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Seminars in Cell & Developmental Biology 17 (2006) 503–509

seminars in
CELL & DEVELOPMENTAL
BIOLOGY

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Review

Two different evolutionary origins of stem cell systems and their molecular basis

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Available online 27 May 2006

Abstract

We propose two major evolutionary origins of stem cell systems in the animal kingdom. Adult pluripotent stem cell systems are found in many invertebrates and probably evolved as components of asexual reproduction. Lineage-specific stem cell systems probably evolved later and include neural and hematopoietic stem cell types. We propose that these two types of stem cell systems evolved independently. The *vasa*-like genes regulate reproductive stem cells, but not lineage-specific stem cells, which may be regulated by *gcm* genes. Here, we review the evidence for the molecular basis for the evolutionary origin of these two different stem cell systems.

Keywords: Stem cell; Planarian; Hydra; Sponge; Colonial ascidian

Contents

1. Introduction

Recently stem cells have attracted great interest in many fields of science, particularly in medical areas, in which stem cells can be used for disease therapies [1]. However, the fundamental cellular and molecular basis of stem cell systems still remains unclear. In this review we focus on the evolutionary origin of stem cell systems. Knowledge about their origins may help us to understand the fundamental principles of stem cell systems and may contribute to the development of new strategies for stem cell utilization.

Planarian regeneration is well-suited to investigations into the molecular basis underlying stem cell regulation [2]. The planarian system allows for the possible resolution of the evolutionary origin and fundamental features of stem cell biology. Planarians can proliferate by fission and regenerate from a small piece (Fig. 1). This system has very unique characteristics in that only stem cells maintain proliferative activity, as they do in plants [3]. However, once they have differentiated, planarian stem cells can no longer proliferate. Planarian pluripotent stem cells are capable of giving rise to all cell types [4]. Even epithelial cells can be newly produced from the pluripo-

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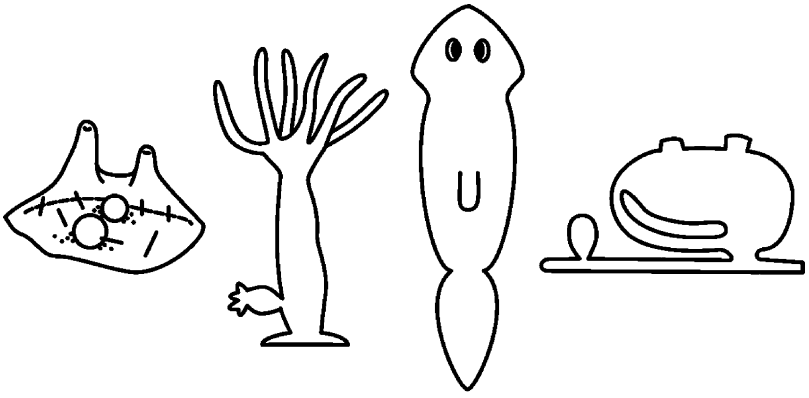
doi:10.1016/j.semcdb.2006.05.004



g. 1. Typical animals possessing pluripotent stem cell systems. Sponge, hydra, planarian, and colonial ascidians (from left to right). Pluripotent stem cells of sponge, dra, planarian, and colonial ascidians are called "archeocytes", "interstitial cells", "neoblasts" and "hemoblasts", respectively. These animals can proliferate by exual reproduction, via processes such as germination, budding and fission (see the text). Interestingly, they also perform sexual reproduction by producing germ ls from pluripotent stem cells.

nt stem cells located within the internal mesenchymal space]. Thus, planarians are unique animals which are ideal for vestigations into the fundamental mechanisms of the stem cell stem.

In previous studies we have identified planarian stem cells rough the use of molecular markers [6,7] and investigated the llular events involved in the process of regeneration using cell-pe-specific markers [8–10]. We found that planarian pluripo-nt stem cells may be present throughout the mesenchymal ace, from head to tail, and that the cells expressing stem ll-specific markers are specifically eliminated by X-ray irra-ation [6]. Interestingly, the stem cells appear committed to anscribe tissue-specific genes in a position-dependent manner ior to migrating to the organ rudiments or blastema [9,10]. ox genes are increasingly expressed along a spatial gradient the posterior region of the intact animal. During regener-on, the expression of Hox genes is rearranged along the terior–posterior axis [11] suggesting that Hox genes may be volved in the regulation of the differentiation of the pluripotent em cells in a position-dependent manner. Our grafting exper-nents clearly demonstrated that intercalation between dorsal d ventral positions induces blastema formation [12] and that terior–posterior intercalation may be essential for rearrange-ent of Hox gene expression [13]. A combination of X-ray adiation and grafting experiments suggested that the posi-onal cues may reside in differentiated cells [14,15].

