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The cellular and molecular bases of the sponge stem cell systems underlying reproduction, homeostasis and regeneration

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ABSTRACT The evolution of multicellular organisms is generally thought (and seems likely) to have been accompanied by the evolution of a stem cell system. Sponges, some of the early-evolved metazoans, have totipotent/pluripotent stem cells.Thus, uncovering the cellular and molecular bases of the sponge stem cells will not only be crucial for understanding the ancestral gene repertoire of animal stem cells, but will also give us clues to understanding the evolution of molecular mechanisms for maintaining multipotency (pluripotency) and differentiation ability during animal evolution. Sponges (Porifera) are a large phylum that includes an enormous number of species, whose cellular compositions and life cycles show striking variations. In the last decade, methodologies for molecular studies and sequencing resources have dramatically advanced and made it possible to clearly define stem cells in sponges in cellular and molecular terms. In this review, together with recent studies of sponges in various classes, the following issues will be discussed: i) recent findings that revealed that the previously proposed model that "archeocytes and choanocytes are the two types of stem cells" originally based on work in demosponges can be applied as a unified view of the stem cell system in sponges that have various cellular organizations, ii) the fact that sponge cells are more plastic than previously thought, as shown by recent studies of sponge regeneration both from dissociated cells and upon injury, and iii) the importance of transdifferentiation in sponge stem cell systems and regeneration.

KEY WORDS: *Archeocyte, choanocyte, epithelial mesenchymal transition, regeneration, transdifferentiation*

Cellular organization of four classes of sponges

Since different sponge species show wide variations of cellular composition and life cycle, in order to understand stem cells in sponges, one has to organize their knowledge in ways that depend on the model system(s) of sponge(s) used for study, including considerations such as i) the class of sponges to which the specimens studied belong, ii) whether the specimens are adult, or embryonic, or juvenile sponges, and whether they have developed through sexual or asexual reproduction, and iii) whether normal developmental processes or processes that occur during regeneration are examined.

Sponges (Porifera) are divided into four classes: Demospongiae, to which more than 90% of sponge species belong, Hexactinellida (Glass sponges), Calcarea, and Homoscleromorpha. The body of a sponge is supported by either the inorganic skeleton (mainly composed of mineralized silica ($SiO_2.nH_2O$) in demosponges, hexactinellids and homoscleromorphs), or calcium carbonate ($CaCo_3$) in calcareous sponges (Uriz, 2006). In some demosponges and homoscleromorphs, the body is supported by collagen fibrils in the inner body space (mesohyl). Modern molecular phylogenetic studies have suggested that demosponges and hexactinellids are sister clades, as are calcareous sponges and homoscleromorphs (Fig. 1A)(Philippe *et al.*, 2009).

Sponges do not have germline stem cells; instead, gametes are produced from two types of somatic stem cells: archeocytes (amoeboid cells in the inner body space) and/or choanocytes (see details in "Archeocytes" and "Choanocytes" sections, respectively (reviewed in Leys and Ereskovsky, 2006; Ereskovsky, 2010). In demosponges, oocytes are generally derived from archeocytes,

Abbreviations used in this paper: GMP gene, germline multipotency program gene; EMT, epithelial-mesenchymal transition.

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and sperm from choanocytes. In hexactinellids, oocytes are derived from archeocyte-like cells. In calcareous sponges (which do not have archeocytes) and homoscleromorphs (classically thought not to have archeocytes), both oocytes and sperm are derived from choanocytes. Thus, these two types of cells (archeocytes and choanocytes) have totipotency, at least during sexual reproduction. In vertebrates, "totipotent" and "pluripotent" stem cells are generally distinguished as "stem cells that can give rise to all types of cells, including extra-embryonic cells" and "stem cells that can give rise to any cell type except extra-embryonic cells", respectively (Mitalipov and Wolf, 2009). In this review, "totipotent" and "pluripotent" stem cells in invertebrates, including sponges, are used to designate "cells that can generate all types of cells" and "cells that can generate multiple types of cells" respectively.

Recent progress in defining and understanding the cellular system of stem cells in each sponge class has led to the realization that the cellular organization of each class of sponges is key to understanding the stem cell systems both in the various classes of sponge as well as in sponges overall. As filter feeders, all classes of sponges have well-developed aquiferous systems constituted

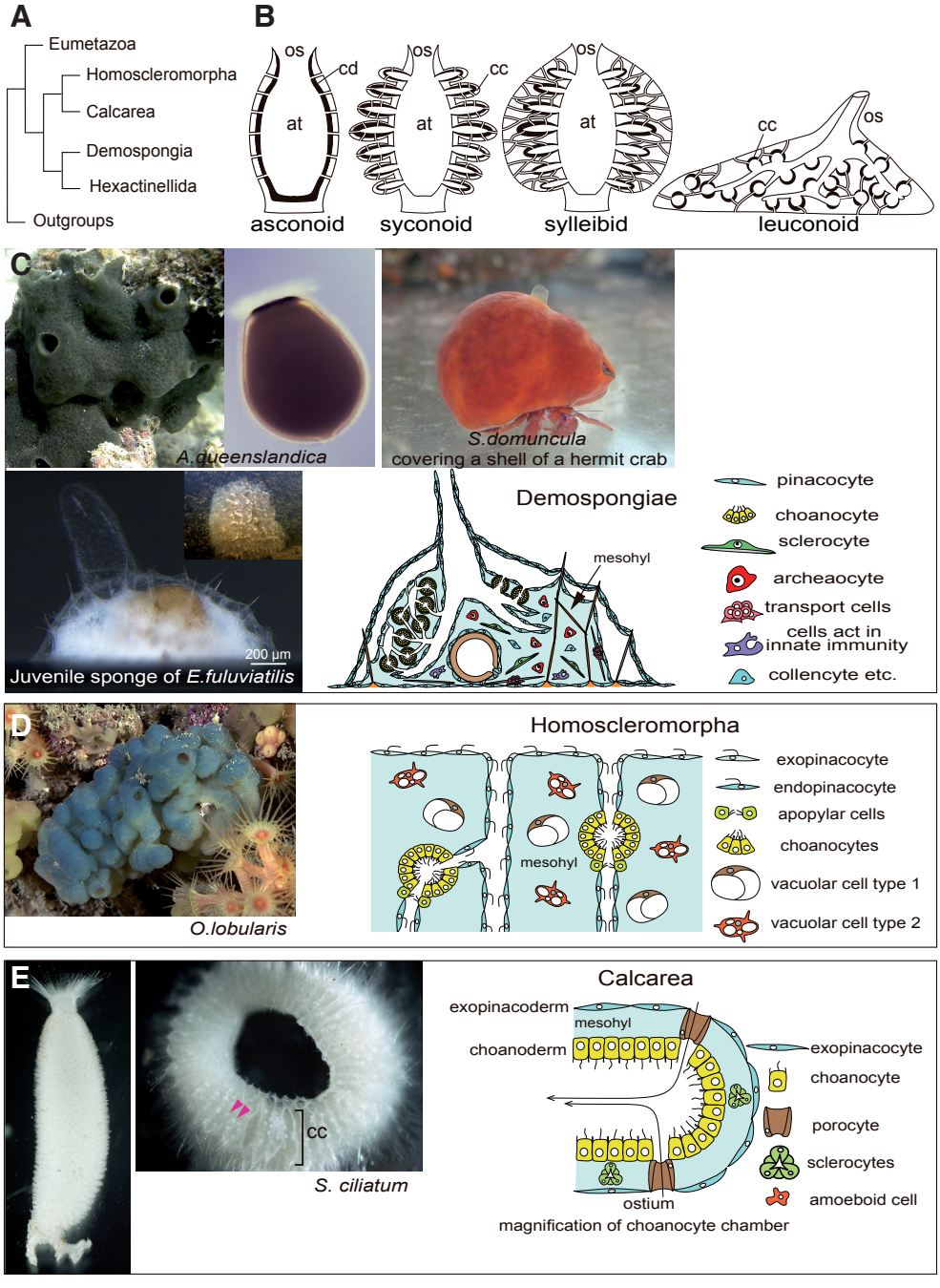

Fig. 1. Schematic drawings of different types of sponge body organization and cell types. (A) *Phylogeny of 4 sponge classes.* **(B)** *Four main types of aquiferous system of sponges. In sponges with an asconoid-type aquiferous system, the internal cavities are entirely lined with choanocytes. In sponges with a syconoid-type aquiferous system, elongated choanocyte chambers pass through the whole sponge body. In sponges with a sylleibid-type aquiferous system, the elongated choanocyte chambers are arranged radially around an invagination of the atrium cavity. In sponges with a leuconoid-type aquiferous system, choanocyte chambers are lined along the network canal system. at: atrium, cc: choanocyte chamber, cd: choanoderm of asconoid, os: osculum (After Hyman 1940 and Ereskovsky 2010).* **(C-E)** *Examples of model sponges.* **(C)** *Examples of model demosponges used for studies of stem cells. Upper panel, from left: marine sponges,* Amphimedon queenslandica *(Adult and larva, courtesy of Dr. Bernard Degnan),* Suberites domuncula *covering a shell of a hermit crab of the genus Paguristes (courtesy of Dr. Roger Revilla-i-Domoingo). Lower panel, from left: Freshwater sponge,* Ephydatia fluviatilis, *juvenile sponge hatched from a gemmule (asexual reproduction), insert: adult sponge. Right: vertically sectioned view of a simplified cellular organization of gemmule-hatched juvenile sponge of* E. fluviatilis. *Note that there are several additional cell types (such as apopylar cells, porocytes and several types of collagen-expressing cells) that are not described in this simplified diagram. Furthermore, the presence of cell types that were not classically described is suggested by their gene expression profiles.* **(D)** *Homoscleromorphs:* Oscarella lobularis. *Live adult sponge (left, courtesy of Dr. Emmanuel Renard, and Christian Marschal (Institut Méditerrannéen de Biodiversité et Ecologie Marine et Continentale)). Right: Simplified diagram of the cellular organization of adult* O. lobularis *(After Ereskovsky* et al., *2015).* **(E)** *Calcareous sponges:* Sycon ciliatum. *Adult sponge (left) and its transverse section (middle) showing radially arranged choanocyte chambers with embryos (magenta arrowheads, cc: choanocyte chamber). Courtesy of Dr. Maja Adamska. Right: Simplified diagram of the cellular organization of* S. ciliatum *(after Adamska* et al., *2011).*

by several types of inner epithelial cells (reviewed in (Ereskovsky, 2010; Simpson, 1984). They are endopinacocytes (endodermal epithelial cells) and choanocytes (water-flow-generating food entrapping cells) forming "chambers" in syconoid, sylleibid and leuconoid-type sponges, or "a continuous layer lining a single cavity" in asconoid-type sponges (Fig.1B). Several types of specialized cells are located in the junction between the endopinacoderm and choanocyte chamber. The term "choanoderm" is used to designate a continuous layer lining a single cavity in asconoid-type sponges, and to designate tissues composed solely of choanocytes lining the choanocyte chambers in syconoid, sylleibid, and leuconoid-type sponges. Choanocyte chambers and choanoderm in asconoid-type sponges are the digestive organ of sponges (Ereskovsky, 2010).

