BioEssays

www.bioessays-journal.com

25 Years
Thinking About Biology

WILEY-BLACKWELL

The Homoscleromorph sponge *Oscarella lobularis*, a promising sponge model in evolutionary and developmental biology

Model sponge *Oscarella lobularis*

Alexander V. Ereskovsky,[1,2]* Carole Borchiellini,[1] Eve Gazave,[1] Julijana Ivanisevic,[1,3] Pascal Lapébie,[1] Thierry Perez,[1] Emmanuelle Renard,[1] and Jean Vacelet[1]

[1]Centre d'Océanologie de Marseille, Station marine d'Endoume, Aix-Marseille Université - CNRS UMR 6540-DIMAR, rue de la Batterie des Lions, Marseille, France

[2]Department of Embryology, Faculty of Biology and Soils, Saint-Petersburg State University, Universitetskaja nab. 7/9, St. Petersburg, Russia

[3]Faculté de Sciences, Université de Nice Sophia-Antipolis – CNRS UMR 6001 LCMBA, Parc Valrose, Nice, France

Sponges branch basally in the metazoan phylogenetic tree and are believed to be composed of four distinct lineages with still uncertain relationships. Indeed, some molecular studies propose that Homoscleromorpha may be a fourth Sponge lineage, distinct from Demospongiae in which they were traditionally classified. They harbour many features that distinguish them from other sponges and are more evocative of those of the eumetazoans. They are notably the only sponges to possess a basement membrane with collagen IV and specialized cell-junctions, thus possessing true epithelia. Among Homoscleromorphs, we have chosen *Oscarella lobularis* as a model species. This common and easily accessible sponge is characterized by relatively simple histology and cell composition, absence of skeleton, and strongly pronounced epithelial structure. In this review, we explore the specific features that make *O. lobularis* a promising homoscleromorph sponge model for evolutionary and developmental researches.

Why sponges?

One of the major questions in the evolution of animals is the transition from unicellular to multicellular organization, which resulted in the emergence of Metazoa through a hypothetical Urmetazoa.[1,2] Sponges are filter-feeders devoid of organs and specialized tissue; they have no nervous system, no digestive cavity. Their relatively simple body plan and the resemblance of their fundamental cell type, the choanocyte, to the choanoflagellates, suggested to some zoologists of the end of the 19th century that they were the first multicellular animals. The others considered a sponge as a colony of unicellular organisms only with loose, labile cell differentiation,[3] but not as a multicellular animal. This question has now been clearly settled, and the inclusion of sponges in the Metazoan clade appears without doubt[4–8] (Fig. 1A). However, the phylogenetic status of sponges is not so obvious. Traditionally, sponges are considered as monophyletic and divided in three lineages: Demospongiae, Hexactinellida and Calcispongiae.[9] However, monophyly of sponges has been recently challenged by number of molecular studies suggesting a paraphyletic arrangement of Porifera at the basis of a metazoan tree (Fig. 1).[10–13] In addition, Homoscleromorpha have been proposed as a fourth Sponge lineage phylogenetically distinct from Demospongiae in which they were traditionally classified (Fig. 1B).[11,14–16] Nevertheless, phylogenies based on different molecular datasets testing the relationships between these sponge lineages on one hand and their relation to non-sponge (eumetazoan) taxa (Ctenophora, Placozoa, Cnidaria and Bilateria) on the other hand, have provided conflicting results[7,10–12,15,17–21] and thus do not permit an unambiguous resolution for these questions. However, sponges still remain, as basal lineages, key organisms for unraveling early metazoan evolution, as illustrated by the growing interest for sponges (and more largely for 'non-bilaterian' animals) in the evolutionary and developmental (Evo–devo) field over the last 5 years.[22–31]

Evo–devo is a domain of research that aims at understanding the evolution of developmental processes and how

*Correspondence to: A. V. Ereskovsky, Centre d'Océanologie de Marseille, Station marine d'Endoume, Aix-Marseille Université - CNRS UMR 6540-DIMAR, rue de la Batterie des Lions, 13007 Marseille, France.
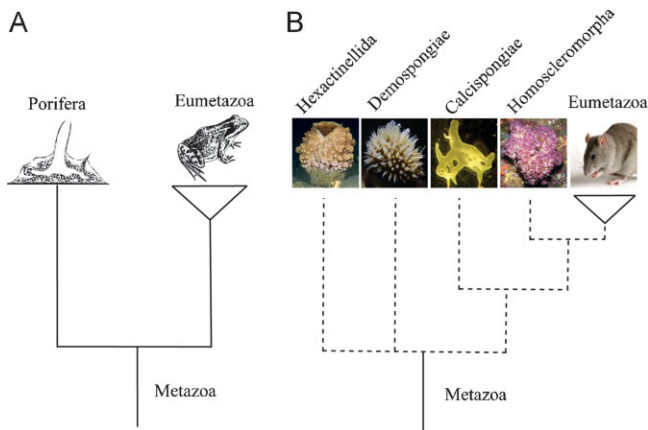E-mail: aereskovsky@hotmail.com

Figure 1. Phylogenetic relationships among sponges and eume-tazoan taxa. (A) Classical view of metazoan evolution scenario: sponges are monophyletic and sister group of Eumetazoa. (B) New phylogenetic hypothesis based on DNAr 18S data according Borchiellini *et al*. 2001, 2004.[10,14] In this new scenario sponges constitutes four independent lineages at the basis of the tree.

Figure 2. *O. lobularis in vivo* and *in situ*. (A) *O. lobularis* (OL) in typical biocoenosis. (B) Photographs of *O. lobularis*. L, lobes; O, osculum.

these developmental changes can explain body plan evolution. One of the challenges of evo–devo is to understand how novelties emerged in evolution. Concerning animal evolution, the current hot topics concern the origins and morphogenesis of polarity axis, 'true' epithelial tissues, nervous system, digestive cavity *etc*. Sponges, despite their morphological simplicity, harbour most of the families of regulatory developmental genes previously identified as actors in bilaterian models development.[23,24,26,27,31–33]

Genetic and expression data remain scarce for sponges. Results were mainly obtained from the study of one lineage— Demospongiae, more precisely *Amphimedon queenslandica*, the only sponge species for which the full genome is available.[23,24,26,27,29,30,32,34] These data gave rise to captivating hypotheses. Nevertheless, the lack of sufficient knowledge of the genes' functions and of their expression diversity in all sponge lineages prompted an occasional over interpretation of their evolutionary implications. Sponges display a huge diversity of growth forms and developmental strategies. Only a comparative approach is suitable to bear out the hypothesis made from *Amphimedon* data. There is, thus, a crucial need to develop new model organisms covering a broader phylogenetic range.[35]