Adult pluripotent stem cells support asexual production

The regenerative ability of planarians is supported by adult uripotent stem cells, called "neoblasts" [16]. These cells are cated in the mesenchymal space and are able to differentiate to all types of cells, including epidermal cells. However, pla-rians did not develop such a cellular system to recover from tificial cutting by humans. Presumably the adult pluripotent em cell system is primarily for asexual reproduction. Prolifer-on is typically by fission and pluripotent stem cells are used to generate missing parts after fission, which is why planarians maintain pluripotent stem cells into adulthood. Pluripotent stem cells are essential for asexual reproduction in planarians.

Similar cellular systems can be observed in other animals. The most famous example is hydra [17]. Hydra proliferate by budding (Fig. 1) and their pluripotent stem cells are called "interstitial cells" since they are located between the ectodermal and endodermal layers [17]. Interstitial cells proliferate and differentiate into neurons, nematocytes and other cell types [18–21]. In contrast to planarian stem cells, hydra pluripotent stem cells are restricted to providing cells other than epidermal and digestive cells [17]. Each of these latter two cell types can proliferate and generate additional cells of the same types. In the process of asexual reproduction, ectodermal and endodermal cells provide epidermal and digestive cells of newly formed buds, respectively, whereas interstitial cells provide all of the remaining cell types. Interestingly, interstitial cell-less mutant hydra have been isolated and maintained by artificial feeding [22,23]. The mutants cannot catch food by themselves because they lack neurons and nematocytes, which are derived from interstitial cells.

Adult pluripotent stem cell systems are not restricted to primitive animals. Similar cellular systems can be observed in some chordates. Most colonial ascidians can proliferate asexually and extend their colony by budding [24] (Fig. 1). In the case of vascular budding, which is one of the budding styles observed in botryllid ascidians, "hemoblasts" participate in bud formation at the base of a tunic vessel, called the ampulla, and the bud generates a new individual [25,26]. The hemoblast has been considered to be an undifferentiated cell that can produce differentiated blood cells, like hematopoietic stem cells in more-derived chordate species [27,28]. In the course of bud formation and asexual reproduction in botryllid ascidians, the hemoblasts can also differentiate into inner epithelia and several tissue types, including neurons, pharynx and heart [29]. The contribution of hemoblasts to other differentiated cells in addition to blood cells during asexual reproduction has been observed in other families of colonial ascidians as well [30–34].

Recently, we have initiated studies into the stem cell system of sponges (phylum Porifera). The freshwater sponge *Ephydatia fluviatilis* has both sexual and asexual reproductive systems [35].

Small particles called gemmules are formed within the sponge tissue throughout the year for asexual reproduction. Thousands of resting stem cells are encapsulated within each gemmule. In the initial stages of gemmule formation, "archeocytes" (which are thought to be sponge pluripotent stem cells), trophocytes (cells that have archeocyte features but also include numerous cytoplasmic lipid inclusions) and spongioblasts (gemmule coat-forming cells) are observed to form primordial gemmules. When the gemmule coat is completed, the gemmules contain only archeocytes filled with vitelline platelets [36]. Hatching from the gemmule is inhibited by a factor or factors from the surrounding sponge tissue. However, when sponge tissue surrounding the gemmule is eliminated, stem cells migrate out from the gemmule coat, proliferate, and differentiate to form a fully functional small sponge (germination; Fig. 1). Our work is focused on archeocyte differentiation during this hatching process [37,38]. This form of asexual reproduction is thought to ensure survival during dry or high-temperature conditions that can severely damage the parent sponge.

As described above, many animals, from sponges to chordates, maintain pluripotent stem cells in adulthood, and these cells play an important role in asexual reproduction. This is one reason why these animals retain high regenerative ability. They can regenerate lost organs and tissues by utilizing pluripotent stem cells. We propose that the pluripotent stem cell system supporting asexual reproduction represents one of the types of origin of stem cell systems. Thus, we believe that we can learn fundamental mechanisms of stem cell systems by studying animals possessing such systems.

3. Germ cells derived from adult pluripotent stem cells

In Section 2, we described the asexual reproduction of animals and how it is supported by the adult pluripotent stem cell system. In this section, we review how sexual reproduction in these animals is also supported by the same adult pluripotent stem cells.