The cellular organization of the inner-body space (mesohyl) differs depending on the class of sponge (Fig.1 C-E). Demosponges have well-developed mesohyl containing various types of cells, which are traditionally defined mostly based on the cell morphology (Flg.1C) (reviewed in Ereskovsky, 2010; Simpson, 1984). These types of cells include types such as archeocytes (which are thought to be totipotent stem cells), several types of collagen-expressing cells, and spicule-producing cells (sclerocytes). In addition, based on gene expression analyses, there seemed to be more types of cells that could not be distinguished based on their cell morphology in earlier studies (Nakayama *et al.,* 2015; Funayama *et al.,* 2005b). The body of hexactinellids consists largely of a single syncytial tissue, but cells including archeocyte-like cells are reported to reside in the mesohyl (Leys *et al.*, 2007). Homoscleromorphs and calcareous sponges have fewer types of cells in the mesohyl compared to demosponges. Homoscleromorphs have two types of vacuolar cells in a thin layer of their mesohyl (Fig. 1D). Calcareous sponges have sclerocytes, and rarely amoeboid cells, in their mesohyl (Flg.1E) (reviewed in Adamska *et al.*, 2011; Adamska, 2016). Neither of these classes of sponge has cells that possess archeocyte cell morphology. Thus, all of the types of cells in calcareous sponges and homoscleromorphs have traditionally been thought to be produced from choanocytes (Simpson, 1984). Accordingly it has been suggested that choanocytes play roles as stem cells in these two classes of sponges. It should be noted that a recent study of gene expression suggested that vacuolar cells type 2 in homoscleromorphs might have multipotency (see details in the section "Ancestral gene repertoire of animal stem cells").

Archeocytes

Nearly all sponge stem cell biology based on classical descriptions in which cell behaviors are inferred based on detailed ultrastructural analysis. Archeocytes of demosponges are totipotent somatic stem cells, as shown by earlier ultrastructural studies (reviewed in (Ereskovsky, 2010; Simpson, 1984) and more recent studies (described below). To date, only studies using freshwater demosponge, *Ephydatia fluviatilis,* revealed archeocytes' direct differentiation into multiple types of cells through characterizing gene expression pattern and profiles (Funayama *et al.,* 2010, Okamoto, 2012, Funayama 2010, 2013). In this section, i) general cell morphological features of archeocytes, ii) studies of archeocytes in freshwater demosponges that were enabled by establishing sequence resources and methodologies, iii) the possible extension to all sponge species of the model that archeocytes and choanocytes constitute the stem cell system of demosponges, and iv) the ancestral gene repertoire of animal stem cells as revealed by comparative transcriptome analysis, will be discussed.

Cell morphology features of archeocytes

In most species, archeocytes are defined by their morphological features. Thus, here "archeocytes" is used to designate cells with archeocytes' morphological features (Fig. 2): large in size compared to other cells, containing a large nucleus with a single large nucleolus (Fig. 2 A,B,E, magenta-bordered white arrowheads), mitotically active (Fig. 2C), motile, and containing numerous vitelline platelets during the early developmental stages of gemmule hatching (Funayama, 2010; Simpson, 1984). In contrast to most stem cells in other organisms, which generally stay in a particular niche, archeocytes are highly motile cells in the mesohyl, and thus it is unusually difficult to trace archeocytes' differentiation and self-

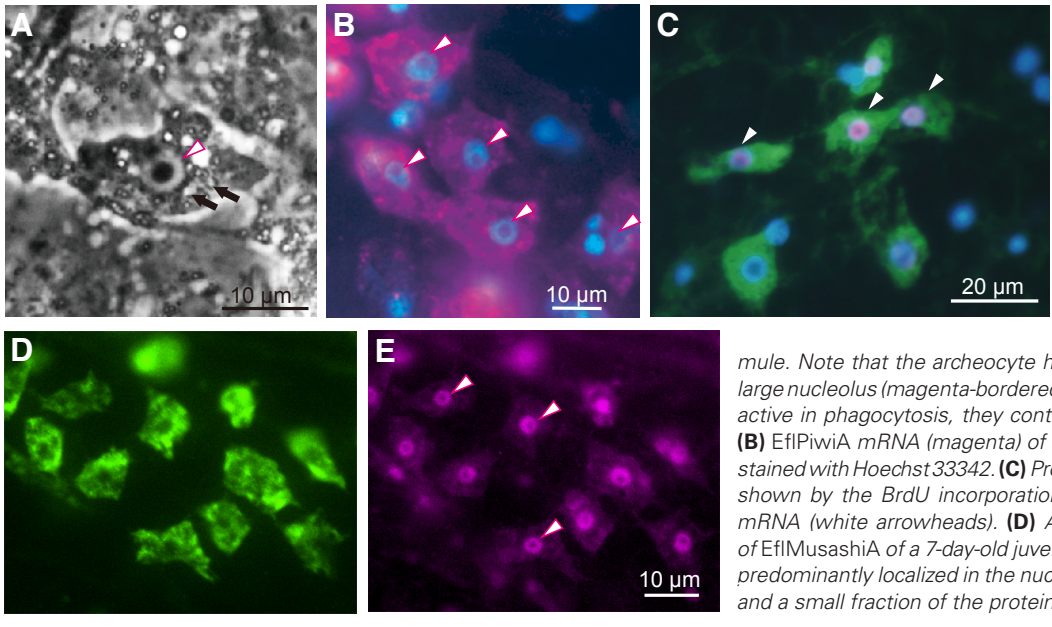

Fig. 2. Archeocytes, which are identified by their morphology or specific expression of *EflpiwiA* and *EflMusashiA* in *E. fluviatilis*. (A) *Archeocyte in mesohyl of a 7-day-old juvenile sponge hatched from a gemmule. Note that the archeocyte has a large nucleus containing a single large nucleolus (magenta-bordered white arrowhead). As archeocytes are active in phagocytosis, they contain many phagosomes (black arrows).* **(B)** EflPiwiA *mRNA (magenta) of a 7-day-old juvenile sponge. Nuclei are stained with Hoechst 33342.* **(C)** *Proliferative ability of archeocytes is clearly shown by the BrdU incorporation of archeocytes expressing* EflPiwiA *mRNA (white arrowheads).* **(D)** *Archeocyte-specific mRNA expression of* EflMusashiA *of a 7-day-old juvenile sponge.* **(E)** *EflMusashiA protein is predominantly localized in the nucleus (black-bordered white arrowhead) and a small fraction of the protein is localized in the cytoplasm.*

renewal. In addition, the morphology of archeocytes seems to be variable in single sponge individual, probably because of archeocytes' phagocytic activity. Thus, to elucidate the stem cell system of sponges and the molecular bases of stem cells, it was crucial to identify cell types, especially archeocytes and choanocytes, in terms of expressed genes.

Definition of archeocytes according to their gene expression

Using gemmule hatching of a freshwater demosponge, *E. fluviatilis,* pioneering studies to identify archeocytes was achieved. The success in identifying archeocytes and their differentiation by monitoring their gene expression was a breakthrough for understanding the molecular mechanisms of sponge stem cell biology (Funayama *et al.*, 2010). Gemmule hatching is one type of asexual reproduction of sponges, and is mostly used in freshwater sponges. It is the development of a fully functional juvenile sponge from solely a group of thousands of archeocytes that are originally packed inside a small particle of collagenous coat (gemmule coat). Gemmule hatching of several species (mostly *Ephydatia fluviatilis*, *Ephydatia muelleri* and *Spongilla lacustris*) has been one of the well-studied model systems for the cell biology of demosponges (Simpson, 1984). Gemmule hatching is a highly ordered process. In brief, "resting archeocytes in the gemmule" (thesocytes) exit from the resting state and become "archeocytes", and then these archeocytes migrate out from the gemmule coat, proliferate and differentiate to form a functional juvenile sponge around the gemmule coat (reviewed in Funayama, 2010; Simpson, 1984). Since all types of cells constructing the sponge body are derived from the archeocytes originally packed inside the gemmule coat, gemmule hatching is an excellent system for investigating the sponge stem cell system and its regulatory mechanisms during the development of fully functional juvenile sponges.

In the last 15 years, dramatic advances in cell identification and isolation techniques and sequence resources have been achieved in sponges. One of the pioneering studies was done using gemmule hatching of *E. fluviatilis*, and its investigation was made possible by the establishment of methods for high-resolution whole-mount *in situ* hybridization (WISH), which can detect mRNA expression in single cells (Funayama *et al.*, 2005a; Mohri *et al.*, 2008), immunohistochemistry (Okamoto *et al.*, 2012), FACS sorting of choanocytes and archeocytes by ingenious methods of specific fluorescent labeling of each type of cell (Alié *et al.*, 2015; Funayama *et al.*, 2005a), EST sequencing, and transcriptome and proteome analysis (Alié *et al.*, 2015; Funayama *et al.*, 2005a). Archeocytes were identified based on the specific mRNA expression of certain genes (*EflpiwiA*, *EflpiwiB* and *EflMusashiA*) in the cells with archeocyte morphology and on other specific features of the cells, namely, the abilities to directly differentiate into multiple types of cells, and to proliferate (likely by symmetric cell division) (Fig. 2 B-E) (Funayama *et al.*, 2010; Okamoto *et al.*, 2012). For use in tracing the differentiation of archeocytes and identifying types of differentiated cells, a number of lineage-specific genes of various cell types were identified. Such as for choanocytes (Funayama *et al.*, 2005a), sclerocytes (spicule-producing cells) (Funayama *et al.,* 2005b; Mohri *et al.*, 2008) and cells thought to act in innate immunity Funayama *et al.,* 2005b). More recently, lineage-specific genes were identified for basopinacocytes, spicule-transport cells and collagen-expressing cells in the mesohyl (Nakayama *et al.*, and Takagi and Funayama, manuscripts in preparation) together with some yet-unidentified cells (Funayama, unpublished). Similarly, advances in sequence resources have been achieved for archeocytes and choanocytes (Alié *et al.*, 2015; Funayama, 2013), (see details in section "Ancestral gene repertoire of animal stem cells").

