11) support the hypothesis that they are active in this organism (Adamska et al. 2007b; Richards

and Degnan 2009). In this context, only Wnt, Notch, and TGF- $\beta$  signaling pathways can at present be considered as metazoan synapomorphies. In these three cases, it has been shown that these metazoan innovations consist mainly of a new combination of oldest elements since most of the components and their parts predate the metazoan emergence. These innovative recombinations are the result of domain shuffling, gene duplications, and divergences but are also the result of lateral gene transfers (Putnam et al. 2007; Gazave et al. 2009; Richards and Degnan 2009; Adamska et al. 2011). The later additions of components in Eumetazoa are interpreted as the acquisition a more finely-tuned method of regulating of the pathways (Gazave et al. 2009; Richards and Degnan 2009). Except in the case of Wnt, described earlier, for which the first—albeit preliminary—functional data have been published, we can hardly propose concrete roles for these pathways in sponges.

It was pointed out that in other animals there are strong interrelations between EMT/MET, stem cell regulation and Wnt, Notch, Hh, and TGF- $\beta$  interconnections. Moreover, according to data obtained during *Amphimedon* development, it seems that the interaction of key developmental pathways—such as Wnt, TGF- $\beta$ , and Hedgehog—are also of ancient origin and could have emerged in the metazoan ancestor (Adamska et al. 2011).

#### Main conclusions

In recent years, the first draft genome sequences from representatives of the earliest branching animals as well as non-metazoan lineages have significantly altered our understanding of early animal evolution and the genomic complexity of the metazoan ancestor. A large majority of transcription factors and signaling pathways have their evolutionary origin at the base of the animal tree, including the acquisition of new genes (some of them via domain shuffling), gene cooption, or diversification of ancestral domains, in accordance with the acquisition of the multicellularity in this taxon.

Even if a very limited amount of expression and functional data is available for sponges, the initial data demonstrate that these developmental gene toolkits play a role in sponge development and morphogenesis (Larroux et al. 2006, 2007, 2008; Adamska et al. 2007a, b, 2010, 2011; Fahey et al. 2008; Gauthier and Degnan 2008; Gazave et al. 2008; Degnan et al. 2009; Lapébie et al. 2009; Richard and Degnan 2009; Windsor and Leys 2010; Hill et al. 2010; Holstein et al. 2010; Srivastava et al. 2010a, b).

It is only through the sequencing of additional basal metazoan and non-metazoan Opisthokont genomes that we will be able to expand our understanding of the origin and evolution of TFs and signaling pathways, keeping in mind that the reconstruction of the evolutionary history of

developmental processes would depend on still contentious nonbilaterian phylogenetic relationships. Another challenge is to establish tools for studying their function in sponges. The first published encouraging RNAi approaches are far from being routinely under control in each sponge model (Rivera et al. 2011).

## Conclusions and perspectives

Mechanistic similarities with eumetazoans: sponges are not so simple

Finally, our review has attempted to show that morphogenetic processes found in sponges are as diverse and complex as those found in other animals. In true epithelial sponges (Homoscleromorpha), as well as in others, we find similar cell/layer movements, cellular shape changes involved in major morphogenetic processes such as embryogenesis, larval metamorphosis, or regeneration processes. Thus, sponges can provide information enabling us to better understand early animal evolution at the molecular level as well as at the cell/cell layer level. Indeed, comparison of molecular tools will only be of value if accompanied by functional data and expression study during morphogenetic processes.

More than one sponge morphogenesis: The importance of models diversification

By the variety of examples cited in the previous text, we have shown that each sponge lineage presents morphogenetic specificities. Homoscleromorpha are of special interest for comparison with eumetazoans because of their similar epithelial organization. Nevertheless, as previously demonstrated, even in the absence of a basement membrane, other sponge taxa also use very similar cellular mechanisms (even if epithelial morphogenesis is less represented). Thus, the comparison between different sponge taxa may help to determine the precise role of each type of cell adhesion tool or molecular element involved in morphogenetic mechanisms.

