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Radial Glia in Echinoderms

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Abstract

Radial glial cells are crucial in vertebrate neural development and regeneration. It has been recently proposed that this neurogenic cell type might be older than the chordate lineage itself and might have been present in the last common deuterostome ancestor. Here, we summarize the results of recent studies on radial glia in echinoderms, a highly regenerative phylum of marine invertebrates with shared ancestry to chordates. We discuss the involvement of these cells in both homeostatic neurogenesis and post-traumatic neural regeneration, compare the features of radial glia in echinoderms and chordates to each other, and review the molecular mechanisms that control differentiation and plasticity of the echinoderm radial glia. Overall, studies on echinoderm radial glia provide a unique opportunity to understand the fundamental biology of this cell type from evolutionary and comparative perspectives.

Keywords

Radial glia; Echinoderms; Regeneration; Neurogenesis

Introduction

Echinoderms have been important models in developmental biology for over a century. For example, sea urchin embryos have been extensively used as models to understand the cellular and gene regulatory mechanisms involved in the formation of the early embryo (McClay, 2011). In addition, echinoderms have long been known for extraordinary plasticity of their adult tissues, manifested in indeterminate life-long growth (Bodnar and Coffman, 2016), homeostatic cell turnover (Mashanov et al., 2014a, 2015b), and post-traumatic regeneration of various body parts. One of the most impressive examples of this phenomenon is continuous homeostatic production of new cells in the adult echinoderm central nervous system (CNS) and quick regeneration of even major parts of the CNS after autotomy or surgical removal. Both homeostatic and post-traumatic neurogenesis in echinoderms are driven by a phylogenetically conserved radial glial cell type, which echinoderms share with chordates. Understanding of the echinoderm glial biology and neurogenesis had long been hampered by the lack of cell type-specific markers and the lack of functional genomics tools applicable to adult individuals. However, new methodological developments in the field have allowed us and other groups to overcome at least some of

those obstacles. Here, we present an overview of the recent progress in our understanding of the biology and functions of the echinoderm radial glia.

Organization of the echinoderm CNS

Echinoderms are a phylum of exclusively marine invertebrates with shared ancestry to chordates. Together with hemichordates (acorn worms and pterobranchs), the phylum Echinodermata is classified as a sister group to chordates within the monophyletic superphylum Deuterostomia (Fig. 1). All currently living echinoderms comprise five classes: Echinoidea (sea urchins), Holothuroidea (sea cucumbers), Ophiuroidea (brittle stars), Asteroidea (sea stars), and Crinoidea (sea lilies). A comprehensive review of the comparative anatomy and cellular composition of the nervous system in these echinoderm classes is available elsewhere (Mashanov et al., 2016). Therefore, here we provide the reader with only the relevant essential background on general features of the organization of the central nervous system (CNS) common to all echinoderms.

The body plan of extant adult echinoderms is characterized by multiradial symmetry, where several (usually five) sets of major radial organs are arranged around a common oral-aboral axis that passes through the mouth and the anus. The anatomical organization of the adult echinoderm CNS follows this radial pattern (Fig. 2A). Each body axis is supplied with a major CNS component called the radial nerve cord (RNC), which runs the length of the radius in the proximodistal (in asteroids, ophiuroids, and crinoids) or oral-aboral (in holothuroids and echinoids) direction. Along its course, the RNC gives off numerous segmented peripheral nerves that innervate muscles, locomotory podia, connective tissue components, and the integument. Around the mouth, the five radial nerve cords are joined together by the circumoral nerve ring.