Proposed model of a stem cell system consisting of archeocytes and choanocytes, and its possible extension to all sponge species

In addition to archeocytes having been identified as multipotent stem cells in demosponges based on monitoring of their gene expression, the possible totipotency (pluripotency) of choanocytes is also suggested. During the ordinary differentiation process of archeocytes into various cell types, *EflpiwiA* and *EflpiwiB* (from here on denoted as *EflpiwiA/B*) expression eventually ceases while lineage-specific gene expression is induced (Fig. 3 A-C). In contrast, *EflpiwiA/B* mRNA expression is exceptionally maintained in choanocytes (Fig. 3 D,E) (Funayama *et al.*, 2010). Early ultrastructural

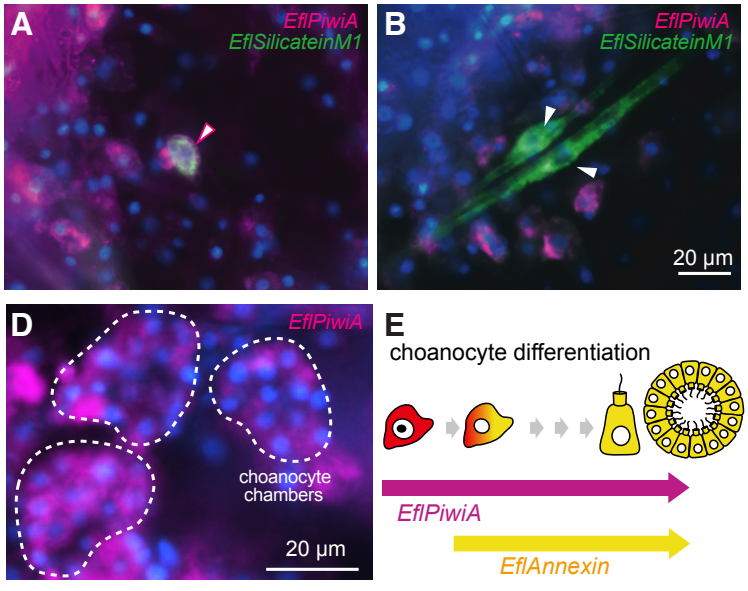

Fig. 3. Continuous expression of *EflPiwiA* mRNA in mature choanocytes forming chambers suggests the possible pluripotency of choanocytes. **(A,B)** *Gene expression transition during the process of sclerocyte differentiation and spicule production. Magenta,* EflPiwiA*; green,* EflSilicatein-M1 *(sclerocyte-lineage-specific gene); blue, nuclei were detected using Hoechst 3334.* **(A)** *An archeocyte committed to the sclerocyte lineage coexpresses* EflPiwiA *(magenta-bordered arrowhead) and* EflSilicatein-M1*.* **(B)** *Spicule-containing sclerocytes express only* EflSilicatein-M1*, not* EflPiwiA *mRNA (white arrowheads).* **(C)** *Schematic drawing of the gene transition during differentiation of archeocytes to sclerocytes.* **(D)** EflPiwiA *mRNA is detected in all choanocytes in mature-sized chambers in functional juvenile sponges.* **(E)** *Schematic drawing of the gene expression during archeocyte differentiation to the choanocyte lineage. Modified from Funayama* et al.*, Evo. Devo. 2010 12: 275-287.*

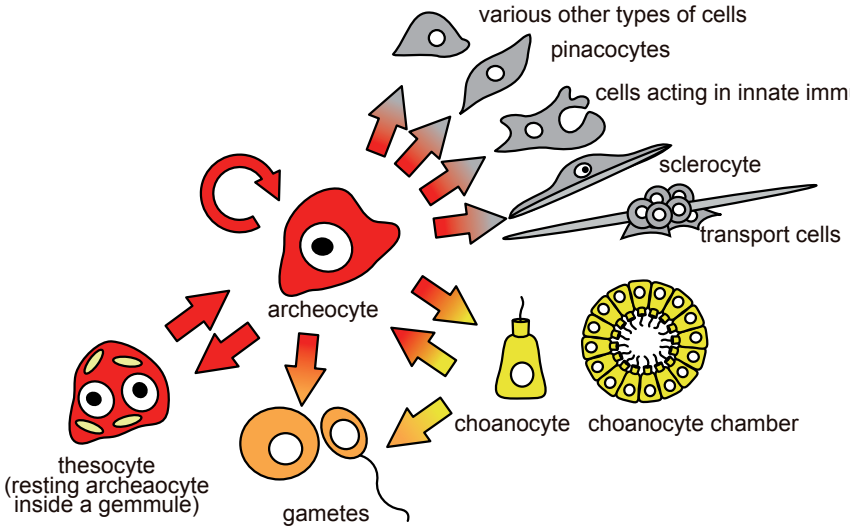

Fig. 4. Possible model of the stem cell system in demosponges. *Archeocytes are active stem cells that differentiate into various types of cells (including sclerocytes, spicule-transport cells, cells that presumably act in innate immunity, and choanocytes) and also undergo self-renewal. In sexual reproduction, gametes are derived from archeocytes and/or choanocytes. Choanocytes transform into archeocytes under specific circumstances. In most species of demosponges investigated thus far, oocytes are derived from archeocytes and sperm are derived from choanocytes. Archeocytes have a special form when they are in the resting state inside a gemmule coat (thesocytes) of freshwater sponges. Modified from Funayama* et al.*, Evo. Devo. 2010 12: 275-287.*

studies also showed that the choanocyte in demosponges has cellular plasticity, or possibly totipotency (pluripotency), in certain conditions as described below. Those earlier studies included studies showing that: a) choanocytes transform into pinacocytes (reviewed in Simpson, 1984), b) choanocytes undergo EMT (epithelial mesenchymal transition) to become archeocyte-like amoeboid cells upon disruption of sponge tissue and subsequent remodeling (Diaz, 1977) and asexual reproduction (Connes *et al.*, 1974), and c) choanocytes produce gametes (Ereskovsky, 2010; reveiwed in Leys and Ereskovsky, 2006; Riesgo and Maldonado, 2009). In addition, a precise recent study in a marine demosponge, *Halisarca dujardini*, using EM (electron microscopy) and EdU incorporation experiments also showed that choanocytes undergo EMT and transdifferentiate (de-differentiate and re-differentiate) to another type of epithelial cells (exopinacocytes) during regeneration after injury. It was shown that choanocytes, archeocytes and rarely endopinacocytes were the three main sources of exopinacocytes that covered a wound (Borisenko *et al.*, 2015). The importance of transdifferentiation in sponges will be discussed in detail in the section "Transdifferentiation". Based on these earlier histological studies indicating the totipotency (pluripotency) of archeocytes and choanocytes, and the specific expression of *EflPiwiA/B* in archeocytes and choanocytes in juvenile of *E. fluviatilis*, we proposed a model that the stem cell system of demosponges is constructed with two types of cells, archeocytes and choanocytes (Fig. 4) (Funayama *et al.*, 2010). The fact that archeocytes and choanocytes share certain numbers of genes that are known to have roles in maintaining pluri or multiporency was further revealed by our cell-type specific transcriptomic analysis (Alie *et al.,* 2015).

We further suggested that the possible model of stem cell system we proposed for demosponges could be extended to all classes of sponges, and that which type of cells (archeocytes or choanocytes) mainly acts as stem cells depends on the different cell organization of each class of sponges. In demosponges, archeocytes generally act as stem cells, whereas choanocytes are likely to exert their totipotency (pluripotency) in particular circumstances, such as reproduction and regeneration, as described above (additional recent studies are discussed in the section "Regeneration"). In hexactinellids, clusters of archeocyte-like cells in mesohyl are considered very likely to be pluripotent cells that produce other types of cells (Leys *et al.*, 2007). In calcareous sponges and homoscleromorphs, which do not have archeocytes, choanocytes are most likely be the totipotent (pluripotent) stem cells, since they are known to act as the source of other types of cells (see details in "Choanocytes, possible totipotent (pluripotent) stem cells in calcareous sponges and homoscleromorphs").

Ancestral gene repertoire of animal stem cells

About a decade ago was a time of excitement when it started to be revealed that genes that had been originally identified as being expressed in germline cells in *Drosophila* (namely, *piwi*, *vasa*, and *nanos*) are expressed specifically in pluripotent somatic stem cells that have the ability to give rise to germ cells in some highly regenerative animals (hydra, hydractinia and flatworms) (hydra: Juliano *et al.*, 2014; reviewed in Bosch, 2009; Bosch *et al.*, 2010; Juliano and Wessel, 2010; David, 2012; Hobmayer *et al.*, 2012; Nishimiya-Fujisawa and Kobayashi, 2012, hydractinia: Rebscher *et al.*, 2008, reviewed in Plickert *et al.*, 2012, flatworms: Shibata *et al.*, 1999; Wagner *et al.*, 2012; reviewed in Gehrke and Srivastava, 2016; Shibata *et al.*, 2010; Shibata *et al.*, 2012; Solana, 2013) The finding of *EflpiwiA/B* expression in archeocytes and choanocytes (see above) then revealed that that notion could be applied to an earlier-emerging metazoan, sponge. Based largely on those findings together with their own findings that homologues of *piwi*, *vasa* and *nanos* are expressed in multipotent precursors and germ cells in sea urchin embryo, Julliano and Wessel proposed the likely existence of a highly conserved germline multipotency program (GMP) that operates in both multipotent cells and germ cells (Juliano *et al.*, 2010; Juliano and Wessel, 2010).

Metazoans use stem cells for their development and tissue homeostasis. Thus, one important question is, what is the gene toolkit that was associated with the emergence of stem cells in a common ancestor of metazoans? Together with extensive studies about the genes expressed in the pluripotent stem cells of highly regenerative animals, especially cnidarians and flatworms (Hemmrich *et al.*, 2012; Solana *et al.*, 2012), recent studies of sponge stem cells opened a way to getting insight into the ancestral gene repertoire of animal stem cells and its evolution (Alié *et al.*, 2015) The number of genes considered to be involved in GMP might increase in the future based on further increases of our knowledge,

but the originally proposed genes were: *piwi*, *vasa*, *nanos*, *pumillio*, *tudor*, *bruno*, *germ cell-less* and *maelstrom* (Juliano *et al.*, 2010). Transcriptome analysis of flatworm neoblasts showed elevated expression of RNA-binding proteins that act in maintenance of stem–cell identity (Rouhana *et al.*, 2010; reveiwed in Shibata *et al.*, 2012). In pluripotent stem cells of hydra (interstitial stem cells, I cells), in addition to some of the GMP genes, it was revealed a set of transcription factors whose expression is conserved in later-evolved animals, including vertebrates (Boehm *et al.*, 2012; Hemmrich *et al.*, 2012) in addition to some of the GMP genes. We had to wait for the cell-type-specific transcriptome analysis of sponge stem cells to learn whether there is also similar conserved expression of such genes in sponges.