One must keep in mind that the multiplication of models in an evo–devo approach is not at all a weakness but a powerful tool for 'reconstructing' the Urmetazoan genetic toolkit. Indeed, the presently abundant background on Bilateria evo–devo must be a lesson to us in the way that an a priori chosen model can a posteriori appear to possess very derived features, and that a single model cannot be considered as representative of its taxon.[36–39]
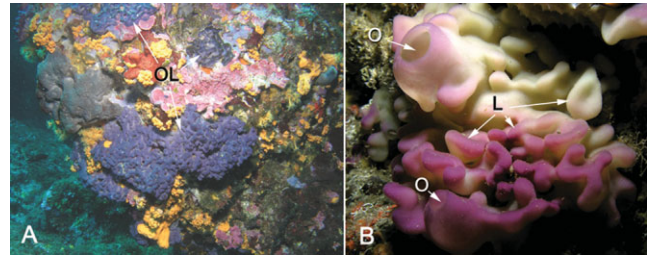
For this reason, our group chose to focus on another promising sponge model—*Oscarella lobularis* (Fig. 2) which belongs to a clade distant from the Demospongiae, and for which many data are presently available in the various fields of taxonomy, ecology, biochemistry, morphology, cytology and development. *O. lobularis*, thus could provide a suitable model, in a comparative context within sponges, for inferring the ancestral states from which the living animal phyla were derived.

Why choose a Homoscleromorpha species?

Among the approximately 8,000 sponge species described to date,[9] we have chosen a representative of a small group, the Homoscleromorpha, which could provide, as emphasized before, a valuable comparison with the distantly related Demospongiae models such as *Amphimedon*.

The anatomy of Homoscleromorpha is limited to what some authors imagined to represent a 'minimum functional sponge.'[40] with a choanoderm (surface lined by choano-cytes) composed of large choanocyte chambers, no cortex (Fig. 3A), and a non-organized skeleton made of siliceous spicules of a peculiar type (calthrops and its derivatives through reduction: diods and triods) that may even be absent. The thin, unspecialized ectosome and aquiferous system canals are lined by a regular layer of pinacocytes (pinaco-derm), which is composed of relatively flat, uniflagellated cells (Figs. 3B, C). Between the choanoderm and pinacoderm epithelia a mesohyl layer contains several types of symbiotic bacteria and a few types of scattered cells, including cells with inclusions called vacuolar cells (Figs. 3G, H), and rare amoeboid cells called archaeocytes.[41–43]

Another interest of Homoscleromorpha is that they are the only sponge lineage in which cell layers, both in larvae and adults, are very similar to those of eumetazoans and can be considered as a true epithelium. They possess a basement membrane including collagen IV fibrils (Figs. 3C, D)[44–46] and the pinacocytes and larval cells are connected by
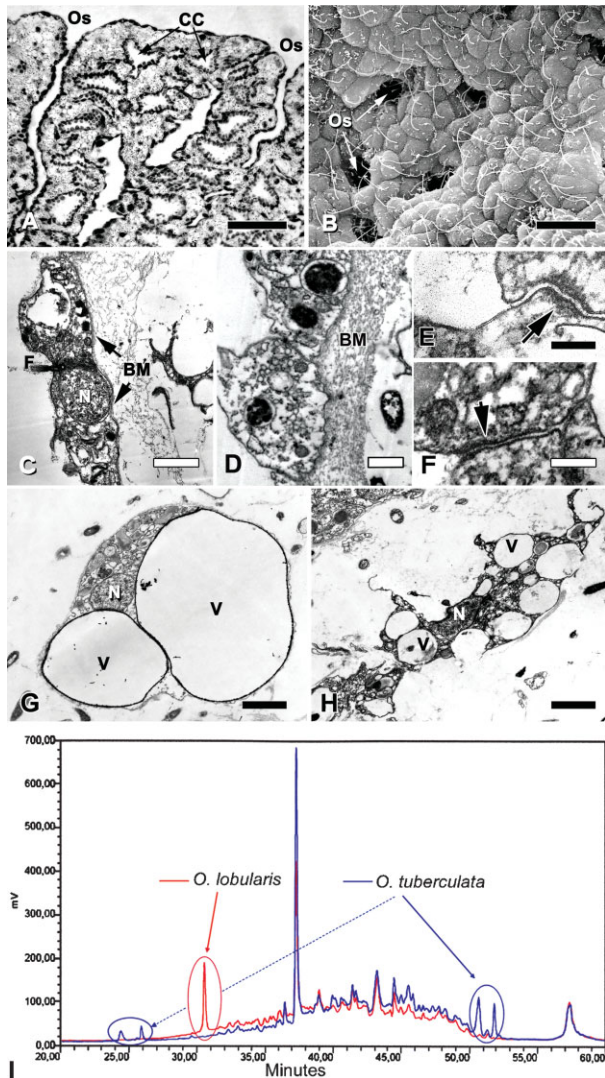
Figure 3. Histology and ultrastructure of *O. lobularis*. (A) Semi-thin section through the sponge body (anatomy). CC, choanocyte chambers; Os, ostia. Scale bar: 10 μm. (B) SEM micrograph of external surface with flagellated exopinacoderm and ostia. Os, ostia. Scale bar: 20 μm. (C) TEM micrograph of flagellated endopinacocyte with the basement membrane. F, flagellum; BM, basement membrane. Scale bar: 2 μm. (D) TEM micrograph of basement membrane under the choanocytes. BM, basement membrane. Scale bar: 1 μm. (E): TEM micrograph of cell junctions (zonula adhaerens) between the ciliated cells of cinctoblastula larva (arrow). Scale bar: 2.5 μm. (F) TEM micrograph of cell junctions (zonula adhaerens) between the endopinacocytes (arrow). Scale bar: 3 μm. (G) View of vacuolar cell type I (TEM). N, nucleus; V, vacuole. Scale bar: 2 μm. (H) View of vacuolar cell type II (TEM). N, nucleus; V, vacuole. Scale bar: 2 μm. (I) Chromatogramme (SPE-HPLC-ELSD) of crude extracts of *O. lobularis* (red line) and *O. tuberculata* (blue line). The extracts of both species contain the fatty acid C24:2 (specific for the *Oscarella* genus) as a major compound. A large number of minor compounds are also shared. Encircled compounds are specific to each species. HPLC conditions: Standard gradient from CH3CN/H2O/HCOOH 10:90:0.1 to CH3CN/H2O/HCOOH 100:0:0.1, after Ivanisevic *et al.* 2007.[64]

specialized cell junctions, an unusual feature in sponges (Figs. 3E, F).[47,48]

These characteristics probably explain that Homoscleromorpha exhibit a true epithelial morphogenesis–morphogenetic movements of cells united with their neighbours in a layer.[49] Epithelial folding is one of the basic morphogenetic processes reiterated throughout embryonic development in Eumetazoa. Interactions between epithelial cells and the extracellular matrix play a fundamental role in this morphogenesis.[50] In Homoscleromorpha, this process is observed during the egg's follicle formation,[51] during the metamorphosis in a rhagon (earliest developmental stage with a functional aquiferous system),[52] during the sponge growth starting as formation of projections of the exopinacoderm,[53] during asexual reproduction by budding,[47] during ostia formation and reparative regeneration (Ereskovsky *et al.*, in preparation).

