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The Evolutionary Origins of Glia

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ABSTRACT
The evolutionary origins of glia are lost in time, as soft tissues rarely leave behind fossil footprints, and any molecular footprints they might have been left we have yet to decipher. Nevertheless, because of the growing realization of the importance glia plays in the development and functioning of the nervous system, lessons we can draw about commonalities among different taxa (including vertebrates) brought about either from a common origin, or from common adaptational pressures, shed light on the roles glia play in all nervous systems. The Acoelomorpha, primitive interstitial flatworms with very simple cellular organization and currently at the base of the bilaterian phylogeny, possess glia-like cells. If they indeed represent the ancestors of all other Bilateria, then it is possible that all glias derive from a common ancestor. However, basal taxa lacking convincing glia are found in most major phyletic lines: urochordates, hemichordates, bryozoans, rotifers, and basal platyhelminths. With deep phylogenies currently in flux, it is equally possible that glia in several lines had different origins. If developmental patterns are any indication, glia evolved from ectodermal cells, possibly from a mobile lineage, and even possibly independently in different regions of the body. As to what functions might have brought about the evolution of glia, by-product removal, structural support, phagocytic needs, developmental programming, and circuit modulation may be the more likely. Explaining possible cases of glial loss is more difficult, as once evolved, glia appears to keep inventing new functions, giving it continued value even after the original generative need becomes obsolete. Among all the uncertainties regarding the origin of glia, one thing is certain: that our ideas about those origins will change with every rearrangement in deep phylogeny and with continued advances in invertebrate molecular and developmental areas.

INTRODUCTION

The question, "Where did glia come from?" in an evolutionary sense has a simple answer: "we don't know." The flip side of such a question—"Where did it go?"—might be extrapolated back to help answer the first part. Most of the available detailed information on this—morphological, physiological, and molecular—resides in the much-studied vertebrates. However, the evolutionary emergence of glia considerably predates the emergence of vertebrates—an already ancient group with half a billion years of evolutionary history. Additional clues lie with two model organisms, *Drosophila* and *C. elegans*, details of which will be treated in other articles in this issue. However to gather as much relevant information as possible on the topic, we must examine the more sparsely-covered "nonmodel" invertebrates as well. Previous reviews of invertebrate nervous systems start with the classic *magnus opus* by Ted Bullock and Adrian Horridge *Structure and Function in the Nervous Systems of Invertebrates* (Bullock and Horridge, 1965) which remains relevant to this day. In this, the authors make a taxon-by-taxon analysis of the neural architecture of the invertebrates, including the glial situation, drawing heavily on the classical histological literature. Modern molecular marker approaches had not been invented at that time, nor even had a great many ultrastructural studies been published. The general but exhaustive Wiley series *Microscopic Anatomy of Invertebrates* (Harrison et al., 1991 ff), should be consulted for additional ultrastructural information, as should more recent reviews specifically focused on invertebrate glia by Lane (1981) and by Pentreath (1989) and the comprehensive review on the glia of more advanced invertebrate taxa by Radojcic and Pentreath (1979).

Before reviewing the more recent literature on the subject of glia's origins, we should ask first why we should care. Part of the answer to this lies in the observation that has been made repeatedly of how similar the appearances are between vertebrate and invertebrate glia (e.g. Barres, 2008). One is tempted thereby to imagine similar functions, insight into which might be obtained from a comparative study. Is the similarity a result of a common ancestor for all glias in all modern bilaterians, or might it be the result of convergent evolution? Indeed, did all glial types in one taxon evolve from a single ancestral type, or were there more than one? Either way, the answer to the question in invertebrates may illuminate important processes at work in vertebrate glia and vice versa. Another point to make at the outset is that whatever roles glia may play in more advanced nervous systems, such as are seen in vertebrates, arthropods, and molluscs, the same roles may not be those that were instrumental in inducing the initial evolution of the character. As time went on other opportunities to contribute to organism fitness by

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assuming additional roles likely appeared, making glia what we see today. We might barely recognize the original form were we to encounter it.

WHERE ARE WE IN OUR UNDERSTANDING OF INVERTEBRATE GLIA?
What Do We Actually Know, versus What Do We Think We Know?