Radial glia in echinoderms

Both major components of the echinoderm CNS – the nerve ring and the RNCs – have neuroepithelial organization (Fig. 2B). The supporting scaffold of the neuroepithelium is formed by tall multifunctional glial cells, which bear a striking similarity to radial glia of chordates. Unlike in chordates though, whose nervous tissue contains a plethora of glial cell types (Dimou and Götz, 2014), radial glia is the only known major morphological glial cell type in the echinoderm CNS (Mashanov et al., 2006, 2010). The organization of radial glial cells is stereotypical in all echinoderm classes studied so far (Fig. 2C–D'). They span the entire height of the neuroepithelium between its apical and basal surfaces and exhibit clear epithelial features. The cells are characterized by a clear apicobasal polarity with a single cilium on the apical surface. The apicolateral surfaces of adjacent glial cells are joined by intercellular junctions either to other glial cells or to neurons, whose perikarya reach the apical surface of the neuroepithelium.

Most glial cells are unipolar (Fig. 2B–D'). Their cell bodies containing the nucleus are often localized at the apical region of the neuroepithelium and give off a long basal process, which occasionally branches and anchors to the basal lamina by hemidesmosomes. The spaces between glial cell bodies and processes are filled with neuronal perikarya and areas of neuropil. Some radial glia are, however, bipolar, with their cell bodies localized deep in

the neuroepithelium and the two processes extending in opposite directions to the apical and basal surfaces of the neuroepithelium, respectively (Fig. 2C). As in chordates, the cytoplasm of the echinoderm radial glia contains extensive bundles of intermediate filaments. The exact molecular composition of these cytoskeletal structures is yet unknown (Mashanov et al., 2006, 2010).

Besides morphological features, the echinoderm radial glial cells also share with their chordate counterparts certain molecular and functional properties. One of them is the ability to produce and secrete a glycoprotein called subcommissural organ (SCO)-spondin (also known as Reissner's substance). This substance has been implicated in a multitude of functions, including modulation of cell adhesion, migration, neuronal survival, differentiation, axonal growth, CNS morphogenesis, and facilitation of the cerebrospinal fluid flow (Meinzel, 2007; Huh et al., 2009; Vera et al., 2013). In vertebrate embryos, SCO-spondin is transiently produced by the radial glia of the floor plate during CNS development. In adult vertebrates, it is secreted by a subset of specialized radial glia of the subcommissural organ, a small excretory gland positioned at the point where the third ventricle is connected to the Sylvian aqueduct (Meinzel, 2007). In adult echinoderms, similar to the embryonic floor plate of chordates, expression of SCO-spondin is ubiquitous, as most, if not all, radial glia of both the RNCs and nerve ring appear to be secretory (Viehweg et al., 1998; Mashanov et al., 2009). The function of SCO-spondin in echinoderms remains unknown, but its potential neurogenic role is of particular interest in the context of the ability of these animals to quickly regenerate their CNS after injury.

Radial glial cells constitute between ~45% and ~70% of the total cell mass in the echinoderm nervous tissue, depending on the CNS region (Mashanov et al., 2010). Close numbers were reported for the human brain (Azevedo et al., 2009). They appear to be similar in brains throughout the vertebrate lineage, except in certain regions, such as the pacemaker nucleus in electric fish, which is characterized by much higher predominance of glia over neurons (Zupanc, 2017). The latter unusually high glia-to-neuron ratio appears to be related to the high frequency firing of this oscillatory brainstem nucleus. Nevertheless, the glia-to-neuron ratio in many other vertebrate brain regions are similar to those in echinoderms. This might indicate that a certain number of glial cells per neuron are required for proper neuronal functioning even in the relatively simple echinoderm CNS. Thus, the glia-to-neuron ratio does not necessarily correlate with the level of CNS complexity.

Very little is currently known about the origin of radial glial cells in echinoderm development. However, it is clear that there are some similarities in neural development between echinoderms and chordates. In vertebrates, radial glia are produced very early in development by neuroepithelial cells of the primitive neural tube (Chanas-Sacre et al., 2000). The neuroepithelium is itself induced to segregate from the embryonic ectoderm by signals from the mesodermally derived notochord. In echinoderms, the CNS also originates from the embryonic ectoderm, specifically from the restricted oral region called the vestibule (Minsuk and Raff, 2002; Mashanov et al., 2007). Microsurgery experiments suggest that the development of the neuroepithelium from the vestibular ectoderm is also induced by signals from the mesoderm (hydrocoel), as in vertebrates (Minsuk and Raff, 2002). Radial glial cells

appear in the echinoderm neuroepithelium shortly after its separation from the floor of the vestibule (Mashanov et al., 2007).