Epithelial morphogenesis is quite rare in other sponge clades. In *Halisarca* (Demospongiae) invagination was described during disphaerula larva development and epithelization of larval posterior pole during metamorphosis.[54,55] In *Sycon* sp. (Calcaronea), stomoblastula excurvation,[56] invagination of larval anterior pole and epithelization of larval posterior pole during metamorphosis were described.[57]

Selection of *O. lobularis*

Homoscleromorpha consists of a single family Plakinidae with seven genera, including 77 species. Our favourite animal, *O. lobularis* (Schmidt, 1862) (Fig. 2), belongs to the genus which is characterized by the absence of skeleton, a great advantage for techniques such as *in situ* hybridization (ISH) on adults or dissociation-re-aggregation experiments.

A good taxonomic knowledge renders determination quite easy

The absence of a skeleton, the main morphological character for sponge taxonomy, explains the very complicated history of the species status of *O. lobularis*. It was considered for a long time as the only species of the genus *Oscarella*, displaying different consistency and colour morphotypes.[58] In fact, *Oscarella* appears as a good example of a single 'cosmopolitan' sponge species which turned out to be a highly diversified complex of species. At the present time, 13 species are listed in the World Data Base (http://www.marinespecies.org/porifera/index.php), including six Mediterranean species.[41,59] All these species can be distinguished by a careful examination of field, histological, cytological and biochemical characters. Since a clear discrimination of our model is needed for the benefit of the international scientific community and in the interests of the repeatability of results, our team

is at present studying the relationships within the *Oscarella* genus by combining various characters including subtle histological, cytological and biochemical ones, which, combined with molecular analysis, may help in resolving the phylogeny of this sponge group.

Oscarella ecology and availability for experimental research

Sponges of the genus *Oscarella* only grow on rocky bottoms. In the Mediterranean, which exhibits a high diversity of homoscleromorph sponges. *Oscarella* species are dwellers in sciaphilic hard substratum communities, such as the coralligenous, semi-dark and dark submarine caves, and are found from the Gibraltar Strait to the Eastern Mediterranean. They are mainly located in shallow waters from 4 to 35 m, making the sampling as well as *in situ* monitoring easy. *O. lobularis* is one of the most common and abundant homoscleromorphs in the Mediterranean, conditioning specific facies in some places. As other species of the same genus, *O. lobularis* seems to be a strong competitor for space, overgrowing massive sponges, sea fans and bryozoans (Fig. 2A). This strong, out-competing ability may be due to a particularly efficient secondary metabolism, and the biochemical defence it confers. This hypothesis is also supported by the absence of a well-known predator or epibiotic organisms.

Contribution of biochemical fingerprints in understanding Homoscleromorpha relationships

The biochemical characteristics of several homoscleromorph sponges have been described by several authors,[60] but most of these studies have been focused on species or genera offering the best bioactivity and potential value for the biomedical field. The first chemical analyses of *O. lobularis* were probably performed on misidentified individuals of at least two distinct species, *O. lobularis* and *O. tuberculata*.[60–63] Because we believe that secondary metabolites can be useful complementary taxonomical markers, our team has recently developed a 'chemical fingerprint approach' that will allow rapid assessment of the chemical diversity and clear discrimination of Homoscleromorpha species. In general, all analysed Homoscleromorpha species show a high level of expression and diversity of apolar metabolites. The chemical analysis performed on two sibling species, *O. tuberculata* and *O. lobularis* clearly demonstrates distinct chemical signatures (Fig. 3I): some compounds are common to both species (and possibly to the genus) while others are species-specific.[64] These results give a classification of the group quite congruent with those obtained with traditional characterization. The chemical fingerprint approach is presently applied not only to *Oscarella*, but also to all Mediterranean homoscleromorphs in order to contribute to a phylogenetic interpretation.

Good knowledge of its reproduction and development: some specific features

Sexual and asexual reproduction and development of several *Oscarella* were studied in details. *O. lobularis* is ovoviviparous (Fig. 4A) with various reproductive strategies: most individuals are gonochoric, but some are hermaphroditic.

Spermatogenesis proceeds inside of spermatic cysts (Fig. 4B), formed through choanocyte chamber transdifferentiation.[65] Spermatogonia derive directly from choanocytes (Fig. 4C).[65,66] Spermatogenesis is asynchronous and there is a gradient in cell differentiation along the spermatic cysts, as in eumetazoans, whereas in Demospongiae spermatogenesis is synchronous in a cyst.[67] As in eumetazoans, the mature spermatozoa harbour an acrosome,[65,66] a feature also observed in few Demospongiae.[67,68]

The mature egg is isolecithal and polylecithal. One of the most remarkable characteristics of *Oscarella* oogenesis is the synthesis of yolk due to its own metabolism,[69] as it is in eumetazoans, whereas in the other sponges with polylecithal eggs, vitellogenesis is accompanied by phagocytosis of somatic cells or/and bacteria.

The embryonic development of Homoscleromorpha has been described as a special cinctoblastular type on the basis of its differences from the development of other sponges.[70] Cleavage in *O. lobularis* is holoblastic, equal, asynchronous and results in a solid apolar morula (stereoblastula) (Fig. 4D).[51] A coeloblastula with a large central cavity is formed by means of centrifugal migration of cells from the centre to the periphery of the morula (Fig. 4E). This embryonic morphogenesis is unique within sponges and Metazoa and has been termed multipolar egression.[51] The differentiation and proliferation of cells in the coeloblastula lead to the formation of a cinctoblastula larva with well pronounced anterio-posterior polarity (Figs. 4F, G). The columnar ciliated cells of the larval epithelium are closely linked by zonula adhaerens junctions (Fig. 3E).[48,52] The extracellular matrix forms a basement membrane immediately below the epithelial cells of larva. Thus, the larval epithelium of homoscleromorphs is very similar to the eumetazoan columnar epithelium.[48]