Planarians can convert from an asexual state to a sexual state if their food or feeding conditions are changed [6,39]. This phenomenon is called "sexualization" or "sexual conversion". Recently, a substance inducing sexualization was partially purified by a Japanese group [40]. Although Orii and his colleagues observed that germ-line-committed stem cells pre-exist in asexual planarians before their conversion into the sexual state, they also found that adult pluripotent stem cells can differentiate into germ cells in the process of sexualization (Orii et al., unpublished observation).

Differentiation of germ-line cells from adult pluripotent stem cells is also observed in hydra and other animals. In the case of hydra, it is well documented that germ cells differentiate from interstitial cells in the process of sexualization [41,42]. Poriferans lack true sexual organs and develop germ cells throughout their body during the sexually reproductive period. In the freshwater species *Spongilla lacustris* [43] and *E. fluviatilis* [43–45], detailed cytological studies suggested that germ cells differentiate from archeocytes (oocytes) and from choanocytes (spermatocytes). A similar origin of germ cells has been described for the marine demosponges *Aplysilla rosea* [46] and *Halichondria panacea* [47,48]. The choanocyte is a type of cell which is specialized for the entrapment of small particles as nutrients. Choanocytes are however, pluripotent and can transform into archeocytes under particular conditions; for example, upon spontaneous disorganization and reorganization of sponge tissue [35]. Although it has been proposed that the germ cells are derived from archeocytes, choanocytes, or archeocytes transformed from choanocytes, there is no direct proof of the development of germ cells from these cell types. Recently, in the demosponge *Sberites domuncula*, several genes that are expressed in the area in which gametogenesis begins were reported [49]. Selective cell labeling and tracing during gametogenesis combined with molecular studies will be needed to clarify whether germ cells are derived from archeocytes/choanocytes, and to determine the developmental pathways of germ cells.

In the case of colonial botryllid ascidians, the germ cells and gonadal cells also originate from aggregates of hemoblasts [50,51]. The loose cell mass of the hemoblasts in the gonadal space expresses *Botryllus primigenus Vasa* homolog (*BpVas*) and differentiates into both male and female germ cells [52]. However, recent work suggests that the hemoblasts may separate into somatic and germ-line-lineages early during embryonic development [53].

Model animals such as *Drosophila*, *C. elegans* and mouse reproduce only by sexual reproduction. In these animals, germ cells segregate from somatic cell lineages at early stages of embryonic development, which makes it possible to conduct genetic analyses. It is commonly assumed that germ cell segregation during the early stages of embryonic development is a general rule in the animal kingdom. However, there are many animals in which germ cells are formed in adulthood, which is common in plants. In plants, stem cells in the meristem differentiate germ cells at the last stages of development, with the formation of flowers [54].

4. Common molecules involved in regulation of pluripotent stem cell systems

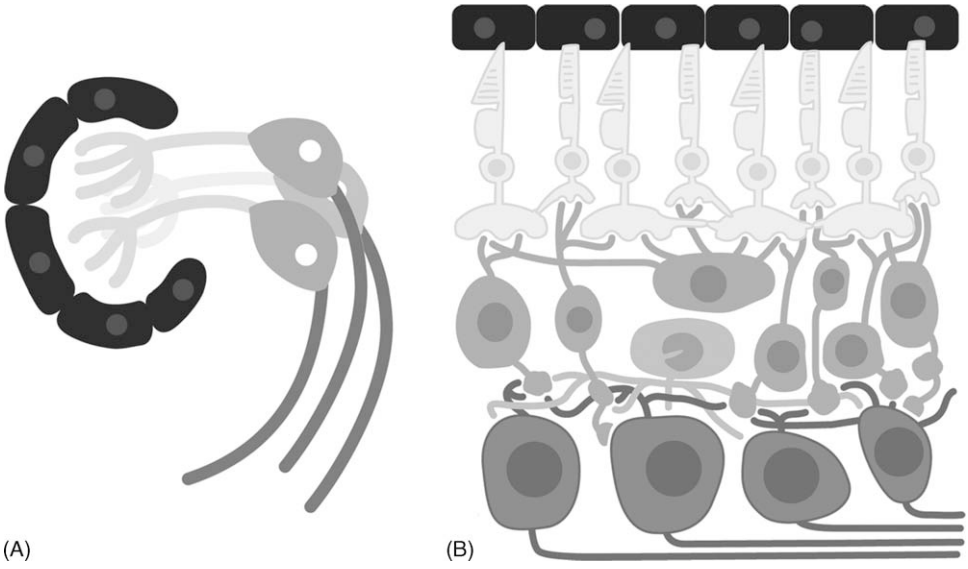
What kind of molecules might account for the regulation of the pluripotent stem cell systems supporting reproduction in the widely diverse animals described in the previous sections? The most notable commonly expressed genes in pluripotent stem cells in these animals are the *vasa*-family genes. The *vasa* gene was initially identified as an essential gene for germ cell formation in *Drosophila*. We isolated a *vasa*-family gene (*DjvlgA*) from the planarian *Dugesia japonica* as the first molecular marker for planarian neoblasts [6,55]. It encodes a DEAD box type RNA helicase. After our report, *vasa*-family genes were isolated from many animals and it was found that they are specifically expressed in both pluripotent stem cells and germ cells in them [42]. In colonial ascidians, one *vasa* homologue was identified and shown to be expressed specifically in germ-line cells [52]. We expect that additional *vasa*-family genes will be found in ascidians and may be expressed in pluripotent stem cells. In sponges, several *vasa*-family genes have been isolated, but their expression patterns have not yet been characterized [42].