The successful isolation of archeocytes and choanocytes of juvenile sponges of *E. fluviatilis* enabled by the invention of methods to fluorescently label archeocytes (by staining of vitelline platelets) and choanocytes (by feeding fluorescent beads) enabled cell-type-specific transcriptome analysis of sponge cells for the first time (Alié *et al.*, 2015; Funayama *et al.,* 2005a, respectively). Transcriptome profiling of archeocytes and choanocytes made it possible to trace shared molecular signatures of these cells with those of flatworm and hydra stem cells (Alié *et al.*, 2015). 180 genes (orthology groups) were thus revealed to be ancestral stem cell genes shared by pluripotent (totipotent) somatic stem cells in flatworms, hydra, and sponges (archeocytes). As expected, the repertoire of 180 genes is strongly enriched in cell cycle, DNA replication and DNA repair genes. Regulators of DNA transcription are underrepresented among the ancestral stem cell genes. In sharp contrast, a wide range of RNA binding proteins were highlighted as genes that are highly expressed in archeocytes as well as included among ancestral stem cell genes. The expression of genes encoding GMP proteins (*piwi*, *vasa*, *bruno*, *pl-10*, and of all of the genes encoding Tudor domain, *ddx6*, and *mago-nashi*) and genes encoding RNA helicase and proteins involved in mRNA splicing is elevated in *Ephydatia* archeocytes (Fig. 5 A,B)(Alié *et al.*, 2015). GMP gene expression in totipotent (pluripotent) cells was further confirmed by a recent study in the homoscleromorph *O. loburalis*: mRNA expression of 11 GMP genes examined was analyzed in various stages of the life cycle: embryogenesis, non-sexual adult stage, and during spermatogenesis and oogenesis. Throughout these stages, choanocytes and germline cells expressed most of the 11 GMP genes examined, suggesting the pluripotency of choanocytes in Homoscleromorpha (Fierro-Constaín *et al.*, 2017).

Unexpectedly and interestingly, it was revealed that orthologues of several mammalian RNA binding proteins known to regulate the balance between ES cell self-renewal and differentiation are also highly expressed in *E. fluviatilis* archeocytes (25 out of 44 RNA binding proteins included in the ancestral stem cell genes). Thus, the comparative transcriptome analysis suggest that post-transcriptional regulators played a decisive role in the emergence of stem cells (Fig. 5C) (Alié *et al.*, 2015). Previous studies revealing that *EflpiwiA/B* and *EflMusashiA* are specifically expressed in *Ephydatia* archeocytes also support this notion (Funayama *et al.,* 2010; Okamoto *et al.*, 2012).

How can we explain the fact that the stem-cell specificity of so many RNA binding proteins has been conserved during metazoan evolution? Considering the recent understanding of fluctuating and oscillatory gene expression is the stem-cell state (Abranches *et al.*, 2014; Furusawa and Kaneko, 2012; Imayoshi and Kageyama, 2014) and the fact that these pluripotent (totipotent) stem cells of demosponges, hydra, planarian and ES cells actively proliferate, post-transcriptional regulation might be more advantageous than transcriptional regulation. Taking these findings all together, we proposed that the acquisition of stem cells in the last common metazoan ancestor was essentially supported by the use of eukaryotic RNA binding proteins as an efficient tool to control the balance between self-renewal and differentiation, and to orchestrate precisely timed gene regulation within coexisting lineages of multiple cell types (Alié *et al.*, 2015).

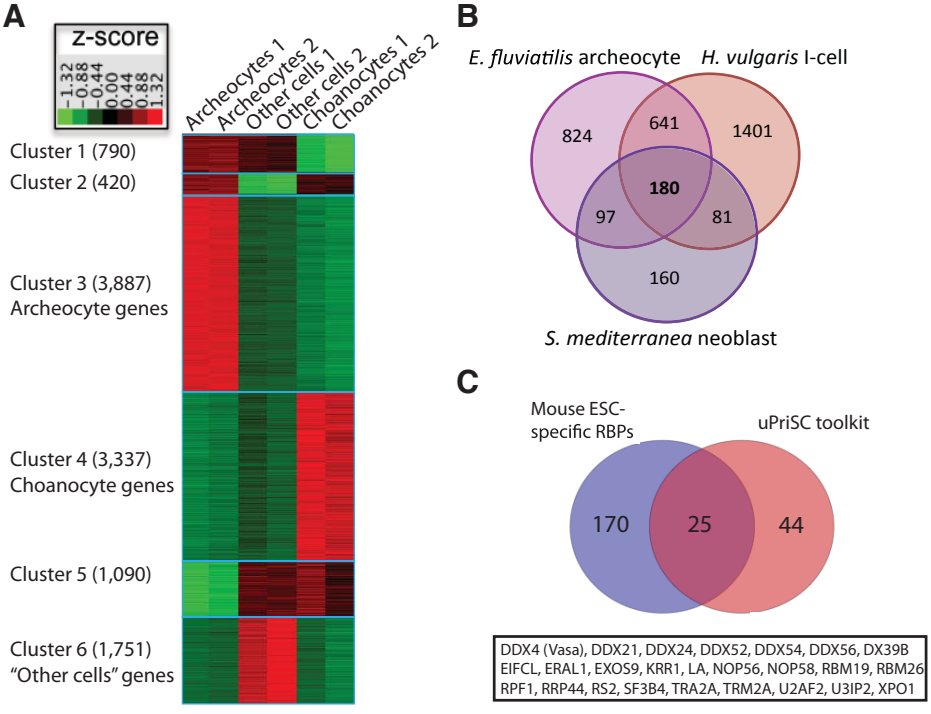

Fig. 5. Transcriptomic signature of archeocytes and unlimited primordial stem cells (uPriSCs). **(A)** *Heat map of the 11,275 genes differentially expressed between archeocytes, choanocytes, and other cells.* **(B)** *Venn diagram showing the 180 OrthoMCL groups overexpressed in representative uPriSCs, the interstitial stem cells of* Hydra vulgaris, *the neoblasts of* S. mediterranea *(planarian), and the archeocytes of juvenile sponges of* E. fluviatilis. *uPriSCs are adult stem cells that retain the potential to produce both the germ-line and at least several somatic cell types.* **(C)** *Venn diagram showing the 25 OrthoMCL groups corresponding to RBPs from mouse ESCs that belong to the uPriSC repertoire. Modified from Alié* et al., *Proc. Natl. Acad. Sci. USA 2015 E7093-E7100.*

Choanocytes

Totipotency (pluripotency) of choanocytes in certain situations in demosponges, and in two other classes of sponges (calcareous sponges and homoscleromorphs), was

strongly suggested by earlier precise histological studies. In this section, i) general cell morphological features of choanocytes, ii) earlier studies that suggested that choanocytes have the ability to exert possible totipotency (pluripotency) by transforming into cells that morphologically resemble archeocytes in demosponges in certain conditions, and iii) studies that suggest that choanocytes are the source of other types of cells in homoscleromorphs are introduced.

Cell morphological features of choanocytes

Sponges are filter-feeding organisms, and consequently one of their most important types of cells is the flagellated choanocyte. Choanocytes have a single flagellum, which is surrounded by a laterally connecting microvillous collar. Choanocytes form chambers that are one of the prominent structures of the sponge body (Figs. 1,6). Choanocytes construct the choanoderm (in asconoid or syconoid-type sponges) or face inward to form chambers that are connected to the canal system (in sylleibid-, or leuconoid-type sponges) (Fig. 1B). Choanocytes forming chambers along the canals generate unidirectional water currents by moving their flagella, ingest nutrients from the incurrent, and then pass these nutrients to other cells via vesicles.

The proliferative ability of choanocytes was clearly shown by histological analyses and EdU or BrdU incorporation studies. Choanocytes proliferate for maintaining chambers in adult sponges (Borisenko *et al.*, 2015; Ereskovsky, 2010; Ereskovsky *et al.*, 2015; De Goeij *et al.*, 2009; reviewed in Ereskovsky, 2010;Simpson, 1984). Additionally, it was also revealed that presumably immature choanocytes proliferate within a cluster of choanocytes during the process of chamber formation in gemmule hatching (Funayama *et al.,* 2005a; Tanaka and Watanabe, 1984; Weissenfels, 1981) and also during metamorphosis of larvae (Sogabe *et al.*, 2016).

It should be noted that choanocytes have also garnered attention from the point of view of the evolution of multicellular animals, because of their general morphological similarity to choanoflagellates. Several species of choanoflagellates form colonies, and the protists situated closest to metazoans in molecular phylogenetic trees (King, 2004; King and Carroll, 2001; Li *et al.*, 2008; Suga *et al.*, 2008). Both sponge choanocytes and choanoflagellates have a flagellum and microvilli surrounding this flagellum in a collar-like ring. It was revealed that sponges have conserved homologs of most vertebrate microvillar genes (Peña *et al.,* 2016). Some researchers have called attention to the fact that the similarity between these two collar-flagellum systems could be either evolutionarily maintained or convergent, based on detailed structural analysis showing several differences in the two collar-flagellum systems (Mah *et al.*, 2014). Actually, there are other protists that form colonies, such as capsaspora (Suga *et al.*, 2013) and the proposed model that the urmetazoan was something like a conglomerate of choanoflagellates-like cells is one of the possible models. At least, choanocytes possess some of the fundamental features of unicellular organisms, namely, the abilities to proliferate and to take up nutrients, Thus, comparison between the cell-type-specific transcriptome of choanocytes and the genomes of choanoflagellates might give us some clues for understanding the evolution of metazoans.

Choanocytes, possible totipotent (pluripotent) stem cells in calcareous sponges and homoscleromorphs

Studies using a homoscleromorph marine sponge, Oscallera lobularis

The body of homoscleromorph sponges consists of two epithelial layers, exopinacoderm and endopinacoderm + choanoderm, with a thin mesohyl layer that includes two types of vacuolar cells (type 1 and type 2, Flg.1D). So far as I know, until recently, choanocytes were thought to be the source of other types of cells in homoscleromorphs based solely on the fact that both female and male gametes

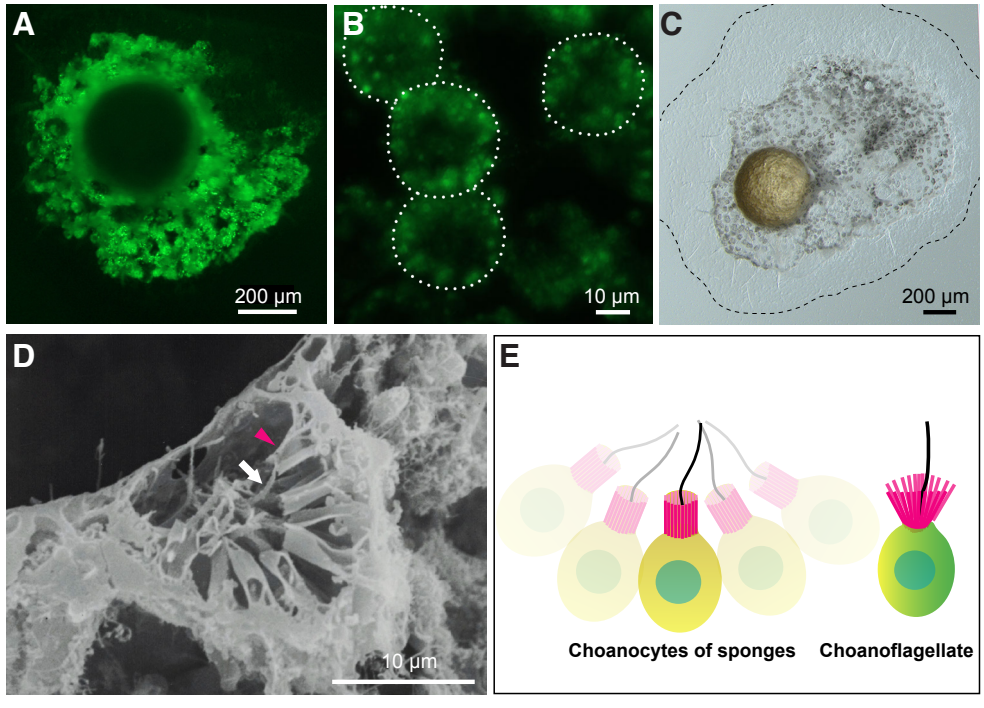

Fig. 6. Choanocytes of demosponges. (A-C) *Abundance of spherical choanocyte chambers. Choanocyte chambers of juvenile sponges (*E. fluviatilis*) hatched from gemmules were visualized by fluorescence of the beads fed to the sponges* **(A,B)** *or the ink fed to them* **(C)**. *At higher magnification, individual choanocyte chambers can be seen to be constructed from clustered structures containing green fluorescent beads. Aggregates of ingested fluorescent beads indicate individual choanocyte-forming spherical chambers* **(B)**. **(D)** *Scanning electron microscopic image of choanocytes forming a chamber (courtesy of Dr. Yoshiki Masuda). Flagellum (white arrow), microvillus collar (magenta arrowhead).* **(E)** *Schematic drawing shows that choanocytes of sponges and the choanoflagellate (a protist) share morphological features: a single flagellum (black) and microvilli (magenta) surrounding the flagellum like a collar. Note that the collar of choanocytes is generally cylindrical because the microvilli are laterally connected. Nucleus is shown in green. (A, B) are taken from Funayama* et al., *2005a Dev. Growth. Differ. 47:243-253.*