The metamorphosis occurs after 12–36 hours of larval swimming. The larva attaches to substrate by the anterior pole, its anterio-posterior axis corresponding to the basal–apical axis of the adult sponge.[52] The presence of an intranuclear crystalloid in posterior-lateral larval cells[48] is a natural marker that allows monitoring the fate of cells during metamorphosis, a highly disputable issue in sponge embryology. In all sponge species studied so far, metamorphosis is accompanied by a major disorganization of the external layer of larval ciliated cells, and the formation of pinacoderm and choanoderm occurs by a new association of
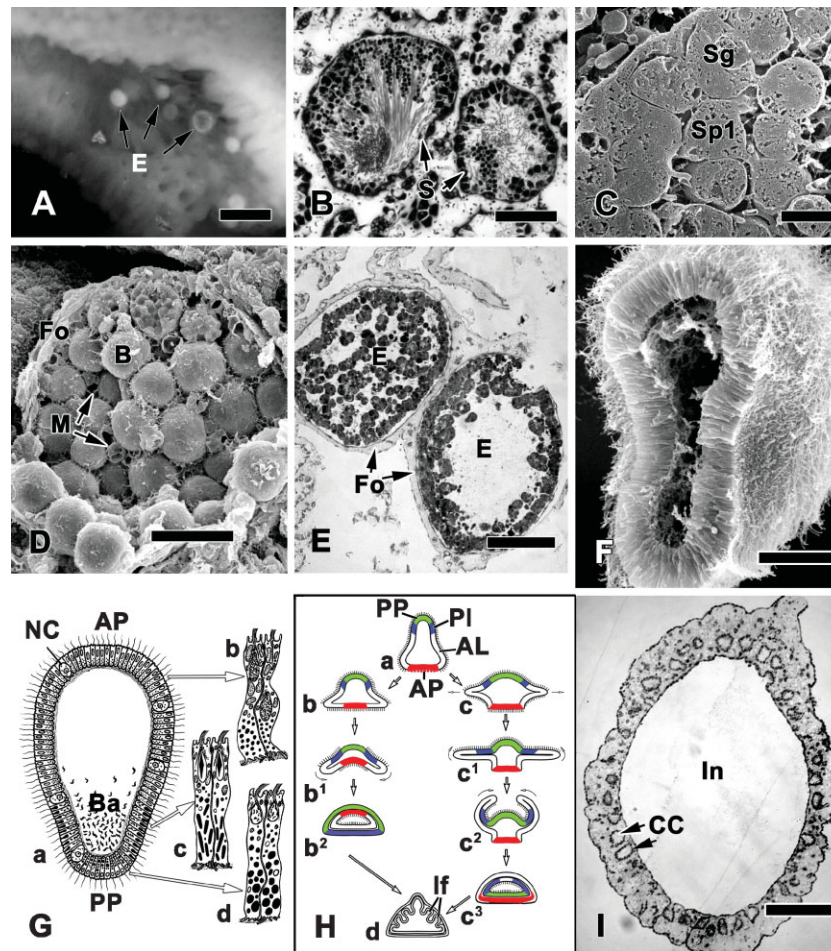
Figure 4. Development of *O. lobularis*. (A) Internal part of *O. lobularis in vivo* with the embryos. E, embryos. Scale bar: 250 μm. (B) Semi-thin section through spermatocysts. S, spermatocysts. Scale bar: 10 μm. (C) SEM micrograph of freeze-fracture through spermatocyst—evidence of choanocyte origin of male sexual cells. Sg, spermatogonia; Sp1, spermatocyte 1. Scale bar: 1.25 μm. (D) SEM micrograph of morula (stereoblastula). B, blastomere; Fo, follicle; M, maternal vacuolar cells. Scale bar: Scale bar: 40 μm. (E) Semi-thin section through the morulae during morphogenesis by multipolar egression–two stages. E, embryo; Fo, follicle. Scale bar: 60 μm. (F) SEM micrograph of a longitudinal section through a released, free-swimming cinctoblastula. Scale bar: 40 μm. (G) Diagram of cinctoblastula; after Boury-Esnault *et al*. 2003.[48] (a) Free-swimming larva. (b) Flagellated cell of anterior pole. (c) Flagellated cell of postero-lateral zone. (d) Flagellated cell of posterior pole. Ap, anterior pole; Ba, bacteria, NC, non-flagellated cells, Pp, posterior pole. (H) Diagram of the different paths of formation of the rhagon during the metamorphosis of *O. lobularis* larvae, after Ereskovsky *et al*. 2007.[52] a: Cinctoblastula. b–b²: Basal invagination of anterior pole larval ciliated epithelium. c–c³: Extension and folding up of lateral sides of larval ciliated epithelium. d: Young sponge, rhagon. AL, antero-lateral cells; AP, anterior pole cells; If, internal folds of post-larva epithelium; PL, postero-lateral cells with intranuclear paracrystalline inclusions; PP, posterior pole cells. (I) Semi-thin section of floating bud of *O. lobularis*. CC, choanocyte chambers; In, internal cavity. Scale bar: 200 μm.

separate cells.[68] In contrast, in *Oscarella*, the formation of adult epitheliums, pinacoderm and choanoderm during metamorphosis occurs through the trans-differentiation of the larval epithelium (Fig. 4H).

The fate of larval cells during metamorphosis depends on their position in the post-larva (Fig. 4H: b–b², c–c³).[52,71] Larval epithelium forms invaginations and evaginations accompanied by ingression of some cells.[52] Vertical transmission of symbionts from embryo to juvenile sponges has been also confirmed by our studies.[48,51]

Asexual reproduction in *Oscarella* occurs via budding that finished from 2 to 4 days (Fig. 4I).[47] The bud attached to the substrate has a syconoid-like organization similar to the rhagon developing after larva metamorphosis. The bud development in *Oscarella* differs from that of other sponges. It does not involve migration of cells from the mesohyl and their integration into the forming bud, but is based on epithelial morphogenesis by evagination. Similar morphogenesis is characteristic of palleal, stolonial and vascular budding in Ascidiacea[72] and is also well known in Cnidaria.[73]

Interest of O. lobularis as a promising sponge model in Evo–Devo compared to other models, and first encouraging results

We chose *O. lobularis* to study genetic mechanisms involved in morphogenetic processes because of its clear systematic description, its availability, its well-described histology, ultra-structure and development. Furthermore, as representative of Homoscleromorpha, it displays unique eumetazoan-like features absent in other sponges and phylogenetically may constitute an independent lineage distant from demosponge models (*A. queenslandica, Ephydatia* and *Suberites domuncula*).