Origins of neural and hematopoietic stem cell systems

We propose a novel hypothesis regarding the evolutionary igin of another type of stem cell system, which has developed vertebrates. This idea was derived from the study of brain generation in planarians. Planarians possess an inverted U-aped brain with nine branches on each outer side. A pair of es is located on the dorsal side of the third branch and visual ons form an optic chiasma on the dorsal-inner region of the verted U-shaped brain [8,56–58]. The sixth to ninth branches uster closely together and form auricles on the surface which ay function as the sensory organ of taste [8,59–61]. We found at these structurally distinct and functionally diverse domains e defined by the discrete expression of three evolutionarily nserved homeobox genes, *DjotxA*, *DjotxB*, and *Djotp* [62,63]. e found that the gross structure of the planarian CNS along the terior–posterior (A–P) axis is strikingly similar to the distribu-on pattern of the 'primary' neurons of vertebrate embryos [8] hich differentiate at the neural plate stage to provide a funda-ental nervous system (although the vertebrate CNS is located rsally rather than ventrally as in planarians). These data sug-st that the basic plan for the CNS development along the A–P is might have been acquired at an early stage of evolution hereas the location of the CNS on the ventral or dorsal side olved later.

To provide further support for this notion, we conducted a rge-scale EST project using a planarian brain cDNA library 4,65]. We identified 14 and 15 different glutamate recep-rs and acetylcholine receptor genes, respectively (Tarui et al., published data), suggesting that planarians possess a set of ural genes similar to that of vertebrates. We also extensively studied the genetic programs involved in planarian brain regeneration by combinatory approaches using cDNA chip screening and RNAi [66–69]. The results clearly indicate that similar genetic programs are used in the development of brains in both planarians and vertebrates [69]. From these studies, we conclude that not only the gene sets (hardware) but also the genetic cascades (software) for brain formation were acquired at an early stage of brain evolution. The question remains however, as to what differences have arisen during the evolution of complex brains.

Knowledge about the cell-type complexity of neural cells in higher animals will provide an opportunity to understand brain evolution. The evolution of visual systems is one good example (Fig. 2). The planarian retina is composed of a single type of cell which not only extends microvilli into the pigment cup, but also an axon toward the visual center of the brain [70,71]. That is, in planarians, a single cell type functions as both a retinal ganglion cell and a photoreceptor cell (Fig. 2A). In the course of evolution, these cells have diversified into photoreceptor cells, interneurons, glial cells, and retinal ganglions, forming complex retinal layers and acquiring functions for light adaptation [72] (Fig. 2B). This implies that a primitive neural cell might have had the potency for multiple functions, and different cell types able to perform each distinct function might have arisen from this multifunctional cell during evolution. This could be the same mechanism or the evolutionary basis for the fact that a variety of cell types are produced from a single neural stem cell in higher vertebrates. The development of the neural stem cell system in vertebrates plays a crucial part in producing a variety of cell types within the neural cell lineage. One way of looking at brain development may be to regard the differentiation of many types



g. 2. Evolution of the neural stem cell system in eyes. (A) Planarian visual neurons are composed of a single type of visual neuron, which has multiple functions: ingle neuron receives photons in the microvilli, and transmits photo-signals to the brain through a visual axon. (B) In vertebrates, a variety of neurons diversified deal with photo-signals. Rod and cone cells receive signals with different wavelengths. There are several different interneurons, forming a multi-layered structure. sual ganglions transmit signals to the brain after contact with interneurons. Interestingly, these different types of neurons have a clonal cell origin. They are rived from a single neural stem cell by asymmetric division during development. The neural stem cell system may have been evolutionarily established by dividing ltiple-functions, which were dealt with by a single neuron in the primitive animal, into specialized neurons during evolution. In contrast to the nervous system, gment cells have continued as a monolayer of pigment cells during the long history of eye evolution in all animals. They have not diversified during evolution.