are derived from choanocytes (reviewed in Ereskovsky, 2010)). Recently, a study showing that GMP genes (*piwi*, *argonaute*, *vasa*, *nanos*, *pl10*, *tudor*, *pumillo*, *boule*) are expressed in choanocytes of adult *O. loburalis* during gametogenesis, regeneration after injury, and budding (asexual reproduction) strongly indicated the multipotency of choanocytes in these sponges (Fierro-Constaín *et al*., 2017). In addition, a recent precise ultrastructural analysis showed that choanocytes transform at least into ciliated exopinacocytes in the wound upon regeneration after injury (Ereskovsky *et al*., 2015); this will be further discussed in " Regeneration"). In addition to choanocytes, vacuolar cells type 2s have also been suggested to have multipotency based on their expression of most of the GMP genes (except for one of two *piwi* genes (*piwiA*)). The authors of that study suggested that these cells are archeocyte-like cells (Fierro-Constaín *et al*., 2017). It should be noted that the authors carefully avoided describing choanocytes or vacuolar cells type 2 (archeocyte-like cells) as stem cells, since the capacity of self-renewal of these two types of cells in *O. lobularis* had not been proved yet. Since sequence resource (transcriptome) and other advanced tools have now been established in *O. lobularis*, we can expect that the stem cell system and its molecular machineries in Homoscleromorpha will be clarified in the near future.

Studies using a calcareous sponge

Nowadays, it is accepted that both female and male gametes are derived from choanocytes in Calcareous sponges, an issue that was resolved after having been controversial for a long time (Gallissian and Vacelet, 1990; Lanna and Klautau, 2010, reviewed in Ereskovsky, 2010). By ultrastructural analysis, the cell plasticity of choanocytes was shown as choanocytes transdifferentiate into exopinacocytes during the regeneration of a part of body in the adult sponge (Ereskovsky *et al*., 2017). Although it is strongly implied that choanocytes might be the source of the other types of cells, the question of which cells are stem cells and their cellular and molecular bases in calcareous sponges are still open questions to be answered. Among calcareous sponges, *Sycon ciliatum* will be a powerful model system for stem cell biology, since sequence resources are abundant, covering the maturation of oocytes, fertilization, embryogenesis, swimming larvae, and the three parts of non-reproductive adults (top, middle and bottom of the body) (Adamska, 2016; Fortunato *et al*., 2016; Leininger *et al*., 2014). In addition, techniques of molecular biology have established in this sponge, and many types of cells can be identified by their gene expression profiles (Fortunato *et al*., 2012; Fortunato *et al*., 2016; Leininger *et al*., 2014; Voigt *et al*., 2014; reviewed in Adamska *et al*., 2011; Adamska, 2016). Furthermore, because *Sycon* has a vase-like shape with a clear body axis, and the body plan and the mRNA expression of developmental regulatory genes have been extensively studied in *S. ciliatum*, this would be an excellent model system to explore the cellular and molecular mechanisms of regeneration after amputation, i.e., how the lost part could be regenerated, in studies like those that have advanced our understanding of regeneration mechanisms in planarian or hydra. Such studies are indeed underway (Adamska and Ereskovsky, personal communication).

Regeneration of sponges

Sponges have remarkable regenerative ability enabling them to recover upon loss or disruption of a certain fraction of their body. Experimental regeneration of explants (in which the explants adhere to a new substrate), transection of whole animals, and tissue ablation have been studied since the 1960s (reviewed in Simpson 1984). Furthermore, sponges' dissociated cells have the ability to adhere to each other (re-aggregate) to form a new individual. This latter ability is particularly well known from the studies of Wilson carried out more than 100 years ago (Wilson, 1907). Regeneration of both modes, i.e., recovery after injury and after dissociation into single cells, had been investigated in earlier studies. In the past couple of years, new light has been shed on the regeneration of sponges as a result of several ultrastructural studies that aimed to revisit the cellular mechanisms underlying these phenomena using advanced EM. Regeneration after injury has thus been studied in a demosponge (Borisenko *et al*., 2015) and in a homoscleromorph (Ereskovsky *et al*., 2015), and regeneration from dissociated cells has also been studied in various demosponge species and a calcarea spices (Lavrov and Kosevich, 2016, Eerkes-Medrano *et al*., 2015).

What types of cells participate in the regeneration as sources of newly formed tissue or body parts is an important issue (reviewed in Elliott er al., 2013; King and Newmark, 2012; Tanaka and Reddien, 2011). Studies in organisms that have regenerative ability (for example, hydra, flatworm, and salamander) showed that the source of cells for regeneration is i) totipotent/pluripotent cells, or ii) transdifferentiation of some types of cells, or iii) division of terminally differentiated cells (Brockes, 1997; King and Newmark, 2012; Tanaka and Reddien, 2011; Weissman *et al*., 2001). In this section, the importance of transdifferentiation (with and without EMT) and the plasticity of sponge cells, which is even greater than previously thought, will be discussed, together with an introduction of the classical observational studies.

Transdifferentiation

The term "transdifferentiation" was first introduced to describe a switch in cell differentiation that occurs in the development of the silk moth (Selman and Kafatos, 1974). Then, Okada (1976) and Eguchi (1979) used "transdifferentiation" to denote the switching of pigment cells to lens cells of newt, which was clearly demonstrated using *in vitro* cell cultures. In his book *Transdifferentiation*, Okada (1991) used the term "transdifferentiation" to denote flexibility in cell differentiation or a possible reprogramming of already differentiated cells based on his group's studies of newt lens regeneration. It is impressive that his view of this phenomenon fits surprisingly closely to our current understanding of the differential plasticity of cells based on genome reprogramming. The transdifferentiation process often includes cell division, but not in all cases, as T.S. Okada noted.

Regeneration after injury

Earlier histological studies done in the 1960's and 1970's described observations showing that several types of cells (pinacocytes, choanocytes etc.) transform into mesohyl cells (reviewed in Simpson, 1984). Recently, several studies have strongly claimed the importance of transdifferentiation during regeneration upon disruption of sponge tissue and subsequent remodeling in a demosponge *(Halisarca dujardini*), a homoscleromorph (*O. lobularis*), and a calcareous sponge (*Leucosolenia complicata*) (Borisenko *et al*., 2015; Ereskovsky *et al*., 2015, Ereskovsky *et al.,* 2017,

respectively*).* In adult *H. dujardini,* exopinacoderm is regenerated mainly from choanocytes and archeocytes, and rarely from endopinacocytes. It was clearly shown that choanocytes undergo EMT and migrate toward a wound. Archeocytes together with the mesenchymal cells which were originated from choanocytes form an undifferentiated cell gathering beneath the wound (which the authors described as a blastema-like structure). They then regenerates new exopinacoderm by MET (mesenchymal epithelial transition). Thus, the authors claimed that transdifferentiation is the driving force of regeneration in this sponge. The fact that actively proliferating choanocytes seemed to be the main source of regenerated exopinacoderm in this demosponge species *(Halisarca dujardini*) supports the proposed model of the sponge stem cell system (described above in this review) and the proposed scenario in which the type of cells (archeocytes or choanocytes) that acts as the main source of totipotent (pluripotent) stem cells depends on the cellular organization of sponges. Additionally, it was indicated that the density of archeocytes and choanocytes, and their activity (probably related to their proliferative ability) might be one of the features that determine whether they act as stem cells (Borisenko *et al*., 2015).

In contrast, in an adult homoscleromorph (*O. lobularis)* and in an adult calcareous sponge (*L. complicata*), regeneration is epithelial-cell driven. None of the below phenomenon, EMT, local proliferation, local dedifferentiation and formation of a blastema-like structure could be found. Instead, three epithelial tissues contribute to the regenerated exopinacoderm: i) exopinacoderm surrounding the wound surface, ii) endopinacoderm from peripheral canals, and iii) and endopinacoderm facing the wound (Ereskovsky *et al*., 2015). Thus, in Homoscleromorpha, regeneration seemed to depend entirely on the transdifferentiation ability (differential plasticity) of epithelial cells and remodeling of the remaining tissue. This might be because the body of *O. lobularis* and *L. complicata* is mainly constructed from two epithelia.

Regeneration of whole sponge body from dissociated cells

Two issues about sponge regeneration from dissociated cells should be noted. First, the process is not just like an assembly of LEGO blocks (cells) that originally constructed a toy house (the sponge body) breaking down into individual blocks, and then the blocks getting reassembled into their original places in the house. Rather, dissociated cells seem to de-differentiate and re-differentiate during regeneration, probably accompanied by the differentiation of stem cells. Thus, it is likely that transdifferentiation occurs as if a type of assembled LEGO blocks are disassembled, then lose their original form, and become transformed into other type of LEGO block. Second, as pointed out by Eerkes-Medrano *et al.,* (2014), complementation by some other cells seems to be required. Using juvenile sponges hatched from gemmules of *E. fluviatilis*, it was clearly shown that without archeocyte cell fractions, dissociated cells could form cell aggregates, but could not regenerate into functional individuals (De Sutter and Van de Vyver, 1977). Recently systematic ultrastructural analysis of regeneration from the dissociated cells of adults of seven sponge species (a species of calcareous sponge, and six species of demosponges covering a range of taxa) was performed. In contrast to the previous widely held notion, it was clearly shown that the ability to regenerate their body from dissociated cells varied among the species (Eerkes-Medrano and Leys, 2006). Actually, two out of the seven species (*Spongilla lacustris* and *Haliclona cf. permollis*) could develop functional individuals from the dissociated cells. The other species remained as aggregates, or as a cell mass attached to the substratum, but could not become organized into tissue(s). Supporting the notion of different abilities of regeneration from dissociated cells, it was also recently reported that even among the same genus, this ability varies. One of three species of *Haliclona* could reconstitute a functional body, but the others only could form cell aggregates (primorphs) (Lavrov and Kosevich, 2016). Eerkes-Medrano *et al.,* defined steps of the regeneration process and formalized the following 4 steps (the author described as checkpoints) after dissociated cells formed aggregates: i) sorting of cells and removal of debris, ii) adhesion of aggregates to the substrate and cell differentiation, iii) organization of cells into tissues, and iv) regionalization of tissues (Eerkes-Medrano *et al.,* 2015).