In *O. lobularis*, degenerated PCR approach and a collection of 2000 EST resulted in the characterization of various genes of the Antennapedia (ANTP) class of homeobox genes. This class of genes is generally important for various developmental processes in Eumetazoa and is supposed to have played an early role in bauplan evolution.[23,74,75] The various families of ANTP genes, distributed into four subclasses (Hox, Parahox, EHGbox and NKL),[23] are found in the three major clades of Bilateria (deuterostomes, lophotrochozoans and ecdysozoans) and with a few exceptions they are also present in cnidarian genomes.[76–78] All previously characterized sponge ANTP homeobox genes belong to the NKL subclass.[23] A comparison of *NK*-related genes present in the genome of the demosponge *A. queenslandica* with their orthologues in other metazoans, led Larroux *et al.*[23] to propose that the last common ancestor of Metazoa probably possessed only six or seven *NK*-related genes (with reservation due to possible secondary loss in this species).[74] Among them at least five (*NK5-7, Tlx, Hex, Msx* and *NK2-4*) might have composed a hypothetic 'protoNK' cluster.[23] Nevertheless, expression data remain scarce and almost nothing is known concerning their role in sponge development. Thus, we focused on the expression characterization by ISH of some of them.[31]

Five partial homeobox gene fragments were isolated from five homoscleromorph species (*O. lobularis*, *O. imperialis*, *Pseudocorticium jarrei*, *Plakina jani* and *Plakortis simplex*). These five new sequences form a strongly supported clade phylogenetically related to the *NK6* and *NK7* families from cnidarians and bilaterians, possibly also to the *AmqNK567* gene from *A. queenslandica*, although feebly supported. Our ISH analysis of the *OlobNK* gene, in whole mount specimens and tissue sections of *O. lobularis* (containing gametes and various developmental stages), revealed exclusive expression in the choanocytes[31] (Fig. 5).

Structural similarities between choanocytes and eumetazoan sensory cells such as mecanoreceptors[79] have been already evocated. Furthermore, major expression of the *NK6* and *NK7* families of bilaterian homeobox genes (the closest relatives of homoscleromorphs *NK* genes in our phylogene-
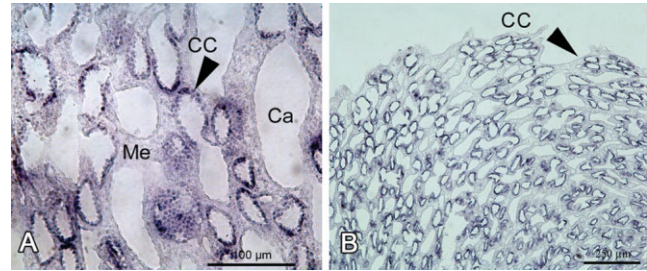

Figure 5. Expression pattern of *OlobNK* observed on sections at (A) high and (B) low magnifications; only the choanocytes are stained, non-reproductive specimen. (CC) Ch: choanocyte chamber; Ca: canal; Me: mesohyl.

tical analyses) are neural. Thus, our expression result[31] is in line with other works suggesting possible homology between choanocytes and neurosensory cells.[13,79]

In addition to the great diversity of *NK*-like genes, members of important pathways were evidenced in the sponges *A. queenslandica*, *S. domuncula* and *O. carmela*.[23,26–28,33,80] In our *O. lobularis*, various genes coding for molecules of the Notch signalling pathway are under study by our team (unpublished data). Preliminary results suggest that this pathway may form a complex network in the last common ancestor of Metazoa.

Concerning the famous Wnt signalling pathway involved in primordial developmental events in Eumetazoa such as gastrulation and axis patterning, we characterized in *O. lobularis Wnt* genes and genes coding for other members of this pathway[81] (Lapébie *et al.*, in preparation). As far as its role is concerned, initial expressional data in *A. queenslandica* are consistent with an ancestral implication of Wnt signalling in embryonic axis patterning.[75] Preliminary expression analysis of the two *O. lobularis Wnt* genes on adult specimens revealed complementary regionalized expression patterns in an outer epithelial layer, the exopinacoderm (Lapébie *et al.*, in preparation), suggesting that employment of WNT signalling in epithelial patterning and morphogenesis is an ancient metazoan feature.

As genetic and genomic data on sponges are becoming more extensive, the idea of the 'simplicity' of sponges and of the common ancestor of Metazoa seems less and less convincing.[24,33,75,82,83] However, data on developmental gene expression must be accumulated and compared between different sponge lineages and eumetazoans in order to understand the mechanisms involved in the evolution of metazoan body plans and genomes.

Conclusion and perspectives

In this paper, we have shown the reasons why we feel that our knowledge of *Oscarella* and our experience of working with it

justify offering our favourite animal as a candidate as a new sponge model for evolutionary developmental studies. The phylogenetic position and distinctive features of the organization and development of *Oscarella* will provide a basis for answering a number of relevant questions in modern biology.

Homoscleromorph sponges display many morphological, cytological, biochemical and embryological features that distinguish them from other sponges and are more evocative of the Eumetazoa.[44,48,51,68,70] For example, they are the only sponges to possess a basement membrane with collagen IV, tenascin and laminin,[44,45] presently considered as a synapomorphy of eumetazoans. Thus, Homoscleromorpha is a unique sponge group that could be called 'epitheliosponges,'[47] since only this sponge group has a true epithelial structure and an epithelial morphogenesis. Three explanations are possible for the presence of basement membrane in homoscleromorphs and eumetazoans: (i) a convergent development in homoscleromorphs and eumetazoans, (ii) its presence in the common ancestor of Homoscleromorpha–Eumetazoa clade or (iii) its presence in metazoan ancestor, with loss in sponges except in Homoscleromorpha. The choice among these alternative scenarios requires a more complete phylogenetic framework. But, even if the homology between basement membranes of Homoscleromorpha and Eumetazoa remains to be confirmed,[84] Homoscleromorpha appear to be a model of particular interest for comparing cell movement processes occurring during morphogenesis with those of eumetazoan, and to understand the origin and evolution of epithelia.

Our second centre of interest concerns the origin of the polarities. Bilaterians have two principal body axes, dorso-ventral and anterio-posterior, while sponges have a single main axis of polarity (clear in sponge larvae, but not always in adults). The relationships between the polarities of bilaterian and non-bilaterian animals remain a fundamental unresolved issue in evolution/developmental biology. Morphological research can only reveal external similarities. To what extent are these axes defined and patterned by shared molecular and cellular mechanisms in sponges? Were polarity generating mechanisms derived from a common metazoan ancestor, or have they been adopted independently in different evolutionary lineages?

Nowadays, molecular data clearly indicate that sponges and bilaterians share highly conserved homologies of basic genetic machineries involved in cell differentiation, axis formation and development control. In the light of the concept that results from only one sponge clade, which cannot be generalized to all sponges,[35] future researches on *O. lobularis*, will be an important contribution to the knowledge of the early metazoan evolution events.