of cells from a single stem cell during early development as mimicking the evolutionary processes. According to this notion, the cells comprising the planarian brain may have the potential for all the basic functions to process various external signals, despite the simple composition of cell types.

A similar idea could also explain the evolution of the hematopoietic stem cell system. Multifunctional cells, circulating throughout the entire body to transfer oxygen and eliminate invaders, may have been present in primitive animals. These cells may have separated into two specialized types of cells, oxygen-transporting cells and defensive cells. The cell type free from the obligation of transporting oxygen then started to evolve into a complex cellular system to eliminate invaders, resulting in the establishment of a complex immune system. This may be the reason why red blood cells, phagocytic cells, and lymphoid cells are derived from a common stem cell (hematopoietic stem cell). They may have a common evolutionary origin.

6. Molecular basis of cellular diversification

Interestingly, the *glial cells missing* (*gcm*) and *gcm2* genes are required to make a binary cell fate decision not only in the neural cell lineage (neuron versus glial cells) but also in the hematopoietic cell lineage (plasmatocytes versus crystal cells) in the *Drosophila* stem cell system [73–76]. This dual role of *gcm* in both lineages is controlled, via *cis*-regulatory elements of *gcm* required for the precise temporal and spatial regulation of its transcription [77,78]. The planarian genome also has a *gcm*-related gene; however, its activity might not be required for the neural cell lineage (Umesono et al., unpublished data) or hematopoietic cell lineage (since planarians have no blood system). This suggests that dynamic changes of *cis*-regulatory elements of these genes might be one of the motive forces for the development of these stem cell systems during evolution.

7. Conclusion

Recently, interest in stem cell systems has rapidly increased. However, fundamental knowledge concerning stem cell biology is still very poor. Here, we introduced a variety of stem cell systems, including both invertebrate and vertebrate systems that provide unique opportunities to investigate basic principles of stem cell systems. In conclusion, we propose that there are two different origins of stem cell systems. One system basically evolved from adult pluripotent stem cells which support both asexual and sexual reproduction. The other system developed by separating multiple functions of primitive cells into specialized cell lineages. Specifically, the nervous and immune systems have achieved high performance by evolving a variety of specialized cells. Thus, it is critical to understand the molecular basis of stem cell systems for the use of stem cells in therapeutic strategies for human disease.

Acknowledgments

This review was written based on studies supported by Special Coordination Funds for Promoting Science and Technology to KA, and Grants-in-Aid for Exploratory Research to NF, Creative Research to KA and Scientific Research on Priority Areas to KA.