Given the lack of direct fossil evidence, indirect approaches to the evolutionary origin of glia must be used. Four such approaches will be considered: (1) a forward phylogenetic approach identifying at what node or nodes in the phylogenetic tree of extant organisms glial cells can first be recognized; (2) a developmental approach, in which the pathways followed by adult glial cells are traced embryologically to suggest possible evolutionary paths; (3) a reverse evolutionary approach in which an ancestral state is inferred from commonalities and backward extrapolation ("retrodiction" in current parlance) from living taxa; (4) examination of possible cases of glial loss to gain insight into the adaptive value of glia, from the other side—when it is no longer needed.

First, we need a definition of "glia" sufficient to determine presence or absence. Furthermore, physiological, molecular, and even morphological properties of the descendants of the first glial cells may be very different from their ancestral properties. The situation is not helped by the difficulties researchers have had in arriving at a consistent set of criteria for the multiplicity of glial cell types found in invertebrates (Radojcic and Pentreath, 1979; Roots, 1986). Different types of nonneuronal cells in greater or lesser association with the nervous system may or may not be referred to as "glial," and these may or may not be related to each other. Such issues make the identification of the "first" glial cell or cells an elusive target. Keeping in mind this caveat, the existence of nonneuronal cells closely associated with neurons and not present outside of the nervous system was recognized clearly long before the physiological or molecular characteristics of glia were known. The broad definition of "neuroglia" advanced by Bullock and Horridge is "Any nonnervous cell of the brain, cords,... ganglia..., and ... peripheral nerves, except for cells comprising blood vessels, trachea, muscle fibers, glands, and epithelia ... roughly ... connective tissue associated with nervous tissue ..." This makes no assumption about homologies nor does it ensure that "glial" characteristics will be uniform among different taxa. This is viable provided the organisms have discrete nerves, brains, cords, or ganglia. It differs from the definition used in vertebrates, in which collagen-secreting connective tissue of the nervous system (e.g. meninges) is excluded as "glia" (Roots, 1978). Glia researchers also currently exclude from that definition cells of demonstrably mesodermal origin, regardless of their degree of association with neurons. With these ideas in mind, then, when and how might glial cells have arisen?

Phylogenetic Approaches: Distribution of Neuroglia

As well stated by Morris et al. (2007): "As we have no direct access to the ancestral organisms that lived during earlier phases of evolution, taking a comparative route and paying attention to seemingly 'primitive' organisms that may have retained more of the original characters of the ancestors appears as the next best approach." Pursuing this principle, I first review the morphological, cytological, and molecular characteristics ("markers") by which we might recognize "glia" and then turn to an accounting of how they play out in different "primitive" taxonomic groups.

Morphological glial markers. Bullock and Horridge (1965; pp 95 ff) set down several unifying morphological characteristics, generally confirmed by subsequent authors, that can be used to identify candidate glial cells on morphological grounds in most of the more "advanced" invertebrates:

One type of glial morphology was flat or cuboidal, with cells arranged around the periphery or central canal of a central nervous structure to generate ensheathments or "capsules." The outer "perilemma" cells (also termed "barrier glial cells" in insects) form a layer interposed between blood and neurons (Pentreath, 1989). Inner "ependymal" cells line the hollow nerve cord of chordates.

A second cellular morphology is one of close investment of neuronal somata, with fine processes infiltrating into the cell rind regions or in nerve bundles within peripheral nerve and central connectives. The processes form complex branching patterns of thin cytoplasmic sheets sandwiched between adjacent neural or glial processes and filling the space between axons. This lends a characteristically irregular shape to the cell, distinct from the rounded form typical of neuronal processes cut in cross section. Thus *close exclusive association* with neurons is a defining character of such glia. So close is the apposition (ca. 20 nm typically) that at low magnification, glial cells appear to "wet" neuronal membrane. For medium to large neurons almost *complete coverage* of the neuronal surface is characteristic. This may go so far as to include formation of invaginations, termed "trophospongium," into the larger somata and even axons.