What causes the difference of regenerative ability to construct an individual from dissociated cells is still an open question. The density of archeocytes and of cells that have ability to transdifferentiate (most likely choanocytes) suggested to be important, since the species with a high density of choanocyte chambers and low density of spicules and collagen were able to regenerate new individuals (Eerkes-Medrano *et al.,* 2015). Studies using juvenile hatched from gemmules of *E. fluviatilis* showed that a certain number of archeocytes seem to be needed to form functional individuals from dissociated cells (Van de Vyver and Buscema, 1981; reviewed in Simpson, 1984). Additionally, transdifferentiation of choanocytes into exopinacocytes during the process of regeneration from dissociated cells was clearly shown by ultrastructural study using a demosponge, *Lubomirska baicalensis* (Ereskovsky *et al*., 2016). Interestingly, Eerkes-Medrano *et al.,* introduced a new view, namely, that the activity of these stem cells, and the capacity of transdifferentiation of other types of cells might differ depending of the adaptive traits of each species. For examples, adaptation to the freshwater habitat where the temperature and/or turbidity fluctuate or seasonally changes, or the capacity of asexual reproduction. It should also be pointed out that these features of stem cells and their density might also differ depending on such factors as whether sponges are in a reproductive state or non-reproductive state, whether they are adult or juveniles, and whether there are growing parts of the body or not.

Transdifferentiation during metamorphosis of sponge larvae

Transdifferentiation accompanied with EMT or MET during metamorphosis of sponge larvae has been shown by cell lineage-tracing using both precise ultrastructural studies (Leys and Degnan, 2002) and live cell labeling of fluorescent dye (Nakanishi *et al*., 2014; Sogabe *et al*., 2016). The fully differentiated ciliated outer epithelial cells simultaneously underwent EMT and internalized to become de-differentiated amoeboid cells. De-differentiation was clearly shown by the fact that those amoeboid cells contained axonemes of former cilia, and they retained the fluorescent dye. Those amoeboid cells are archeocyte-like cells. Since they have archeocytes-like cell morphology, rather large nucleus containing large nucleolus, and at least re-differentiate into choanocytes forming chambers (by undergo MET) in the metamorphosing sponges and juvenile sponges after metamorphosis (Leys and Degnan, 2002; Nakanishi *et al*., 2014; Sogabe *et al*., 2016).

Summary: current view of sponge stem cell system and regeneration

A comprehensive overview of studies of the stem cell system in sponges in various situations, including regeneration, resulted in three important issues becoming apparent. i) The proposed model that two types of cells, archeocytes and choanocytes, constitute the stem cell system of demosponges seemed to be extendable to the general stem cell system of sponges (including members of all of the four classes), ii) Transdifferentiation seemed to be a key for understanding the cellular systems underlying the regeneration, and possibly, development and homeostasis of sponges. iii) The importance of EMT for achieving multipotency or pluripotency (totipotency) was also highlighted. In this section, these issues will be discussed.

Archeocytes and choanocytes may constitute the stem cell system in all four classes of sponges

A generalized possible model of the stem cell system in sponges seems to be that two types of cells, archeocytes and choanocytes, construct a stem cell system, a model that was originally proposed in demosponges. Which type of cells mainly acts as stem cells seems likely to depend on the cellular organization of each class of sponges (Fig. 7). This stem cell type selection might differ even in the same species, since the cellular organization differs among the various stages of the life cycle of sponges (such as larva, juvenile sponge, and adult), and depending on the season.

Now we have some evidence that supports the notion that choanocytes are the source of other types of cells (stem cells) in Homoscleromorpha and calcareous sponges. In a homoscleromorph (*O. lobularis*), choanocytes transdifferentiate into exopinacocytes without undergoing EMT. The pluripotency (totipotency) of choanocytes is strongly suggested by their expression of multiple GMP genes throughout the sponge's life cycle. Interestingly, it was also suggested that vacuolar cells type 2 may be pluripotent based on their expression of a set of GMP genes. Thus, the possible stem cell system scenario of Homoscleromorpha is that choanocytes act as the main stem cells, and vacuolar cells type 2s (possible archeocytes) might have some role as stem cells (Fig. 7). To obtain a clearer view of the stem cell system in homoscleromorphs, further studies, including studies of the self-renewal of choanocytes, will be needed.

Considering the cellular organization of calcareous sponges, it is likely that these sponges might use a choanocyte-main stem cell system. It is tempting to speculate that amoeboid cells, which are relatively rare cells that reside in the mesohyl of calcareous sponges, might be archeocyte-like cells, or that de-differentiated choanocytes produce other types of cells, especially sclerocytes (mesohyle cells) (Fig. 7). The fact that hexactinellids have cells with typical archeocyte morphology in the mesohyl suggests that this type of cell might act as stem cells. It should be noted that the body of hexactinellids consists largely of syncytial tissue. Thus, we will have to wait for further studies to learn whether archeocyte-like cells are a source of other type of cells (such as sclerocytes) and syncytial tissue.

Importance of transdifferentiation in sponge stem cell system

It is intriguing that the transdifferentiation of epithelial cells (choanocytes and endopinacocytes) to other types of epithelial cells (either exopinacocytes or endopinacocytes) was clearly demonstrated to be the driving force of regeneration upon injury in demosponges (Borisenko *et al.*, 2015) a homoscleromorph (Ereskovsky *et al.*, 2015), and a calcareous sponge (Ereskovsky *et al.*, 2017), and also the driving force of regeneration from dissociated sponge cells (Eerkes-Medrano *et al.*, 2015; Lavrov and Kosevich, 2016). These observations were also in line with the findings of

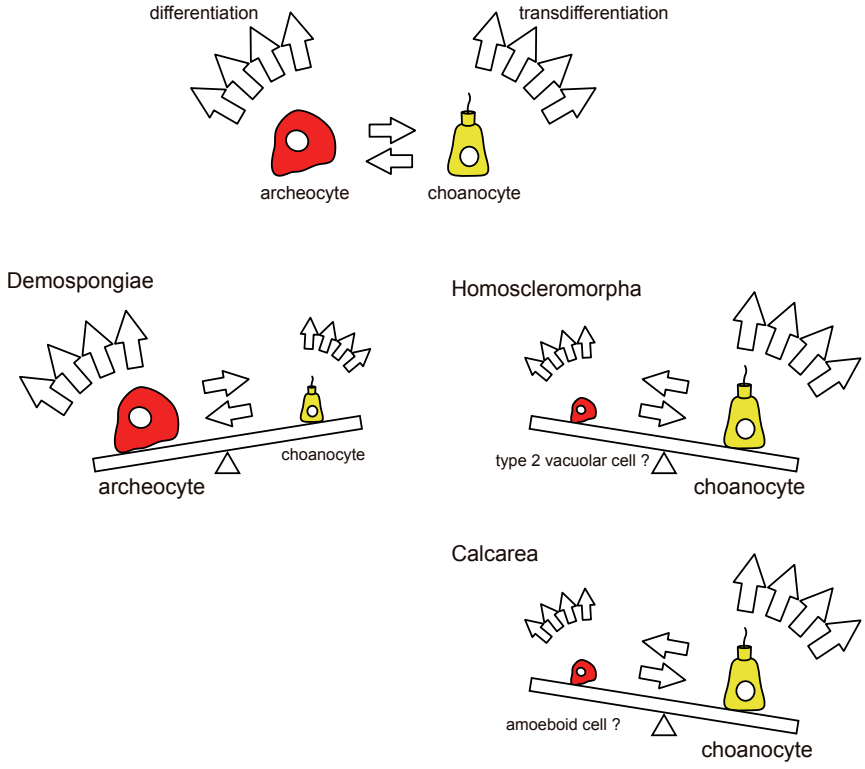

Fig. 7. Generalized possible model of stem cell system in sponges. *Possible model of stem cell system that consists of archeocytes and choanocytes is extended to all (at least three) classes of sponges. In Demospongiae, the archeocyte is a main actor for producing all types of cells, including oocytes (but not sperm). In special circumstances, choanocytes undergo EMT to transform into archeocytes or de-differentiate, then re-differentiate into at least exopinacoderm. In Homoscleromorpha and Calcarea, the choanocyte is thought to be the main actor for producing other types of cells. Both sperm and oocytes are produced from choanocytes. During regeneration upon injury, choanocytes transdifferentiate into exopinacocytes (in Homoscleromorpha) or endopinacocytes (in Calcarea) without EMT. In Homoscleromorpha, vacuolar cells type 2 are suggested to possibly possess pluripotency, based on the expression of a set of GMP genes, and thus they might have the ability to produce other types of cells under special circumstances. In Calcarea, amoeboid cells that are rarely found in the mesohyl can possibly be de-differentiated choanocytes (equivalent to archeocytes transformed from choanocytes in demosponges).*

earlier studies (reveiwed in Simpson, 1984). Transdifferentiation of choanocytes also occurs in the production of sperm in demosponges (Diaz and Connes, 1980; Riesgo *et al.*, 2007), in the production of sperm or oocytes in homoscleromorphs (E Gaino *et al.*, 1986; E. Gaino *et al.*, 1986), and in calcareous sponges (Lanna and Klautau, 2010; reviewed in Ereskovsky, 2010; Leys and Ereskovsky, 2006). Whether transdifferentiation occurs only in these specific circumstances, or generally in various life processes of sponges, that is, during metamorphosis of embryos, gemmule hatching, growth, and homeostasis, is one of the important questions still to be addressed for understanding the stem cell system in sponges.

Interestingly, transdifferentiation has only been reported to occur in epithelial cells so far. One of the reasons for this might be because the relevant studies were histological analyses performed by light microscopy and EM, using which choanocytes can be clearly distinguished due to their specialized cell morphology (a flagella and microvillus collar). Considering the following issues, it is possible to speculate that epithelial cells of sponges might have cell plasticity to transdifferentiate into other types of epithelial cells: i) Epithelia are connected and thus different types of epithelial cells often adhere and sit next to each other, ii) When exopinacoderm of a sponge touches a substrate, it becomes adherent to the substrate (in this situation, exopinacocytes might differentiate into basopinacocytes), iii) The remodeling of sponge tissues probably occurs constantly during both development and tissue regression. Many earlier studies described the disorganization and reorganization (remodeling) of sponge tissues, especially of the aquiferous system, and the remodeling was thought to be related to a) maximizing the efficiency of the aquiferous system, b) reestablishing canals and water pumping after sexual reproduction, during which the canal system becomes greatly reduced, and c) body growth (reviewed in Simpson 1984). Although the cellular and molecular mechanisms of those remodeling events remain unknown, it can be speculated that transdifferentiation is involved in epithelial tissue remodeling.