Radial glia in neural regeneration

Cellular mechanisms of neural regeneration—In the vertebrate lineage, the presence of radial glial cells in the adult CNS usually correlates with the capacity for homeostatic neurogenesis and high regenerative potential of the neural tissue (Chernoff et al., 2003; Dimou and Götz, 2014; Joven and Simon, 2018). The role of radial glia in echinoderms, on the other hand, had not been studied until recently, even though glial cells were first described in the echinoderm CNS as early as in the 19th century (Hamman, 1889). The main impediment had been the lack of reliable cell type-specific markers, as many of the commercial antibodies raised against mammalian antigens do not work in echinoderm tissue samples. Recently, however, two novel monoclonal antibodies, ERG1 and ERG2, were generated to specifically label the radial glia within the echinoderm CNS (Mashanov et al., 2010). The ERG1 antibody proved especially useful, as it labeled radial glia in the four of the five echinoderm classes studied so far (Mashanov et al., 2016). This antibody, in combination with neuronal markers and cell proliferation assays, was used to elucidate the involvement of the radial glia in post-traumatic neurogenesis in sea cucumbers and brittle stars. The best studied experimental model of echinoderm neural regeneration so far has been the radial nerve cord in sea cucumbers (Mashanov et al., 2008; San Miguel-Ruiz et al., 2009; Mashanov et al., 2013, 2012, 2014b, 2015a,c, 2017). The injury paradigm in this case involves complete transection of one of the five RNCs at the mid-body level. Immediately after the injury, the injured body wall muscles pull the wound margins apart, creating a wound gap a few millimeters across. In the course of a few weeks following the injury, each of the two newly made wound surfaces of the RNC forms an outgrowth. The two outgrowths extend across the injury gap towards each other and eventually fuse, bridging the wound gap and restoring the anatomical connectivity of the lesioned RNC. The organization of the nervous tissue in the fully regenerated RNC is indistinguishable from that in the undamaged regions of the CNS.

Radial glial cells play a key role in this regeneration as they form the supporting scaffold for neuronal migration and act as a progenitor cell population that produces both new glia and neurons. Shortly after injury, the glia at the vicinity of the wound drastically change their appearance by undergoing dedifferentiation (Fig. 3). These cells lose their characteristic long basal processes, but never undergo epithelial-mesenchymal transition, as in, for instance, regenerative urodele amphibians (Chernoff et al., 2003). Dedifferentiated glia retain epithelial organization, as their apical cell bodies remain connected to each other by intercellular junctions. About one week post-injury, the activated glia start to extensively proliferate and form a tubular outgrowth that invades the connective tissue that fills the injury gap. Initially, this early rudiment is completely devoid of neurons. As regeneration progresses, neuronal elements start to appear in the newly created segment of the CNS by the third week post-injury.

In response to injury, the rate of cell proliferation in the dedifferentiated glia increases about 10-fold as compared to the glia in the uninjured CNS. These glial cells are the only

proliferating cell types in the regenerating nervous tissue. Combination of pulse-chase labeling with thymidine analogs with cell type-specific markers demonstrated that the dividing glial cells generate both new glia and neurons. In the regenerating RNC of the sea cucumber *Holothuria glaberrima*, almost half (~45%) of the post-mitotic progeny of the radial glia differentiate into neurons. These newly generated neurons were shown to survive in the regenerated RNC for extended periods of time (over 130 days), express markers typical for echinoderm neuronal cell types (Mashanov et al., 2013), and form typical chemical synapses (Mashanov et al., 2008). Similar long-term survival of newly produced neurons was observed in highly regenerative vertebrates, such as teleost fish (Ott et al., 1997; Hinsch and Zupanc, 2007), but not in mammals, in which post-traumatically generated neuroblasts mostly fail to generate functional neurons and largely die (Dimou and Götz, 2014).