A variation on these morphologies is a multilayered one with cells flattened around axon bundles and individually around the larger axons or somata. In central neuropil, such layers may subdivide different regions. Among smaller neuronal processes, these *compartmentalize naked neurites*, with the two margins of the enveloping glial membrane meeting without fusing but typically forming a narrow channel termed a "*mesaxon*" giving the surrounding interstitial fluid access to that within the bundle. Around larger axons, such cells form discrete *sheaths* consisting of multiple layers of glial cytoplasm sometimes but not always interlaminated with fibrous or afibrous, *extracellular matrix* (ECM). In some taxa, tight appositions of membrane between adjacent layers, eliminating virtually all extracellular and in some cases intracellular space, form *myelin sheaths*,

termed by Bullock (2004) "the most dramatic saltation so far known in the evolution of neuroglia."

Using morphological markers for assessing occurrence of glia is not without its difficulties. Among other caveats is that the stereotypical forms laid out above as characterizing "glia" are likely to occur only in cases of better developed glia, and further, that cells other than glial cells may possess similar characters, especially in more basal groups. The extensive studies needed to reduce such uncertainty, including serial electron microscopic sections, are rare among more basal taxa.

Cytological glial markers. Cytological characteristics, particularly cytoplasmic inclusions visible in the electron microscope, are another key to distinguishing glial from neuronal profiles and provide as well an assist in inferring function (Roots, 1978). Axons are characterized by microtubules of some 24 nm diameter. Glia may have microtubules, but in some taxa, are more likely to contain intermediate filaments 5–10 nm in diameter. Glial cells are likely to contain granules some 15–40 nm in diameter identified as glycogen. While both glial and neuronal perikarya have ribosomes, endoplasmic reticulum (ER), and golgi apparatus, glial processes are more likely to contain such organelles They also frequently have vesicles with or without visible contents, termed variously "gliosomes," "lysosome-like bodies," or "lysosomes." The latter are quite variable in appearance and must contain "an appropriate complement of acid hydrolases" to be so identified (Roots, 1978). Other inclusions are "dense bodies," multivesicular bodies and bodies with partially-digested cytoplasmic organelles, some that relate to phagocytic activity or to uptake of material of extracellular origin (Bullock and Horridge, 1965). The distribution of these morphological glial markers among different taxa is useful from an evolutionary perspective. They have been tabulated by Roots (1978) and more extensively by Radojcic and Pentreath (1979). Table 1 presents this tabulation with some additions. Both reviews pointed out that the absence of filaments from the Arthropoda and their presence in the Lophotrochozoa is striking, with the opposite pattern for microtubules. The Deuterostomia possess both: filaments occurring in the ectoneural system of echinoderms and astrocytes and ependyma of chordates, while microtubules occur in echinoderm hyponeural system and chordate oligodendrocytes. Thus, we must expect considerable evolutionary divergence in the distribution of different glial markers among present-day taxa. Altered inclusion content in developing neurons and glia renders the task even more difficult without molecular markers.

Molecular glial markers. Genes controlling body-plan development, including that of the nervous system, are conserved over a broad range of taxa, extending back to (e.g. Ramachandra et al., 2002) and even before the emergence of the basal bilaterians (e.g. Bebenek et al., 2004). Conservation applies not only to genes associated with nervous system layout but to those involved in such specifics as sense-organ construction, e.g., *Pax-6*, *atonal*, and *sine oculis* (Bebenek et al., 2004). One implication of this conservation is that the common ancestor of the Bilateria possessed orthologs with similar function, passing them along to many of the present-day bilaterian clades. Similar commonalities have been sought in investigating glial cells. Studies on both vertebrates and invertebrates have included identification of "glial markers" that are used for determining which cells in a developing organism are destined to become glia, and which to become some other cell type. Establishing the evolutionary emergence of a glial marker and reconstructing a common ancestral form from extant forms should thus be a useful approach in clarifying glial evolution.