References

1. **Müller, W. E. G.,** Review: how was metazoan threshold crossed? The hypothetical Urmetazoa. *Comp Biochem Physiol A Mol Integr Physiol* 2001. **129**: 433–460.
2. **Müller, W. E. G., Wiens, M., Adell, T., Gamulin V., Schroder, H. C.,** *et al.* Bauplan of Urmetazoa: basis for genetic complexity of metazoa. *Int Rev Cyt* 2004. **235**: 53–92.
3. **Minchin, E. A.,** The Porifera and Coelenterata. London, Adam and Charles Black 1900.
4. **Borchiellini, C., Boury-Esnault, N., Vacelet, J., Le Parco, Y.,** Phylogenetic analysis of the Hsp70 sequences reveals the monophyly of Metazoa and specific phylogenetic relationships between animals and fungi. *Mol Biol Evol* 1998. **15**: 647–655.
5. **Borchiellini, C., Chombard, C., Lafay, B. and Boury-Esnault, N.,** Molecular systematics of sponges (Porifera). *Hydrobiologia* 2000. **420**: 15–27.
6. **Kruse, M., Leys, S. P., Müller, I. M. and Müller, W. E. G.,** Phylogenetic position of the Hexactinellida within the phylum Porifera based on the amino acid sequence of the protein kinase C from Rhabdocalyptus dawsoni. *J Mol Evol* 1998. **46**: 721–728.
7. **Dellaporta, S. L., Xu, A., Sagasser, S., Jakob, W. and Moreno, M. A.,** *et al.* Mitochondrial genome of Trichoplax adhaerens supports Placozoa as the basal lower metazoan phylum. *PNAS* 2006. **103**: 8751–8756.
8. **Telford, M.,** Animal phylogeny. *Curr Biol* 2006. **16**: 981–985.
9. **Hooper, J. N. A. and van Soest, R. W. M.** editors. Systema Porifera: a guide to the classification of sponges. New York, Kluwer Academic/Plenum Publishers, 2002.
10. **Borchiellini, C., Manuel, M., Alivon, E., Boury-Esnault, N., Vacelet, J. and Le Parco, Y.,** Sponge paraphyly and the origin of Metazoa. *J Evol Biol* 2001. **14**: 171–179.
11. **Sperling, E. A., Pisani, D. and Peterson, K. J.,** Poriferan paraphyly and its implications for Precambrian palaeobiology. *Geol Soc London Spec Publ* . **286**: 355–368.
12. **Wang, X. and Lavrov, D. V.,** Seventeen new complete mtDNA sequences reveal extensive mitochondrial genome evolution within the Demospongiae. *PLoS ONE* 2008. **3**(7), e2723. doi:10.1371: 1-11.
13. **Nielsen, C.,** Six major steps in animal evolution: are we derived sponge larvae? *Evol Dev* 2008. **10**: 241–257.
14. **Borchiellini, C., Chombard, C., Manuel, M., Alivon, E. and Vacelet, J.,** *et al.* Molecular phylogeny of Demospongiae: implications for classification and scenarios of character evolution. *Mol Phyl Evol* 2004. **32**: 823–837.

15. **Nichols, S. A.,** An evaluation of support for order-level monophyly and interrelationships within the class Demospongiae using partial data from the large subunit rDNA and cytochrome oxidase subunit I. *Mol Phyl Evol* 2005. **34**: 81–96.

16. **Dohrmann, M., Janussen, D., Reitner, J., Collins, A. G. and Wörheide, G.,** Phylogeny and evolution of glass sponges (Porifera, Hexactinellida). *Syst Biol* 2008. **57**: 388–405.

17. **Medina, M. N., Collins, A. G., Silberman, J. D. and Sogin, M. L.,** Evaluating hypothesis of basal animal phylogeny using complete sequences of large and small subunit rRNA. *PNAS* 2001. **98**: 9707–9712.

18. **Manuel, M., Borchiellini, C., Alivon, E., Le Parco, Y., Vacelet, J. and Boury-Esnault, N.,** Phylogeny and evolution of calcareous sponges: monophyly of Calcinea and Calcaronea, high level of morphological Homoplasy, and the primitive nature of axial symmetry. *Syst Biol* 2003. **52**: 311–333.

19. **Wang, X. and Lavrov, D. V.,** Mitochondrial genome of the Homoscleromorph Oscarella carmela (Porifera, Demospongiae) reveals unexpected complexity in the common ancestor of sponges and other animals. *Mol Biol Evol* 2007. **24**: 1–11.

20. **Dunn, C. W., Hejnol, A., Matus, D. Q., Pang, K., Browne, W. E.,** *et al.* Broad phylogenomic sampling improves resolution of the animal tree of life. *Nature* 2008. **452**: 745–749.

21. **Lavrov, D. V., Wang, X. and Kelly, M.,** Reconstructing ordinal relationships in the Demospongiae using mitochondrial genomic data. *Mol Phyl Evol* 2008. **49**: 111–124.

22. **Wiens, M., Mangoni, A., D'Esposito, M., Fattorusso, E., Korchagina, N.,** *et al.* The molecular basis for the evolution of the Metazoan bodyplan: extracellular matrix-mediated morphogenesis in marine Demosponges. *J Mol Evol* 2003. **57**: 60–75.

23. **Larroux, C., Fahey, B., Degnan, S. M., Adamski, M., Rokhsar, D. S.,** *et al.* The NK homeobox gene cluster predates the origin of Hox genes. *Curr Biol* 2007. **17**: 1–5.

24. **Larroux, C., Fahey, B., Liubicich, D., Hinman, V. F., Gauthier, M.,** *et al.* Developmental expression of transcription factor genes in a demosponge: insights into the origin of metazoan multicellularity. *Evol Dev* 2006. **8**: 150–173.

25. **Richelle-Maurer, E., Boury-Esnault, N., Itskovich, V. B., Manuel, M., Pomponi, S. A. and Van de Vyver, G.,** Conservation and phylogeny of a novel family of non-Hox genes of the Antp class in Demospongiae (Porifera). *J Mol Evol* 2006. **63**: 222–230.

26. **Adamska, M., Matus, D. Q., Adamski, D., Green, K., Rokhsar, D. S.,** *et al.* The evolutionary origin of hedgehog proteins. *Curr Biol* 2007. **17**: R836–R837.

27. **Adamska, M., Degnan, B. M., Green, K. M., Amanski, M., Craigie, A.,** *et al.* Wnt and TGF-beta expression in the sponge Amphimedon queenslandica and the origin of Metazoan embryonic patterning. *PLoS ONE* 2007. **2**: e1031.

28. **Adell, T., Thakur, A. N. and Müller, W. E. G.,** Isolation and characterization of Wnt pathway-related genes from Porifera. *Cell Biol Int* 2007. **31**: 939–949.