Importance of epithelial-mesenchymal transition (EMT) as a regulatory mechanism to exert totipotency (pluripotency) or plasticity of choanocytes

EMT is an ordered process in which epithelial cells receive some paracrine signals and accordingly lose those epithelial features (the deconstruction of cell-cell junctions and apico-basal polarity of cells), secrete proteases to destroy extracellular matrix, and then become mesenchymal cells (Lamouille *et al.*, 2014). Since interactions among neighboring cells have important roles in maintaining the epithelial cell state, EMT not only results in cells physically leaving the epithelium, but also releases epithelial cells from the constraint to remain so and enables them to have a different identity as mesenchymal cells. Thus, EMT can be described as a process that dramatically changes cell features.

It seemed likely that this knowledge could be extended to choanocytes of sponges. It was well known that choanocytes undergo EMT to produce sperm in demosponges, and to produce sperm and oocytes in homoscleromorphs and calcareous sponges. Thus, EMT could be suggested to have important roles in controlling pluripotency (totipotency). Additionally, it was recently revealed that during regeneration upon injury of a demosponge, choanocytes undergo EMT to de-differentiate (retract flagella and microvillus collar) and become mesenchymal cells (They then re-differentiate into exopinacocytes through MET) (Borisenko *et al.*, 2015). Since transdifferentiation of choanocytes into exopinacoderm was the main focus in that study, it is still possible that choanocytes also transdifferentiate into types of cells other than exopinacocytes. EMT and MET are also revealed by cell lineage/fate/tracing experiments using labeling of ciliated outer epithelial cells of larvae during and after the metamorphosis of larvae (Leys and Degnan 2002, Nakanishi *et al.,* 2014, Sogabe *et al.,* 2016). Taken together, the above considerations indicate that EMT of choanocytes seems to be a general mechanism for exerting choanocytes' pluripotency (totipotency) or at least cell plasticity. Thus, elucidating the molecular and cellular mechanisms of EMT and its regulation will give us clues for further understanding the stem cell system in sponges. Furthermore, comparing those mechanisms with those of other animals will not only reveal the ancestral mechanisms of EMT, but will also give us clues about the molecular bases of the origin of stem cells and the origin of mesenchymal cells. One of the widely accepted scenarios of the evolution of multicellular organisms is: the ancestral metazoan (urmetazoan) was like a conglomerate of ciliated cells, as are several extant species of choanoflagellate that form colonies without cells inside their body. Then, mesenchymal cells should have evolved via EMT. Thus, it is tempting to speculate that the ancestral stem cells were sponge's choanocyte-like epithelial cells, and then choanocyte-like stem cells somehow gained the ability to undergo EMT to become archeocyte-like multipotent stem cells or to directly produce mesenchymal cells in the inner body space, and that led to the evolution of multicellular organisms with mesenchymal cells.

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References

ABRANCHES E, GUEDES AM V., MORAVEC M, MAAMAR H, SVOBODA P, RAJ A, HENRIQUE D (2014). Stochastic NANOG fluctuations allow mouse embryonic stem cells to explore pluripotency. *Development* 141: 2770–2779.

ADAMSKA M (2016). Sponges as models to study emergence of complex animals. *Curr Opin Genet Dev* 39: 21–28.

ADAMSKA M, DEGNAN BM, GREEN K, ZWAFINK C (2011). What sponges can tell us about the evolution of developmental processes. *Zool* 114: 1–10.

ALIÉ A, HAYASHI T, SUGIMURA I, MANUEL M, SUGANO W, MANO A, SATOH N, AGATA K, FUNAYAMA N (2015). The ancestral gene repertoire of animal stem cells. *Proc Natl Acad Sci* USA: 14789.

BOEHM AM, KHALTURIN K, ANTON-ERXLEBEN F, HEMMRICH G, KLOSTERMEIER UC, LOPEZ-QUINTERO JA, OBERG HH, PUCHERT M, ROSENSTIEL P, WITTLIEB J, BOSCH TC (2012). FoxO is a critical regulator of stem cell maintenance in immortal Hydra. *Proc Natl Acad Sci USA* 109: 19697–19702.

BORISENKO IE, ADAMSKA M, TOKINA DB, ERESKOVSKY A V. (2015). Transdifferentiation is a driving force of regeneration in *Halisarca dujardini* (Demospongiae, Porifera). *PeerJ* 3: e1211.

BOSCH TC, ANTON-ERXLEBEN F, HEMMRICH G, KHALTURIN K (2010). The Hydra polyp: nothing but an active stem cell community. *Dev Growth Differ* 52: 15–25.

BOSCH TCG (2009). Hydra and the evolution of stem cells. *BioEssays* 31: 478–486.

BROCKES JP (1997). Amphibian Limb Regeneration: Rebuilding a Complex Structure. *Science* 276: 81–87.

CONNES R, PARIS J, ARTIGES JM (1974). L'origine des cellules blastogenetiques chez Suberites domuncula Nardo. L'e´ quilibre choanocytes-archeocytes chez les spontiaires. *Ann Sci Natur Zool* 16: 111–118.

DAVID CN (2012). Interstitial stem cells in Hydra: Multipotency and decision-making. *Int J Dev Biol* 56: 489–497.

DIAZ J-P (1977). Transformation histologiques et cytologiques post-traumatiques chez la demosponge Suberites massa. *Nardo Bull Mus Nat Hist Natur* 445: 375–396.

DIAZ JP, CONNES R (1980). E´ tude ultrastructurale de la spermatogenése d'une démosponge. *Biol Cell* 38: 225–230.

EERKES-MEDRANO D, FEEHAN CJ, LEYS SP (2015). Sponge cell aggregation: Checkpoints in development indicate a high level of organismal complexity. *Invertebr Biol* 134: 1–18.

EERKES-MEDRANO DI, LEYS SP (2006). Ultrastructure and embryonic development of a syconoid calcareous sponge. *Invertebr Biol* 125: 177–194.

ELLIOTT SA, SÁNCHEZ ALVARADO A (2013). The history and enduring contributions of planarians to the study of animal regeneration. *Wiley Interdiscip Rev Dev Biol* 2: 301–326.

ERESKOVSKY A (2010). *The Comparative Embryology of Sponges.* Springer, Netherlands.

ERESKOVSKY A V., BORISENKO IE, LAPÉBIE P, GAZAVE E, TOKINA DB, BORCHIELLINI C, SINGH SR (2015). Oscarella lobularis (Homoscleromorpha, Porifera) Regeneration: Epithelial morphogenesis and metaplasia. *PLoS One* 10: 1-19.

ERESKOVSKY A V., CHERNOGOR LI, BELIKOV SI (2016). Ultrastructural description of development and cell composition of primmorphs in the endemic Baikal sponge Lubomirskia baicalensis. *Zoomorphol.* 135: 1–17.

ERESKOVSKY A V., LAVROV AI, BOLSHAKOV F V., TOKINA DB (2017). Regeneration in White Sea sponge Leucosolenia complicata (Porifera, Calcarea). *Invertebr Zool* 14: 108–113.

FIERRO-CONSTAÍN L, SCHENKELAARS Q, GAZAVE E, HAGUENAUER A, ROCHER C, ERESKOVSKY A, BORCHIELLINI C, RENARD E (2017). The Conservation of the Germline Multipotency Program, from Sponges to Vertebrates: A Stepping Stone to Understanding the Somatic and Germline Origins. *Genome Biol Evol*: evw289.

FORTUNATO S, ADAMSKI M, BERGUM B, GUDER C, JORDAL S, LEININGER S, ZWAFINK C, RAPP HT, ADAMSKA M (2012). Genome-wide analysis of the sox family in the calcareous sponge Sycon ciliatum: multiple genes with unique expression patterns. *Evodevo* 3: 14.

FORTUNATO SA V, VERVOORT M, ADAMSKI M, ADAMSKA M (2016). Conservation and divergence of bHLH genes in the calcisponge Sycon ciliatum. *Evodevo*: 1–12.

FUNAYAMA N (2010). The stem cell system in demosponges: Insights into the origin of somatic stem cells. *Dev Growth Differ* 52: 1–14.

FUNAYAMA N (2013). The stem cell system in demosponges: Suggested involvement of two types of cells: Archeocytes (active stem cells) and choanocytes (food-entrapping flagellated cells). *Dev Genes Evol* 223: 23–38.

FUNAYAMA N, NAKATSUKASA M, HAYASHI T, AGATA K (2005). Isolation of the choanocyte in the fresh water sponge, Ephydatia fluviatilis and its lineage marker, Ef annexin. *Dev Growth Differ* 47: 243–253.

FUNAYAMA N, NAKATSUKASA M, KURAKU S, TAKECHI K, DOHI M, IWABE N, MIYATA T, AGATA K (2005). Isolation of Ef silicatein and Ef lectin as molecular markers for sclerocytes and cells involved in innate immunity in the freshwater sponge Ephydatia fluviatilis. *Zoolog Sci* 22: 1113–1122.

FUNAYAMA N, NAKATSUKASA M, MOHRI K, MASUDA Y, AGATA K (2010). Piwi expression in archeocytes and choanocytes in demosponges: Insights into the stem cell system in demosponges. *Evol Dev* 12: 275–287.

FURUSAWA C, KANEKO K (2012). A Dynamical-Systems View of Stem Cell Biology. *Science* 338: 215–217.

GAINO E, BURLANDO B, BUFFA P (1986). Contribution to the study of egg development and derivation in Oscarella lobularis (Porifera, Demospongiae). *Int J Invertebr Reprod Dev* 9: 59–69.

GAINO E, BURLANDO B, BUFFA P, SARÁ M (1986). Ultrastructural study of spermatogenesis in Oscarella lobularis (Porifera, Demospongiae). *Int J Invert Rep Dev* 10: 297–305.

GALLISSIAN M-R, VACELET J (1990). Fertilization and Nutrition of the Oocyte in the Calcified Sponge Petrobiona massiliana. In *New perspectives in Sponge Biology* (Ed. K Rützler). Smithonian Institution Press, Washington DC, p. 533.

GEHRKE AR, SRIVASTAVA M (2016). Neoblasts and the evolution of whole-body regeneration. *Curr Opin Genet Dev* 40: 131–137.

DE GOEIJ JM, DE KLUIJVER a, VAN DUYL FC, VACELET J, WIJFFELS RH, DE GOEIJ a FPM, CLEUTJENS JPM, SCHUTTE B (2009). Cell kinetics of the marine sponge Halisarca caerulea reveal rapid cell turnover and shedding. *J Exp Biol* 212: 3892–3900.