Formation of the tubular outgrowth via extensive local cell proliferation of dedifferentiated radial glia within the rudiment is only one of the key mechanisms implicated in the RNC regeneration across the wound gap. Not all cells contributing to neural regeneration are generated this way. Some of them migrate into the regenerate from the stump regions as far as ~2 mm (about 400 cell body diameters) away from the injury site. This has been demonstrated by directly labeling the cells of the RNC with the non-diffusible lipophilic fluorescent dye DiI at various distances from the wound at the time of injury and then tracing them at a range of time points as regeneration progressed. These two processes – generation of new glial and neuronal cells through cell proliferation of radial glia and recruitment of already existing cells via migration – appear to be functionally cross-regulated. When cell division is experimentally suppressed with an inhibitor of S-phase DNA replication, migration becomes more extensive to compensate for the deficiency in the production of new cells by the proliferating radial glial progenitors (Mashanov et al., 2017). The molecular mechanisms of this cross-talk between glial proliferation within the regenerate and migration of distant cells from the stump into the regenerate remain to be established. The identity of the cells that translocate into the regenerate from the stump is not known yet either. They can be migrating neuronal precursors, postmitotic neurons, or both. A similar hybrid strategy to populate the regenerating segment of the CNS is employed in salamanders. In the injured spinal cord, the new cells are generated both through proliferation of radial glial progenitors in the vicinity of the wound and through migration of neurons from the stump (Zhang et al., 2003; Joven and Simon, 2018).

In addition to forming the scaffold of the regenerating RNC and functioning as the main progenitor cell population, echinoderm radial glial cells are involved in clearing the cell debris in the injured neural tissue. Shortly after the injury, many neurons in the vicinity of the wound undergo programmed cell death. Radial glial cells rapidly phagocytose these apoptotic bodies (Mashanov et al., 2008). This function of echinoderm radial glia is similar to post-traumatic glial phagocytosis performed by diverse cell types in other animals (Barres, 2008).

Molecular underpinnings of radial glia function in neural regeneration—Only recently have we started to obtain insights into the molecular mechanisms regulating the function of the radial glia in post-traumatic neurogenesis in echinoderms. A high-throughput

transcriptome-wide gene expression analysis identified 11 orthologs of vertebrate pluripotency and stem cell maintenance genes expressed in the regenerating RNC of the sea cucumber *H. glaberrima* (Mashanov et al., 2014b, 2015a). In both uninjured and regenerating radial nerve cords, many radial glial cells express stem cell markers, including homologs of the mammalian c-Myc, Sox2, Klf4 and Oct4 genes (Mashanov et al., 2015a). After injury, expression of these genes largely persists at the physiological levels. However, expression of the transcription factor *Myc* significantly increases. Importantly, *Myc* becomes upregulated immediately after the injury, i.e. during the phase characterized by extensive dedifferentiation of radial glia (see above), and then continues to remain highly expressed throughout the course of regeneration (Mashanov et al., 2008, 2013). To get insight into the functional significance of *Myc* overexpression, an RNA interference-mediated gene knockdown was performed. As a result, targeted 2-fold depletion of the *Myc* mRNA resulted in the inhibition of radial glial dedifferentiation in the vicinity of the injury. The radial glial cells in these animals retained their typical palisade morphology, whereas cells in the control vehicle-treated animals lost their basal processes. We thus concluded that *Myc* upregulation was required for the acquisition of the dedifferentiated state by echinoderm radial glial cells in response to injury (Mashanov et al., 2015c).