A long list of molecular "glial markers" has been assembled for both vertebrates and *Drosophila* (Roots, 1981, Stork et al., 2010). Unfortunately, few of them have been searched for in other invertebrate taxa. The few that have been will be considered. Three vertebrate glial markers have been investigated broadly in invertebrates (Wang and Bordey, 2008): *glial fibrillary acidic protein* (GFAP) makes up intermediate filaments that are well-expressed in fibrillar astrocytes and transiently in ependymal and tanycytic glia; *glutamine synthetase* (GS) is expressed in glial cells, especially astrocytes, that take up glutamate released by neurons, convert it to glutamine, and supply it back to neurons; and *S100B* is a calcium-binding protein expressed in some astrocytes (Roots, 1981). Genes for canonical S100 have failed to turn up in *Drosophila* and *Caenorhabditis* (Donato, 2001), but immunoreactivity to it has been reported in some invertebrate groups (see below). Two markers used in *Drosophila* are of particular interest: *repo* (reversed polarity) is a homeobox gene found in nearly all adult as well as embryonic glial tissue except for midline glia (Xiong et al., 1994); *gcm* (glial cells missing) is a transcription factor that controls *repo* expression and determines whether a progenitor cell will become glial rather than neural (Akiyama et al., 1996; Freeman et al., 2003)—however, it can be expressed in cells other than glia (Lee and Jones, 2005).

Glial phylogenetics. The phylogenetic approach asks "Which extant taxa possess 'glia'?" It utilizes the characteristics enumerated above to examine the purportedly more basal taxa to establish a taxonomic divide between the "haves" and the "have nots," thereby locating the phylogenetic origin of the innovation (Fig. 1). Among the Metazoa basal to the Bilateria, neither Poriphera (including sponges) nor Placozoa have nervous systems (e.g. Miller and Ball, 2006). The Ctenophora (comb jellies) have a general subepidermal plexus of neurons with elongate axons but no cells ensheathing the neurons that might qualify as glia (Bullock and Horridge, 1965) The Cnidaria are also diploblastic, with a well-developed nerve net in the epidermis and another one in the gastrodermis with communication at the stomodeum and across the mesoglea at points (Bullock and Horridge, 1965; p. 475; Mackie, 2003) They conduct with nerve impulses and transmit with synapses but neither axons nor somata are closely associated with cells that might be assigned a glial function (Horridge et al., 1962; also see Fig. 2.45 of Bullock and Horridge, 1965; Lentz and Barnett, 1965; Radojcic and Pentreath, 1979). Some

TABLE 1. Glial Markers by Taxon

			Form							Junctions					Organelles						Molecular					
TAXO SUBTAXON	Glia	Reference	Adneuronal	Attenuated processes (lamella	Bundle axons	Secrete ECM	Generate ensheathments	Tropospongium	Reference	Tight (zona occludentes)	Gap	Septate	Desmosome-like (zona adherens)	Reference	Intermediate filaments	Microtubules	Glycogen granules	Lysosomes	Granules (Ribo/Gliosomes etc)	Reference	GFAP	Glutamine synthetase	S100b	Repo	Gcm	Reference
Deuterostomia																										
Chordata																										
Craniata	aemos		+	+	+	–	+			+		+			ae	o	+	+	+	Roots78	ae	ae	a	–	–	Roots81
Cephalochordata	ero	Lane	+	+					Lacalli	+	±	?		Soledad	+	–	–	–	+	Lacalli						
Urochordata	±	Koy, Mein																								
Ambulacraria																										
Echinodermata	?	Mash/Cobb	–	–	–		–		Mash/Cobb						ecto	hypo			+	Roots78						
Hemichordata	?	Benito																	+	Roots78						
PROTOSTOMIA																										
Ecdysozoa																										
Panarthropoda																										
Arthropoda																										
Insecta	+	B&H p97	+	+		+	+	+	Lane, Baner	+	+	+	+	Lane	–	+	+	+	+	Roots78	–		–			Doherty, Endo
Crustacea	+	B&H p97	+	+	+	+	+	+	B&H p902				+	Lane	–	+	+		+	Roots78	+	+	+			Florim, Allodi
Arachnida	+	B&H p97								+	+	–		Lane	–	+	+		+	Roots78						
Xiphosura	+	B&H p97								(+)	+			Lane			+	+	+	Roots78						
Onychophora	+	B&H p97				+	+		Lane 87	–	–	+		Lane94												
Tardigrada	+	Dewel	+	+?	–?	–?	–		Dewel																	
Cycloneuralia																										
Nematoida	+	Wright					+	+	R&P						+	+		+	+	R&P						
Scalidophora																										
Priapula	t	Rehk|Storch.																								
Loricifera	t	Kristensen																								
Kynorhyncha	–																									
Chaetognatha	+	Shinn		+	+		+																			
Spiralia																										
Trochozoa																										
Mollusca	+	B&H p97	+	+	+	+	+	+	R&P						+	rare	+	+	+	Roots78	+	+	–			dosS, Card, R81,Kub
Sipuncula	+	Rice	+		+		+		Rice						+?				+	Rice						
Annelida	+	B&H p97	+	+	+		+	+	Fern		+		+	Cogg/Lane	+	rare	+	+	+	Roots78	+	?	–			Riehl, Niva, Endo
Nemertia	+	B&H p97	+		+				Turb						+				+	Turb	+?					Salnik
Lophophorata																										
Brachiopoda	+	James			+				James										+	James						
Phoronida	+	Temereva					+		B&H						+	+	+		+	Temereva						
Polyzoa																										
Entoprocta	–	Nielsen																								
Bryozoa	–	Guhl		+?			+?		Lutaud																	
Platyzoa																										
Gastrotricha	2	Teuchert	+		+				Teuchert										+	Teuchert						
Platyhelminthes																			+	Roots78						
Cestoda	4	Biserova	+	+	+	+	+		Biserova						+		+	+	+	Biserova			+			Biserova
Tricladida	+	Golubev	+	+	–	–	–	–	Golubev						(+)	+	±		+	Golubev			+ns			Kerschbaum
Polycladida	+	Koop	+		+		+	+	Koop																	
Rhabdocoela	–	Golubev																								
Macrostomida	–	R&G																								
Catenulida	–	R&G																								
Gnathifera																										
Rotifera	–	Clement																								
Acoelomorpha	+	Bery	+	+			(+)		Bery																	
Cnidaria	–	B&H																								
Ctenophora	–	B&H																								