HEMMRICH G, KHALTURIN K, BOEHM AM, PUCHERT M, ANTON-ERXLEBEN F, WITTLIEB J, KLOSTERMEIER UC, ROSENSTIEL P, OBERG HH, DOMAZET-LOSO T, SUGIMOTO T, NIWA H, BOSCH TC (2012). Molecular signatures of the three stem cell lineages in hydra and the emergence of stem cell function at the base of multicellularity. *Mol Biol Evol* 29: 3267–3280.

HOBMAYER B, JENEWEIN M, EDER D, EDER MK, GLASAUER S, GUFLER S, HARTL M, SALVENMOSER W (2012). Stemness in Hydra - a current perspective. *Int J Dev Biol* 56: 509–517.

IMAYOSHI I, KAGEYAMA R (2014). bHLH factors in self-renewal, multipotency, and fate choice of neural progenitor cells. *Neuron* 82: 9–23.

JULIANO C, WESSEL G (2010). Developmental biology. Versatile germline genes. *Science* 329: 640–641.

JULIANO CE, REICH A, LIU N, GOTZFRIED J, ZHONG M, UMAN S, REENAN RA, WESSEL GM, STEELE RE, LIN H (2014). PIWI proteins and PIWI-interacting RNAs function in Hydra somatic stem cells. *Proc Natl Acad Sci USA* 111: 337–342.

JULIANO CE, SWARTZ SZ, WESSEL GM (2010). A conserved germline multipotency program. *Development* 137: 4113–4126.

KING N (2004). The unicellular ancestry of animal development. *Dev Cell* 7: 313–325.

KING N, CARROLL SB (2001). A receptor tyrosine kinase from choanoflagellates: molecular insights into early animal evolution. *Proc Natl Acad Sci USA* 98: 15032–15037.

KING RS, NEWMARK PA (2012). The cell biology of regeneration. *J Cell Biol* 196: 553–562.

LAMOUILLE S, XU J, DERYNCK R (2014). Molecular mechanisms of epithelial-mesenchymal transition. *Nat Rev Mol Cell Biol* 15: 178–196.

LANNA E, KLAUTAU M (2010). Oogenesis and spermatogenesis in Paraleucilla magna (Porifera, Calcarea). *Zoomorphology* 129: 249–261.

LAVROV AI, KOSEVICH IA (2016). Sponge cell reaggregation: Cellular structure and morphogenetic potencies of multicellular aggregates. *J Exp Zool Part A Ecol Genet Physiol* 325: 158–177.

LEININGER S, ADAMSKI M, BERGUM B, GUDER C, LIU J, LAPLANTE M, BRATE J, HOFFMANN F, FORTUNATO S, JORDAL S, RAPP HT, ADAMSKA M (2014). Developmental gene expression provides clues to relationships between sponge and eumetazoan body plans. *Nat Commun* 5: 3905.

LEYS SP, DEGNAN BM (2002). Embryogenesis and metamorphosis in a haplosclerid demosponge: gastrulation and transdifferentiation of larval ciliated cells to choanocytes. *Invertebr Biol* 121: 171–189.

LEYS SP, ERESKOVSKY A V (2006). Embryogenesis and larval differentiation in sponges. *Can J Zool* 84: 262–287.

LEYS SP, MACKIE GO, REISWIG HM (2007). The biology of glass sponges. *Adv Mar Biol* 52: 1–145.

LI W, YOUNG SL, KING N, MILLER WT (2008). Signaling properties of a non-metazoan Src kinase and the evolutionary history of Src negative regulation. *J Biol Chem* 283: 15491–15501.

MAH JL, CHRISTENSEN-DALSGAARD KK, LEYS SP (2014). Choanoflagellate and choanocyte collar-flagellar systems and the assumption of homology. *Evol Dev* 16: 25–37.

MITALIPOV S, WOLF D (2009). Totipotency, Pluripotency and Nuclear Reprogramming. In *Engineering of Stem Cells* (Ed. U Martin). Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 185–199.

MOHRI K, NAKATSUKASA M, MASUDA Y, AGATA K, FUNAYAMA N (2008). Toward understanding the morphogenesis of siliceous spicules in freshwater sponge: Differential mRNA expression of spicule-type-specific silicatein genes in Ephydatia fluviatilis. *Dev Dyn* 237: 3024–3039.

NAKANISHI N, SOGABE S, DEGNAN BM (2014). Evolutionary origin of gastrulation: insights from sponge development. *BMC Biol* 12: 26.

NISHIMIYA-FUJISAWA C, KOBAYASHI S (2012). Germline stem cells and sex determination in Hydra. *Int J Dev Biol* 56: 499–508.

NOORDUIN WL, GRINTHAL A, MAHADEVAN L, AIZENBERG J (2013). Rationally designed complex, hierarchical microarchitectures. *Science* 340: 832–837.

OKAMOTO K, NAKATSUKASA M, ALIÉ A, MASUDA Y, AGATA K, FUNAYAMA N (2012). The active stem cell specific expression of sponge Musashi homolog EfIMsiA suggests its involvement in maintaining the stem cell state. *Mech Dev* 129: 24–37.

PEÑA JF, ALIÉ A, RICHTER DJ, WANG L, FUNAYAMA N and NICHOLS SA (2016) Conserved expression of vertebrate microvillar gene homologs in choanocytes of freshwater sponges *EvoDevo* 7:13.

PHILIPPE H, DERELLE R, LOPEZ P, PICK K, BORCHIELLINI C, BOURY-ESNAULT N, VACELET J, RENARD E, HOULISTON E, QUEINNEC E, *et al.,* (2009). Phylogenomics revives traditional views on deep animal relationships. *Curr Biol* 19: 706–712.

PLICKERT G, FRANK U, MULLER WA (2012). Hydractinia, a pioneering model for stem cell biology and reprogramming somatic cells to pluripotency. *Int J Dev Biol* 56: 519–534.

REBSCHER N, VOLK C, TEO R, PLICKERT G (2008). The germ plasm component Vasa allows tracing of the interstitial stem cells in the cnidarian Hydractinia echinata. *Dev Dyn* 237: 1736–1745.

RIESGO A, MALDONADO M (2009). Ultrastructure of oogenesis of two oviparous demosponges: Axinella damicornis and Raspaciona aculeata (Porifera). *Tissue Cell* 41: 51–65.

RIESGO A, MALDONADO M, DURFORT M (2007). Dynamics of gametogenesis, embryogenesis, and larval release in a Mediterranean homosclerophorid demosponge. *Mar Fresh Res* 58: 398–417.

ROUHANA L, SHIBATA N, NISHIMURA O, AGATA K (2010). Different requirements for conserved post-transcriptional regulators in planarian regeneration and stem cell maintenance. *Dev Biol* 341: 429–443.

SELMAN K, KAFATOS FC (1974). Transdifferentiation in the labial gland of silk moths: is DNA required for cellular metamorphosis? *Cell Differ* 3: 81–94.

SHIBATA N, HAYASHI T, FUKUMURA R, FUJII J, KUDOME-TAKAMATSU T, NISHIMURA O, SANO S, SON F, SUZUKI N, ARAKI R, ABE M, AGATA K (2012). Comprehensive gene expression analyses in pluripotent stem cells of a planarian, Dugesia japonica. *Int J Dev Biol* 56: 93–102.

SHIBATA N, ROUHANA L, AGATA K (2010). Cellular and molecular dissection of pluripotent adult somatic stem cells in planarians. *Dev Growth Differ* 52: 27–41.

SHIBATA N, UMESONO Y, ORII H, SAKURAI T, WATANABE K, AGATA K (1999). Expression of vasa(vas)-related genes in germline cells and totipotent somatic stem cells of planarians. *Dev Biol* 206: 73–87.

SIMPSON TL (1984). *The Cell Biology of Sponges*. Springer-Verlag New York Inc.

SOGABE S, NAKANISHI N, DEGNAN BM (2016). The ontogeny of choanocyte chambers during metamorphosis in the demosponge Amphimedon queenslandica. *Evodevo* 7: 6.

SOLANA J (2013). Closing the circle of germline and stem cells: The Primordial Stem Cell hypothesis. *Evodevo* 4: 2.

SOLANA J, KAO D, MIHAYLOVA Y, JABER-HIJAZI F, MALLA S, WILSON R, ABOOBAKER A (2012). Defining the molecular profile of planarian pluripotent stem cells using a combinatorial RNA-seq, RNA interference and irradiation approach. *Genome Biol* 13: R19.

SUGA H, CHEN Z, DE MENDOZA A, SEBÉ-PEDRÓS A, BROWN MW, KRAMER E, CARR M, KERNER P, VERVOORT M, SÁNCHEZ-PONS N, TORRUELLA G, DERELLE R, MANNING G, LANG BF, RUSS C, HAAS BJ, ROGER AJ, NUSBAUM C, RUIZ-TRILLO I (2013). The Capsaspora genome reveals a complex unicellular prehistory of animals. *Nat Commun* 4: 1–9.

SUGA H, SASAKI G, KUMA K, NISHIYORI H, HIROSE N, SU ZH, IWABE N, MIYATA T (2008). Ancient divergence of animal protein tyrosine kinase genes demonstrated by a gene family tree including choanoflagellate genes. *FEBS Lett* 582: 815–818.

DE SUTTER D, VAN DE VYVER G (1977). Aggregative properties of different cell types of the fresh-water sponge Ephydatia fluviatilis isolated on ficoll gradients. *Roux's Arch Dev Biol* 183: 151–161.

TANAKA EM, REDDIEN PW (2011). The Cellular Basis for Animal Regeneration. *Dev Cell* 21: 172–185.

TANAKA K, WATANABE Y (1984). Choanocyte Differentiation and Morphogensis of Choanocyte Chambers in the Fresh-Water Sponge, Ephydatia fluviatilis, after Reversal of Developmental Arrest Caused by Hydroxyurea. *Zool Sci* 1: 540–561.

URIZ M-J (2006). Mineral skeletogenesis in sponges. *Can J Zool* 84: 322–356.

VOIGT O, ADAMSKI M, SLUZEK K, ADAMSKA M (2014). Calcareous sponge genomes reveal complex evolution of α-carbonic anhydrases and two key biomineralization enzymes. *Evol Biol* 14: 230–247.

VAN DE VYVER G, BUSCEMA M (1981). Capacités morphogènese des cellules d'éponges dissociéés. *Ann, Soc royZoolBelg* 111: 9–19.

WAGNER DE, HO JJ, REDDIEN PW (2012). Genetic regulators of a pluripotent adult stem cell system in planarians identified by RNAi and clonal analysis. *Cell Stem Cell* 10: 299–311.

WEISSENFELS N (1981). Bau und Funktion des Siiflwasserschwamms EphydatiafluviatUis L. (Porifera) VIII. Die Entstehung und Entwicklung der KragengeiBelkammern und ihre Verbindung mit dem ausfiihrenden Kanalsystem Norbert.

WEISSMAN IL, ANDERSON DJ, GAGE F (2001). S TEM AND P ROGENITOR C ELLS : Origins, and Transdifferentiations. *Cell Dev. Biol.* 387–403.

WILSON H V. (1907). On some phenomena of coalescence and regeneration in sponges. *J Exp Zool* 5: 245–258.

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