29. **Sakarya, O., Armstrong, K. A., Adamska, M., Adamski, M., Wang, I.-F.,** *et al.* A post-synaptic scaffold at the origin of the animal kingdom. *PLoS ONE* 2007. **2**(6), e506.

30. **Fathey, B., Larroux, C., Woodcroft, B. J. and Degnan, B. M.,** Does the high gene density in the sponge NK Homeobox gene cluster reflect limited regulatory capacity? *Biol Bull* 2008. **214**: 205–217.

31. **Gazave, E., Lapébie, P., Renard, E., Bézac, C., Boury-Esnault, N.,** *et al.* A novel family of NK homeobox genes with choanocyte-specific expression in homoscleromorph sponges. *Dev Gen Evol* 2008. **218**: 479–489.

32. **Larroux, C., Luke, G. N., Koopman, P., Rokhsar, D. S., Shimeld, S. M. and Degnan, B. M.,** Genesis and expansion of metazoan transcription factor gene classes. *Mol Biol Evol* 2008. **25**: 980–996.

33. **Nichols, S. A., Dirks, W., Pearse, J. S. and King, N.,** Early evolution of animal cell signaling and adhesion genes. *PNAS* 2006. **103**: 12451–12456.

34. **Richards, G. S., Simionato, E., Perron, M., Adamska, M., Vervoort, M. and Degnan, B. M.,** Sponge genes provide new insight into the evolutionary origin of the neurogenic circuit. *Curr Biol* 2008. **18**: 1156–1161.

35. **Collins, A. G., Caertwright, P., McFadden, C. S. and Schierwater, B.,** Phylogenetic context and basal metazoan model systems. *Integr Comp Biol* 2005. **45**: 585–594.

36. **Korschak, R. D., Samuel, G., Saint, R. and Miller, D. J.,** EST analysis of the cnidarian Acropora millepora reveals extensive gene loss and rapid sequence divergence in the model invertebrates. *Curr Biol* 2003. **13**: 2190–2195.

37. **Hughes, A. L. and Friedman, R.,** Loss of ancestral genes in the genomic evolution of Ciona intestinalis. *Evol Dev* 2005. **7**: 196–200.

38. **Jenner, R. A. and Wills, M. A.,** The choice of model organisms in evo–devo. *Nat. Rev. Genet.* 2007. **8**: 311.

39. **Zou, S. M., Jiang, X. Y., Yuan, J., Yuan, X. N.,** *et al.* Hox gene clusters in blunt snout bream, Megalobrama amblycephala and comparison with those of zebrafish, fugu and medaka genomes. *Gene* 2007. **400**: 60–670.

40. **Lévi, C. and Porte, A.,** Etude au microscope électronique de l'éponge Oscarella lobularis Schmidt et de sa larve amphiblastula. *Cah Biol Mar* 1962. **3**: 307–315.

41. **Muricy, G., Boury-Esnault, N., Bézac, C. and Vacelet, J.,** Cytological evidence for cryptic speciation in Mediterranean Oscarella species (Porifera, Homoscleromorpha). *Can J Zool* 1996. **74**: 881–896.

42. **Muricy, G. and Pearse, J. S.,** A new species of Oscarella (Demospongiae: Plakinidae) from California. *Proceeding Calif Acad Sci* 2004. **55**: 598–612.

43. **Ereskovsky, A. V.,** A new species of Oscarella (Demospongiae: Plakinidae) from the Western Sea of Japan. *Zootaxa* 2006. **1376**: 37–51.

44. **Boute, N., Exposito, J.-Y., Boury-Esnault, N., Vacelet, J., Noro, N.,** *et al.* Type IV collagen in sponges, the missing link in basement membrane ubiquity. *Biol Cell* 1996. **88**: 37–44.

45. **Humbert-David, N. and Garrone, R.,** A six-armed, tenascin-like protein extracted from the Porifera Oscarella tuberculata (Homosclerophorida). *Eur J Biochem* 1993. **216**: 255–260.

46. **Exposito, J.-Y., Cluzel, C., Garrone, R. and Lethias, C.,** Evolution of collagens. *Anat Rec* 2002. **268**: 302–316.

47. **Ereskovsky, A. V. and Tokina, D. B.,** Asexual reproduction in homoscleromorph sponges (Porifera; Homoscleromorpha). *Mar Biol* 2007. **151**: 425–434.

48. **Boury-Esnault, N., Ereskovsky, A. V., Bézac, C. and Tokina, D. B.,** Larval development in Homoscleromorpha (Porifera, Demospongiae). *Invert Biol* 2003. **122**: 187–202.

49. **Keller, R., Davidson, L. A. and Shook, D. R.,** How we are shaped: the biomechanics of gastrulation. *Differentiation* 2003. **71**: 171–205.

50. **Morris, P. J.,** The developmental role of the extracellular-matrix suggests a monophyletic origin of the kingdom animalia. *Evolution* 1993. **47**: 152–165.

51. **Ereskovsky, A. V. and Boury-Esnault, N.,** Cleavage pattern in Oscarella species (Porifera, Demospongiae, Homoscleromorpha), transmission of maternal cells and symbiotic bacteria. *J Nat Hist* 2002. **36**: 1761–1775.

52. **Ereskovsky, A. V., Tokina, D. B., Bézac, C. and Boury-Esnault, N.,** Metamorphosis of Cinctoblastula larvae (Homoscleromorpha, Porifera). *J Morphol* 2007. **268**: 518–528.

53. **Gaino, E., Burlando, B. and Buffa, P.,** Structural and ultrastructural aspects of growth in Oscarella lobularis (Porifera, Demospongiae). *Growth* 1987. **51**: 451–460.

54. **Ereskovsky, A. V. and Gonobobleva, E. L.,** New data on embryonic development of Halisarca dujardini Johnston, 1842 (Demospongiae, Halisarcida). 2000. *Zoosystema* 22: 355–368.

55. **Gonobobleva, E. L. and Ereskovsky, A. V.,** Metamorphosis of the larva of Halisarca dujardini (Demospongiae, Halisarcida). *Bull Inst Roy Sci Nat Belgique, Biol* 2004. **74**: 101–115.

56. **Franzen, W.,** Oogenesis and larval development of Scypha ciliata (Porifera, Calcarea). *Zoomorphology* 1988. **107**: 349–357.

57. **Leys, S. P. and Eerkes-Megrano, D.,** Gastrulation in calcareous sponges: in search of Haeckel's gastraea. *Integr Comp Biol* 2005. **45**: 342–351.

58. **Topsent, E.,** Etude monographique des spongiaires de France. II. Carnosa. *Arch Zool expér gén* 1895. **3**: 493–590.