References

[1] Zandonella C. Stem-cell therapies: the first wave. Nature 2005;435:877–8.
[2] Agata K. Regeneration and gene regulation in planarians. Curr Opin Gen Dev 2003;13:492–6.
[3] Brønsted HV. Planarian regeneration. London: Pergamon; 1969.
[4] Baguñà J. Planarian neoblasts. Nature 1981;290:14–5.
[5] Hori I. Possible role of rhabdite-forming cells in cellular succession of the planarian epidermis. J Electron Microsc (Tokyo) 1978;27:89–102.
[6] Shibata N, Umesono Y, Orii H, Sakurai T, Watanabe K, Agata K. Expression of *vasa* (*vas*)-related genes in germline cells and totipotent somatic stem cells of planarians. Dev Biol 1999;206:73–87.
[7] Ogawa K, Kobayashi C, Hayashi T, Orii H, Watanabe K, Agata K. Planarian FGFR homologues expressed in stem cells and cephalic ganglions. Dev Growth Differ 2002;44:191–204.
[8] Agata K, Soejima Y, Kato K, Kobayashi C, Umesono Y, Watanabe K. Structure of the planarian central nervous system (CNS) revealed by neuronal cell markers. Zool Sci 1998;15:433–40.
[9] Kobayashi C, Watanabe K, Agata K. The process of pharynx regeneration in planarians. Dev Biol 1999;211:27–38.
[10] Agata K, Watanabe K. Molecular cellular aspects of planarian regeneration. Semin Cell Dev Biol 1999;10:77–83.
[11] Orii H, Kato K, Umesono Y, Agata K, Watanabe K. The planarian *HOM/HOX* homeobox gene (*Plox*) expressed along anterior–posterior axis. Dev Biol 1999;210:456–68.
[12] Kobayashi C, Nogi T, Watanabe K, Agata K. Ectopic pharynx arise by regional reorganization after anterior/posterior grafting in planarians. Mech Dev 1999;89:25–34.
[13] Agata K, Tanaka T, Kobayashi C, Kato K, Saito Y. Intercalary regeneration in planarian. Dev Dyn 2003;226:308–16.
[14] Ogawa K, Ishihara S, Mineta K, Nakazawa M, Ikeo K, Gojobori T, et al. Induction of a *noggin*-like gene by ectopic D–V interaction during planarian regeneration. Dev Biol 2002;250:59–70.
[15] Kato K, Orii H, Watanabe K, Agata K. Dorsal and ventral position cues residing in differentiated cells are required for the onset of planarian regeneration. Dev Biol 2001;233:109–21.
[16] Wolff E. Recent research on the regeneration of planaria. In: Rudnick D, editor. Proceedings of the 20th growth symposium on regeneration. New York: Ronald Press; 1962. p. 53–84.
[17] Bode HR. The interstitial cell lineage of Hydra: a stem cell system that arose early in evolution. J Cell Sci 1996;109:1155–64.
[18] David CN, Gierer A. Cell cycle kinetics and development of *Hydra attenuata*. III. Nerve and nematocyte differentiation. J Cell Sci 1974;16:359–75.
[19] David CN, Murphy S. Characterization of interstitial stem cells in Hydra by cloning. Dev Biol 1977;58:372–83.
[20] Bode HR, Heimheld S, Chow MA, Huang LW. Gland cells arise by differentiation from interstitial cells in *Hydra attenuata*. Dev Biol 1987;122:577–85.
[21] Bosch TCG, David CN. Stem cells of *Hydra magnipapillata* can differentiate into somatic cells and germ line cells. Dev Biol 1987;121:182–91.
[22] Marcum BA, Campbell RD. Development of Hydra lacking nerve and interstitial cells. J Cell Sci 1978;29:17–33.
[23] Sugiyama T, Wanek N. Genetic analysis of developmental mechanisms in hydra. XXI. Enhancement of regeneration in a regeneration-deficient mutant strain by the elimination of the interstitial cell lineage. Dev Biol 1993;160:64–72.
[24] Nakauchi M. Asexual development of ascidians: its biological significance, diversity, and morphogenesis. Am Zool 1982;22:753–63.
[25] Oka H, Watanabe H. Vascular budding, a new type of budding in Botryllus. Biol Bull 1957;112:225–40.
[26] Oka H, Watanabe H. Vascular budding in Botrylloides. Biol Bull 1959;117:340–6.