a, astrocytic; e, ependimoglial; ecto, ectoneural system; hypo, hyponeural system; o, oligodendrocytic; m, microglia; s, Schwann cells; r, radial glia; t, tanycyte-like glia; +, present; −, absent; ±, sometimes present; ?, assignment uncertain; +ns, in nervous system (location undetermined). Allodi = Allodi et al. (2006); Baner = Banerjee and Bhat (2007); Benito = Benito and Pardos (1997); Biserova = Biserova et al. (2010); B&H = Bullock and Horridge (1965); Card = Cardone and Roots (1990); Cogg = Coggeshall (1974); Cobb = Cobb (1989); Dewel = Dewel et al. (1993); Doherty = Doherty et al. (2009); dosS = dosSantos et al. (2005); Endo = Endo and Endo (1988); Fern = Fernandez et al. (1992); Florim = Florim da Silva et al. (2004); Golubev = Golubev (1988); Guhl = Guhl and Bartolomeus (2007); James = James (1997); Kerschbaum = Kerschbaum and Hermann cited in Reuter and Gustafsson (1995); Koop = Koopowitz (1989); Koy = Koyama and Kusoniki (1993); Kristensen = Kristensen (1991); Kubista = Kubista et al. (1996); Lacalli = Lacalli and Kelly (2002); Lane = Lane (1981); Lane87 = Lane and Campiglia (1987); Lane94 = Lane et al. (1994); Lutaud = Lutaud (1977); Mash = Mashanov et al. (2009); Mein = Meinertzhagen et al. (2004); Nielsen = Nielsen and Jespersen (1997); Niva = Niva et al. (2009); R81 = Roots (1981); Rehk = Rehkämper et al. (1989); R&G = Reuter and Gustafsson (1995); Riehl = Riehl and Schlue (1998); Roots78 = Roots (1978); Salnik = Salnikova and Golubev (2003) = cited in Biserova et al. (2010); Soledad = Soledad and Anadon (1989) (gap jcts: +); Lane (1987) (gap jcts: −); Storch = Storch (1991); Temereva = Temereva and Malakhov (2009); Teuchert = Teuchert 1977 cited in Ruppert (1991); Turb = Turbeville (1991); Wright = Wright (1991).