The role of the Myc family transcription factors in regulation of glial plasticity, potency, and function appears to be evolutionarily conserved in the deuterostome lineage (Tavares et al., 2017). For example, the vertebrate ortholog of Myc, c-Myc, has been demonstrated to control the potency of radial glial cells. Its forced expression was used to convert murine radial glial neural stem cells with limited neurogenic potential into more plastic neuroepithelial progenitor cells (Bung et al., 2016). It was also shown that permanent expression of Myc in embryonic radial glial cells delayed their subsequent differentiation into mature astrocytes upon grafting into the adult CNS environment and allowed the transplanted cells to migrate across spinal cord lesions (Hasegawa et al., 2005).

Radial glia in homeostatic adult neurogenesis

Not only are echinoderms capable of regenerating major injuries to their CNS, but they also continuously generate new neurons in the uninjured adult nerve ring and radial nerve cord under normal physiological conditions.

Adult neurogenesis is a widespread phenomenon that has been documented in both vertebrate and invertebrate taxa (Dimou and Götz, 2014; Pino et al., 2017). Extensive research in the field over the last decades has identified the following general features: (1) neurogenesis is spatially restricted to certain regions within the adult CNS; (2) the neurogenic areas in deuterostomes contain specialized neuronal progenitors that are either radial glial cells themselves or their immediate progeny; (3) newborn neurons often migrate from the place of their birth to their final destination within the CNS.

Among echinoderms, adult neurogenesis in the uninjured CNS has been studied in the RNC of a sea cucumber (Mashanov et al., 2015b) and a brittle star (Mashanov et al., unpublished data). The homeostatic neurogenesis in these animals is thought to serve the function of replacing the neurons lost to programmed cell death and also contributes to the life-long growth of the body. Like in other animal models studied so far, the neurogenic zones in

echinoderms are also clearly spatially restricted, even though radial glia are ubiquitous throughout the CNS. For example, in the RNC of the sea cucumber *H. glaberrima*, the neurogenic zones are organized into two lateral longitudinal domains, one on either side of the RNC. In the arm of the brittle star *Ophioderma brevispinum*, only the radial glia in the few terminal segments of the arm tip continuously generate new cells, whereas the glia elsewhere in the CNS remain quiescent under normal homeostatic conditions (Mashanov et al., unpublished data).

Like in vertebrates, at least some of the new neurons generated in the neurogenic zones in the sea cucumber *H. glaberrima* undergo migration. They leave the place of their origin at the apical regions of the neuroepithelium (where most of the radial glia cell bodies are localized) and move basally into deeper regions of the underlying neural parenchyma (Mashanov et al., 2015b).

One fundamental question is how the neurogenic behavior of radial glia progenitors is similar or different between homeostatic neurogenesis and post-traumatic neural regeneration. As indicated above, only a subset of radial glia is neurogenic in the uninjured echinoderm CNS, whereas injury triggers proliferation in otherwise quiescent glia. For example, in the normal brittle star arm, physiological neurogenesis is restricted to a few terminal arm segments. Only radial glial cells in those segments display neurogenic potential under normal conditions. After arm autotomy, however, at any level along the proximodistal axis of the arm, as many as ~20% of the dormant radial glia rapidly respond, re-enter the cell cycle and generate new neurons and glial cells. This suggests that the same glial cell can have different functions under homeostatic conditions (trophic support of neurons, phagocytosis, etc.) and in neuronal regeneration (i.e., acting as a proliferating progenitor).

Radial glia heterogeneity

In chordates, glia are functionally and phenotypically diverse, with each glial class having a specific function in the nervous tissue. Moreover, each individual glial type (such as astrocytes and oligodendrocytes) is not internally homogeneous and is further subdivided into a number of subtypes. All of them are required for the proper development and function of the nervous system (Barres, 2008). Likewise, a subset of glia that functions as neural stem cells in the adult mammalian brain is highly heterogeneous and includes a number of distinct subpopulations representing different stages of activation and differentiation (Dulken et al., 2017). It was, therefore, unusual to find only a single major glial cell type in the echinoderm CNS.