7] Wright RK. Urochodates. In: Ratcliffe NA, Rowley AF, editors. Invertebrate blood cells. London: Academic Press; 1981. p. 565–626.
] Ermak TH. The hematogenic tissues of tunicates. In: Wright RK, Cooper EL, editors. Phylogeny of thymus and bone marrow-bursa cells. Amsterdam: Elsevier North Holland; 1976. p. 45–56.
9] Satoh N. Developmental biology of ascidians. Cambridge: University Press; 1994.
9] Freeman G. The role of blood cells in the process of asexual reproduction in the tunicate *Perophora viridis*. J Exp Zool 1964;156:157–84.
] Kawamura K, Nakauchi M. Mitosis and body patterning during morphallactic development of palleal bud in ascidians. Dev Biol 1986;116:39–50.
] Kawamura K, Nakauchi M. Development of spatial organization in palleal buds of the compound ascidian, *Symplegma reptans*. Biol Bull 1986;171:520–37.
] Kawamura K, Nakauchi M. Homeostatic integration of stem cell dynamics during palleal budding of ascidians. Zool Sci 1991;8:11–22.
] Kawamura K, Fujiwara S, Sugino YM. Budding-specific lectin induced in epithelial cells is an extracellular matrix component for stem cell aggregation in tunicates. Development 1991;113:995–1005.
] Simpson TL. The cell biology of sponges. New York: Springer-Verlag Inc.; 1984.
6] Langenbruch PF. Zur Entstehung der Gemmulae bei *Ephydatia fluviatilis* L. (Porifera). Zoomorphology 1981;97:263–84.
7] Funayama N, Nakatsukasa M, Hayashi T, Agata K. Isolation of the choanocyte in the fresh water sponge, *Ephydatia fluviatilis* and its lineage marker, *Ef annexin*. Dev Growth Differ 2005;47:243–53.
] Funayama N, Nakatsukasa M, Kuraku S, Takechi K, Dohi M, Iwabe N, et al. Isolation of *Ef silicatein* and *Ef lectin* as molecular markers for sclerocytes and cells involved in innate immunity in the fresh water sponge, *Ephydatia fluviatilis*. Zool Sci 2005;22:1113–22.
9] Kobayashi K, Hoshi M. Switching from asexual to sexual reproduction in the planarian *Dugesia ryukyuensis*: change of the fissiparous capacity along with the sexualizing process. Zool Sci 2002;19:661–6.
9] Kobayashi K, Arioka S, Hase S, Hoshi M. Signification of the sexualizing substance produced by the sexualized planarians. Zool Sci 2002;19:667–72.
] Mochizuki K, Sano H, Kobayashi S, Nishimiya-Fujisawa C, Fujisawa T. Expression and evolutionary conservation of *nanos*-related genes in Hydra. Dev Genes Evol 2000;210:591–602.
] Mochizuki K, Nishimiya-Fujisawa C, Fujisawa T. Universal occurrence of the *vasa*-related genes among metazoans and their germline expression in Hydra. Dev Genes Evol 2001;211:299–308.
] Paulus W. Ultrastructural investigation of spermatogenesis in *Spongilla lacustris* and *Ephydatia fluviatilis* (Porifera, Spongillidae). Zoomorphology 1989;109:123–30.
] Paulus W, Weissenfels N. The spermatogenesis of *Ephydatia fluviatilis* (Porifera). Zoomorphology 1986;106:155–62.
] Saller U. Oogenesis and larval development of *Ephydatia fluviatilis* (Porifera, Spongillidae). Zoomorphology 1988;108:23–8.
6] Tuzet PO, Garrone R, Pavans de Ceccatty M. Annales des Science Naturelles, Zoologie. Paris: Tome XII; 1970. p. 27–50.
7] Barthel D, Detmer A. The spermatogenesis of *Halichondria panicea* (Porifera, Demospongia). Zoomorphology 1990;110:9–15.
] Witte U, Barthel D. Reproductive cycle and oogenesis of *Halichondria panicea* (Pallas) in Kiel bight. In: Van Soest R, Balkema AA, editors. Sponges in time and space. Rotterdam: Brookfield; 1994. p. 297–305.
9] Perovic-Ottstadt S, Cetkovic H, Gamulin V, Schroder HC, Kropf K, Moss C, et al. Molecular markers for germ cell differentiation in the demosponge *Suberites domuncula*. Int J Dev Biol 2004;48:293–305.
9] Mukai H, Watanabe H. Studies on the formation of germ cells in a compound ascidian *Botryllus primigenus*. J Morph 1976;148:337–62.
] Mukai H. Comparative studies on the structure of reproductive organs of four Botryllid ascidans. J Morph 1977;152:363–80.
] Sunanaga T, Saito Y, Kawamura K. Postembryonic epigenesis of *vasa*-positive germ cells from aggregated hemoblasts in the colonial ascidian, *Botryllus primigenus*. Dev Growth Differ 2006;48:87–100.
] Laird DJ, DeTomaso AW, Weissman IL. Stem cells are units of natural selection in a colonial ascidian. Cell 2005;123:1351–60.
[54] Evans MM, Barton MK. Genetics of angiosperm shoot apical meristem development. Ann Rev Plant Physiol Plant Mol Biol 1997;48:673–701.