Pending questions

*When did epithelia emerge?*

The common answer to this question is that epithelia are a eumetazoan innovation. With regard to the Homoscleromorpha, the answer seems less clear. Until as recently as 2009, papers argued either in favor of monophyly (Philippe et al. 2009; Schierwater et al. 2009; Pick et al. 2010) or paraphyly (Hejnol et al. 2009; Sperling et al. 2009; Mallatt et al. 2010) of sponges. Depending on the phylogenetic relationships accepted, the interpretation of Homoscleromorpha

epithelia relative to those of the Eumetazoa is very different: they may either be homoplasic in the first case or homologies (Philippe et al. 2009; Gazave et al. 2012). Even if the last phylogenomic published data are in favor of the former hypothesis, more biochemical and molecular data are expected to indicate whether or not epithelia emerged before the Eumetazoa. In any case, the first results on *O. lobularis* suggest that Eumetazoa and Homoscleromorpha use components from a common molecular toolkit to pattern epithelia (Lapébie et al. 2009). Data on demosponges also suggest that the principal molecular tools needed to produce epithelial cell cohesion predated epithelial emergence (Fahey and Degnan 2010; Srivastava et al. 2010a).

*What about stem cell and cell lineage tracking in sponges?*

Completing morphogenetic processes also relies on the capacity of the organism to mobilize cells to form a new organization. In other words, morphogenesis depends on the ability of cells to proliferate, migrate, differentiate, dedifferentiate, or even transdifferentiate. For this reason, additional knowledge concerning stem cells, cell lineages, and cell differentiation mechanisms is required to fully understand the morphogenesis in sponges. At present, the expression of so-called stem cell-specific genes has confirmed that the archeocyte are the stem cells in the demosponge *Ephydatia*. In addition, choanocytes, though they are specialized cells, seem to conserve pluripotency throughout their life (Funayama 2010; Funayama et al. 2010). Is there a pluripotency equivalent? Are stem cells the same in the different sponge lineages? Further data are needed to answer these questions. Nevertheless, in the genus *Oscarella* where archaeocytes are lacking, we can expect different mechanisms, perhaps relying mainly on transdifferentiation. In order to understand which cells play the role of stem cells and how and to what extent differentiation (or apoptotic) mechanisms are involved in sponge metamorphosis, it is crucial that we develop techniques allowing the study of cells during these processes.

*Can these studies help to shed light on sponge gastrulation?*

We previously emphasized that both epithelial and epithelial-like as well as mesenchymal morphogeneses occur during poriferan ontogenesis, even when they lack true epithelia (with the exception of Homoscleromorpha). According to comparative embryological data on Porifera and Cnidaria, it is clear that the last common ancestor of the Metazoa (Urmetazoa) was already able to form epithelial-like layers and to disaggregate these layers into individual cells. It was also capable of epithelial-like morphogenesis, i.e., possessed mechanisms ensuring formation of various invaginations and evaginations of the cell layer such as that occurring during gastrulation.

The existence of gastrulation and its identification in sponges has long been problematic and open to debate. Indeed depending on the definition used, researchers have argued either in favor of or against the existence of a gastrulation stage during sponge development and even disagree about which stage should be regarded as a gastrulation (see Leys 2004; Ereskovsky and Dondua 2006; Ereskovsky 2007). We have already explained (“[Reproduction and development in sponges](#)” section) that the difficulty of determining clear developmental stages in sponges is mainly due to the simultaneity of various cellular mechanisms. For example, morphogenetic movements of single cells or their layers during embryogenesis in sponges are not directly associated with cytodifferentiation: they may precede differentiation of larval cells, coincide with it, or they may follow it (Ereskovsky and Dondua 2006). The only case where cell differentiation predates morphogenetic movements in sponges is epithelial morphogenesis.

We can expect that exploring more deeply the cellular and molecular mechanisms involved in sponge morphogenetic processes should help us to answer this question more clearly. Thus, because Homoscleromorpha is a unique sponge group with a true epithelial cell layer [named “epitheliosponges” (Ereskovsky and Tokina 2007)] and that epithelial morphogenesis is the main process used throughout homoscleromorph sponges’ lifespan (Ereskovsky 2010), this sponge group appears to be the best candidate to explore the formation and evolution of layers ontogeny.

Thus, the accumulation of cellular and molecular data involved in morphogenesis of homoscleromorphs and comparing them with both other sponge lineages and Eumetazoa should provide important information regarding the origin and early evolution of gastrulation and cell layers in animals.

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