In both the RNC and circumoral nerve ring all radial glial cells exhibit a stereotypical phenotype (Mashanov et al., 2016). Thus, based on morphology alone, it is impossible to distinguish different glial cell types in echinoderms. However, even though all radial glial cells look alike, they exhibit significant molecular and, most importantly, functional heterogeneity. Analysis of the radial glia in the RNC of the sea cucumber *H. glaberrima* at the single-cell level demonstrated that the morphologically homogeneous glia in fact consisted of at least two distinct cohorts of cells, one of which expressed the transcription factor *Myc*, whereas in the second one this gene was transcriptionally silent (Mashanov et

al., 2015b). As indicated above, *Myc* proteins play crucial roles in the regulation of the differentiation status and plasticity of neural progenitors both in echinoderms and other animals (Hasegawa et al., 2005; Fernández-Hernández et al., 2013; Mashanov et al., 2015c).

Similarly, in the terminal segments of the brittle star arm tip, radial glia is the only dividing cell population in the CNS (Mashanov et al., unpublished data). However, not all radial glial cells equally contribute to neurogenesis. A subset of radial glia, along with all neurons, express the transcription factor *Brn1/2/4* (Zueva et al., 2018), a gene of the POU family with an evolutionarily conserved role in the control of neurogenesis (Vierbuchen et al., 2010; Brombin et al., 2011; Garner et al., 2016). Only those radial glial cells in which *Brn1/2/4* was silent were capable of cell division and thus contributing to neurogenesis at the arm tip.

The molecular diversity of the radial glia and their differential involvement in the homeostatic neurogenesis raises a question about the neurogenic function of those glial cells that divide rarely or do not divide at all in the uninjured CNS. As indicated above, one possibility is that these are the quiescent progenitor cells that stay mitotically inactive unless the nervous tissue is injured. The second possibility, which cannot be excluded but has not been tested yet, is that some of the mitotically quiescent radial glia can directly differentiate into neurons without mitotic cell division. Such direct conversion of neuronal stem cells into neurons was demonstrated in intact and injured adult zebrafish telencephalon (Barbosa et al., 2015).

Evolutionary considerations

Radial glia are a phylogenetically ancient cell type that must have been present in the last common ancestor of the superphylum Deuterostomia, before its diversification into the phyla Chordata, Echinodermata, and Hemichordata. A recent study (Helm et al., 2017) even proposed evidence for the existence of radial glial cells in some protostomes.

Echinoderms have been often largely left out of considerations of the evolution of the deuterostome body plan in general and the central nervous system in particular because of their divergent radial body plan (Lowe et al., 2015). Recent evidence discussed elsewhere (Mashanov et al., 2016) strongly suggests that the pentaradial body plan of modern echinoderms has evolved through multiplication of a single body axis of the bilateral ancestor. By extension, this implies a homology between the neural tube of chordates and the echinoderm CNS. This possible homology between neural structures in these two deuterostome phyla put the echinoderm radial glia into a new perspective. Those traits and properties that the glia in echinoderms share with their chordate counterparts can now be considered fundamental to this cell type, as they have been preserved due to evolutionary constraints even after the two taxa diverged from the last common ancestor more than 500 million years ago. On the other hand, unlike chordates, echinoderms have not diversified their glia in the course of evolution. Their radial glial cells therefore might have preserved many of the original features of the glial cells in the last common deuterostome ancestor. Echinoderms therefore provide an interesting opportunity to study the biology of radial glia from the standpoint of both cell type evolution and fundamental neurogenic properties of radial glial progenitors.

The amount of evidence accumulated so far at morphological, functional, and gene expression levels thus strongly suggest a possible homology between radial glia in echinoderms and chordates. However, firmly establishing the evolutionary history of a cell type across species is a non-trivial task. In the current post-genomic era, cell type identity is considered to be determined by a set of regulatory transcription factors that regulate the expression of downstream cell-type specific effector genes. This combination of master regulatory genes is specific for a given cell type and determines the eventual phenotype of the cell and its unique response to extracellular signals (Arendt et al., 2016). It is therefore crucial to compare the topology of gene regulatory networks between the radial glia in chordates and echinoderms to fully resolve the question of homology of this cell type in the two taxa.