59. **Boury-Esnault, N., Solé-Cava, A. M. and Thorpe, J. P.,** Genetic and cytological divergence between colour morphs of the Mediterranean sponge Oscarella lobularis Schmidt (Porifera, Demospongiae, Oscarellidae). *J Nat Hist* 1992. **26**: 271–284.

60. **Kornprobst, J. M.,** Spongiaires (Eponges). In: Kornprobst, J. M. editor. Substances naturelles d'origine marine: chimiodiversité, pharmacodiversité, biotechnologie. Paris, Lavoisier Publisher, 2005. pp. 601– 684.

61. **Aiello, A., Fattorusso, E., Magno, S., Mayol, L. and Menna, M.,** Isolation of two novel 5 alpha,6 alpha -epoxy-7-ketosterols from the encrusting demospongia Oscarella lobularis. *J Nat Prod* 1990. **53**: 487–491.

62. **Aiello, A., Fattorusso, E., Magno, S. and Menna, M.,** Isolation of five new 5-hydroxy-6-keto-7 sterols from the marine sponge Oscarella lobularis. *Steroids* 1991. **56**: 337–340.

63. **Loukaci, A., Muricy, G., Brouard, J. P., Le Guyot, M., Vacelet, J.,** *et al.* Chemical divergence between two sibling species of Oscarella (Porifera) from the Mediterranean sea. *Biochem Syst Ecol* 2004. **32**: 893–899.

64. **Ivanisevic, J., Thomas, O. and Pérez, T.,** Secondary metabolism of the sponge group Homoscleromorpha: diversity and variation of its expression in relation with biotic and abiotic factors. In 5th European conference on marine natural products. 2007. p. 170.

65. **Efremova, S. M., Ereskovsky, A. V., Mukhina, Y. I., Willenz, P. and Boury-Esnault, N.,** Comparative study on spermatogenesis in the Homoscleromorpha. In 7th international sponge symposium. Biodiversity, innovation, sustainability. Buzios, Brazil, Rio de Janeiro, 2006. Ser. Livros 16, p. 70.

66. **Gaino, E., Burlando, B., Buffa, P. and Sara, M.,** Ultrastructural study of spermatogenesis in Oscarella lobularis (Porifera, Demospongiae). *Int J Invert Rep Dev* 1986. **10**: 297–305.

67. **Boury-Esnault, N. and Jamieson, B. G. M.,** 1. Porifera. In: Adiyodi, K. G. and Adiyodi, R. G. editors. Reproductive biology of invertebrates. Part A: Progress in male gamete ultrastructure and phylogeny. New Delhi. Oxford: IBH Publishing Co. 9, 1999. pp. 1–41.

68. **Ereskovsky, A. V.,** Comparative embryology of Sponges (Porifera). Saint-Petersburg, Saint-Petersburg University Press, 2005.

69. **Gaino, E., Burlando, B. and Buffa, P.,** Contribution to the study of egg development and derivation in Oscarella lobularis (Porifera, Demospongiae). *Int J Invert Rep Dev* 1986. **91**: 297–305.

70. **Ereskovsky, A. V.,** Comparative embryology of Sponges and its application for poriferan phylogeny. *Boll Mus Ist Biol Univ Genova* 2004. **68**: 301– 318.

71. **Meewis, H.,** Contribution à l'étude de l'embryogénèse des Myxospongidae: Halisarca lobularis (Schmidt). *Arch Biol Liège* 1938. **50**: 1–66.

72. **Kawamura, K. and Fujiwara, S.,** Cellular and molecular characterization of transdifferentiation in the process of morphallaxis of budding tunicates. *Semin Cell Biol* 1995. **6**: 117–126.

73. **Hoffman, D. K. and Honegger, T. G.,** Bud formation and metamorphosis in Cassiopea andromeda (Cnidaria: Scyphozoa): a developmental and ultrastructural study. *Mar Biol* 1990. **105**: 509–518.

74. **Peterson, K. J. and Sperling, E. A.,** Poriferan ANTP genes primitively simple or secondarily reduced? *Evol Dev* 2007. **9**: 405–408.

75. **Garcia-Fernàndez, J.,** The genesis and evolution of Homeobox gene clusters. *Nat Rev Genet* 2005. **6**: 881–892.

76. **Chourrout, D., Delsuc, F., Chourrout, P., Edvardsen, R. B., Rentzsch, F.,** *et al.* Minimal ProtoHox cluster inferred from bilaterian and cnidarian Hox complements. *Nature* 2006. **442**: 684–687.

77. **Kamm, K. and Schierwater, B.,** Ancient complexity of the non-Hox ANTP gene complement in the anthozoan Nematostella vectensis. Implications for the evolution of the ANTP superclass. *J Exp Zool (Mol Dev Evol)* 2006. **306B**: 589–596.

78. **Putnam, N. H., Srivastava, M., Hellsten, U., Dirks, B., Chapman, J.,** *et al.* Sea Anemone genome reveals ancestral Eumetazoan gene repertoire and genomic organization. *Science* 2007. **317**: 86–94.

79. **Jacobs, D. K., Nakanishi, N., Yuan, D., Camara, A., Nichols, S. A., Hartenstein, V.,** Evolution of sensory structures in basal metazoa. *Integr Comp Biol* 2007. **47**: 712–723.

80. **Adell, T., Nefkens, I. and Müller, W. E. G.,** Polarity factor 'Frizzled' in the demosponge Suberites domuncula: identification, expression and localization of the receptor in the epithelium/pinacoderm. *FEBS Lett* 2003. **554**: 363–368.

81. **Lapébie, P., Gazave, E., Renard-Deniel, E., Boury-Esnault, N. Perez, T.,** *et al.* Wnt pathway putative functions in the metazoan common ancestor. Contribution of a sponge model: Oscarella lobularis (Homoscleromorpha). In 10th evolutionary biology meeting at Marseilles 2006, September. 2006. pp. 20–22.

82. **Manuel, M. and Le Parco, Y.,** Homeobox gene diversification in the calcareous sponge, Sycon raphanus. *Mol Phyl Evol* 2000. **17**: 97–107.

83. **Wang, X. and Lavrov, D. V.,** Mitochondrial genome of the Homoscleromorph Oscarella carmela (Porifera, Demospongiae) reveals unexpected complexity in the common ancestor of sponges and other animals. *Mol Biol Evol* 2007. **24**: 1–11.

84. **Aouacheria, A., Geourjon, C., Aghajari, N., Navratil, V., Deleage, G.,** *et al.* Insights into early extracellular matrix evolution: spongin short chain collagen-related proteins are homologous to basement membrane type IV collagens and form a novel family widely distributed in invertebrates. *Mol Biol Evol* 2006. **23**: 2288–2302.