[55] Kurimoto K, Muto Y, Obayashi N, Terada T, Shirouzu M, Yabuki T, et al. Crystal structure of the N-terminal RecA-like domain of a DEAD-box RNA helicase, the *Dugesia japonica vasa*-like gene B protein. J Struct Biol 2005;150:58–68.
[56] Sakai F, Agata K, Orii H, Watanabe K. Organization and regeneration ability of spontaneous supernumerary eyes in planarians—eye regeneration field and pathway selection by optic nerves. Zool Sci 2000;17:375–81.
[57] Inoue T, Kumamoto H, Okamoto K, Umesono Y, Sakai M, Agata K. Morphological and functional recovery of the planarian photosensing system during head regeneration. Zool Sci 2004;21:275–83.
[58] Okamoto K, Takeuchi K, Agata K. Neural projections in planarian brain revealed by fluorescent dye tracing. Zool Sci 2005;22:535–46.
[59] MacRae EK. The fine structure of sensory receptor processes in the auricular epithelium of the planarian, *Dugesia tigrina*. Z Zellforsch mikrosk Anat 1967;82:479–94.
[60] Pigon A, Morita M, Andbest JB. Cephalic mechanism for social control of fissioning in planarians. II. Localization and identification of the receptors by electron micrographic and ablation studies. J Neurobiol 1974;5:443–62.
[61] Ferrero EA, Bedini C. Chemoreception in Turbellaria. Exp Biol 1989;48:141–8.
[62] Umesono Y, Watanabe K, Agata K. A planarian *orthopedia* homolog is specifically expressed in the branch region of both the mature and regenerating brain. Dev Growth Differ 1997;39:723–7.
[63] Umesono Y, Watanabe K, Agata K. Distinct structure domains in the planarian brain defined by the expression of evolutionarily conserved homeobox genes. Dev Genes Evol 1999;209:18–30.
[64] Tazaki A, Gaudieri S, Ikeo K, Gojobori T, Watanabe K, Agata K. Neural network in planarian revealed by an antibody against planarian synaptotagmin homologue. Biochem Biophys Res Commun 1999;260:426–32.
[65] Mineta K, Nakazawa M, Cebrià F, Ikeo K, Agata K, Gojobori T. Origin and evolutionary process of the CNS elucidated by the comparative genomic analysis of planarian ESTs. Proc Natl Acad Sci USA 2003;100:7666–71.
[66] Cebria F, Kudome T, Nakazawa M, Mineta K, Ikeo K, Gojobori T, et al. The expression of neural-specific genes reveals the structural and molecular complexity of the planarian central nervous system. Mech Dev 2002;116:199–204.
[67] Cebrià F, Nakazawa M, Mineta K, Ikeo K, Gojobori T, Agata K. Dissecting planarian CNS regeneration by the expression of neural-specific genes. Dev Growth Differ 2002;44:135–46.
[68] Nakazawa M, Cebria F, Mineta K, Ikeo K, Agata K, Gojobori T. Search for the evolutionary origin of a brain; planarian brain characterized by microarray. Mol Biol Evol 2003;20:784–91.
[69] Cebrià F, Kobayashi C, Nakazawa M, Mineta K, Ikeo K, Gojobori T, et al. FGFR-related gene *nou-darake* restricts brain tissues to the head region of planarians. Nature 2002;419:620–4.
[70] MacRae EK. Observations on the fine structure of photoreceptor cells in the planarian *Dugesia tigrina*. J Ultrastruct Res 1964;10:334–49.
[71] Carpenter KS, Morita M, Andbest JB. Ultrastructure of the photoreceptor of the planarian *Dugesia dorotocephala*. I. Normal eye. Cell Tissue Res 1974;148:143–58.
[72] Dowling JE. Information processing by local circuits: the vertebrate retina as a model system. In: Schmitt FO, Worden FG, editors. The neurosciences: fourth study program. Cambridge: MIT Press; 1979. p. 163–82.
[73] Jones BW, Fetter RD, Tear G, Goodman CS. *Glial cells missing*: a genetic switch that controls glial versus neuronal fate. Cell 1995;82:1013–23.
[74] Hosoya T, Takizawa K, Nitta K, Hotta Y. *Glial cells missing*: a binary switch between neuronal and glial determination in *Drosophila*. Cell 1995;82:1025–36.

[75] Bernardoni R, Vivancos B, Giangrande A. *Glide/gcm* is expressed and required in the scavenger cell lineage. Dev Biol 1997;191:118–30.
[76] Alfonso TB, Jones BW. *gcm2* promotes glial cell differentiation and is required with *glial cells missing* for macrophage development in *Drosophila*. Dev Biol 2002;248:369–83.
[77] Jones BW, Abeysekera M, Galinska J, Jolicoeur EM. Transcriptional control of glial and blood cell development in *Drosophila*: *cis*-regulatory elements of *glial cells missing*. Dev Biol 2004;266:374–87.
[78] Jones BW. Transcriptional control of glial cell development in *Drosophila*. Dev Biol 2005;278:265–73.