Conclusions

The glial organization of the echinoderm CNS is similar to that in regenerative non-mammalian vertebrates and mammalian embryos in that it contains ubiquitous radial glial cells. The key features shared between the echinoderm and chordate radial glia include:

1. Characteristic morphology. Radial glia are ciliated epithelial cells with clear apicobasal polarity. They stretch throughout the height of the neuroepithelium between the apical and basal surfaces and are joined to their neighbors by intercellular junctions. The cytoplasm of these cells is filled with thick bundles of intermediate filaments.
2. They are multifunctional cells that combine properties of both specialized glia (with known roles in mechanical support of the neuroepithelium, phagocytosis and secretion of the SCO-spondin) and mitotic neurogenic progenitors.
3. Radial glia are molecularly and functionally heterogeneous. Individual cells, depending on their position within the nervous tissue, differ in their molecular signatures (e.g., expression of the transcription factors *Myc* and *Brn1/2/4* in echinoderms) and mitotic activity in the uninjured CNS (i.e., quiescent and mitotic glia).

The key difference between echinoderms and chordates is that radial glia is the only glial cell type in the echinoderm CNS, whereas even basal chordates contain additional specialized glial cell types in their nervous tissue.

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Figure 1:
Echinoderm models of adult neurogenesis. **A.** Phylogenetic position of echinoderms within the Deuterostomia clade. **B.** The sea cucumber *Holothuria glaberrima*. **C.** The brittle star *Ophioderma brevispinum*.

Figure 2:

Organization of radial glia in echinoderms. **A.** Diagram representing the general anatomy of the echinoderm CNS. A sea cucumber is shown as an example. *green* – ectoneural system; *magenta* – hyponeural system. The ectoneural and hyponeural systems represent two anatomically interconnected domains of neural tissue characteristic to echinoderms. Modified after: Mashanov et al. (2009). **B.** Diagram of the cross section through a radial nerve cord of a sea cucumber. Modified after: Mashanov et al. (2006). **C.** Diagram summarizing morphological features of echinoderm radial glial cells. From: Mashanov et al.

(2010). **D** and **D'** Radial glial cells in a cross sectioned radial nerve cord of the brittle star *O. brevispinum* visualized by immunostaining with the ERG1 antibody. **D** – a general view of the radial nerve cord; **D'** – higher magnification view of the boxed area in **D**. Abbreviations: *bl* – basal lamina; *c* – cilium; *ec* – epineural canal; *en* – ectoneural neuroepithelium; *hc* – hyponeural canal; *hn* – hyponeural neuroepithelium; *n1–n3* – three distinct neuronal types; *np* – neuropil; *nr* – circumoral nerve ring; *re* – roof epithelium; *rg* – radial glial cell; *rnc* – radial nerve cord; *s* – radial glial cell somata; *vc* – vacuolar cell (present only in some sea cucumber species).

Figure 3:

Diagram showing changes in the neuroepithelium in response to injury. In the uninjured nervous system and immediately after the injury, radial glial cells have their characteristic long processes that span the height of the neuroepithelium. At the early post-injury stage, the glia in the vicinity of the injury undergoes dedifferentiation. The glial processes are lost to fragmentation, but the cell bodies of the glia persist in the apical region of the neuroepithelium and retain epithelial organization. A subsequent burst in cell division in the dedifferentiated glial cells results in the formation of the outgrowth spanning the injury gap.

Initially, the newly regenerated region of the neuroepithelium contains only glia. As regeneration progresses, the regenerate becomes populated with neurons, which are thought to come from two sources. Some of them are produced by the dividing radial glia in the rudiment, while others seem to migrate from the more distant, uninjured regions of the neuroepithelium.

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