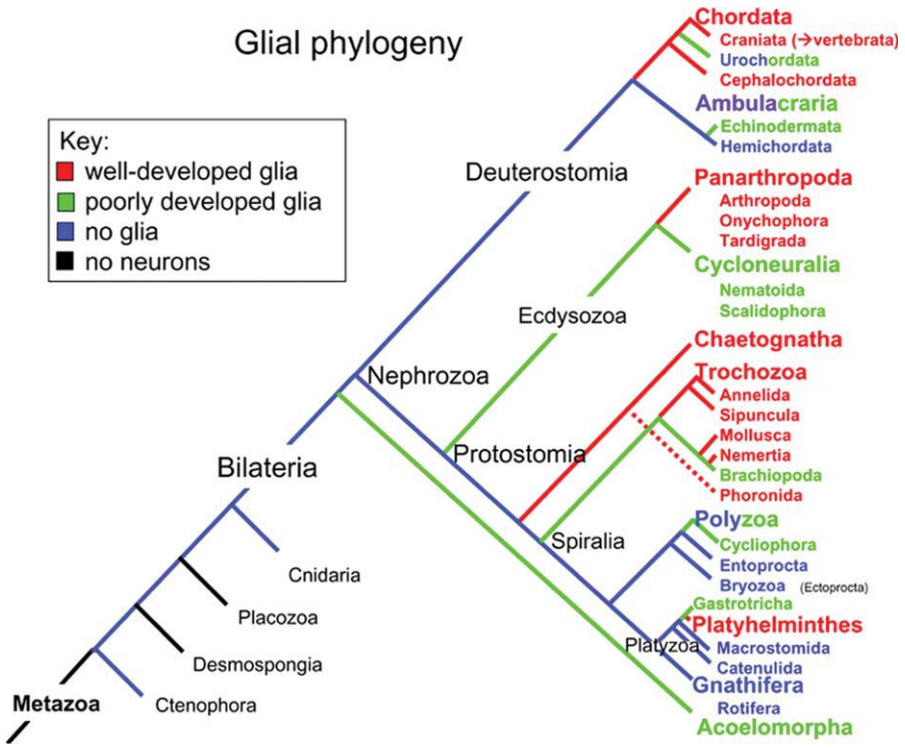

Fig. 1. Glial phylogeny. Phylogenetic "tree" of various living bliaterian taxa, coded for absence of glia (blue), poorly developed glia (glia not as extensively developed and elaborated as in higher taxa, a somewhat subjective measure: green), well-developed glia (red). Where a mixture of glial types is reported in major subtaxa, taxon names are bicolored. Phylogeny based on Hejnol et al. (2009) with the exception that the Phoronida have been placed in their classic position among the Lophophorata, which the cited article did not recognize (but see Brachiozoa: Cavalier-Smith, 1995; Hejnol, 2010). Authority for most of these assignments will be found in Bullock and Horridge (1965; pp. 97–98) and in the taxon-specific chapters. For those not found there, they are as follows: Cephalochordata (Lane et al., 1987), Echinodermata (Mashanov et al., 2009) Onychophora (Bullock and Horridge, 1965, p. 794), Chaetognatha (Shinn, 1997), Annelida no GFAP (Luo et al., 2002), molluscan GFAP (Cardone and Roots, 1990; dosSantos et al., 2005) Gastrotricha (Ruppert, 1991), Rhabdocoela (Golubev, 1988), Rotifera (Clément, 1977), Acoelomorpha (Bery et al., 2010). From www.pbrc.hawaii.edu/~danh/GliaEvolution/with permission.

glia-like cells have been reported in the ganglia of marginal bodies of scyphomeduasae, but their exact relation to glia in the Bilateria, if any, is unclear (Bullock and Horridge, 1965). Thus, the most basic features of nervous system function were present prior to the appearance of glia.

In contrast, the more derived taxa in each of the three eubilaterian branches of the Metazoa have neurons closely associated with nonnervous "supporting cells" or "connective tissue" that are usually referred to as "glia." Bullock and Horridge (1965; p. 101) suggest that taxa possessing intraepithelial nervous systems, being usually viewed as "primitive," are particularly good candidates for lacking glia. Other typically basal features include paucity of different neuronal cell types, multipolar neuronal morphologies and a network-like organization of the nervous system. Several basal taxa have nervous systems that appear to approach those of the preglial state.

Lower Deuterostomia

Cephalochordata. This taxon is currently placed at the base of the Chordata. Its hollow dorsal nerve cord reflects its kinship to vertebrate nervous systems. The nervous system is organized as a neuroepithelium, with the apical poles of cells, neurons and glia, ending on the hollow neurocoel that runs along the middle of the cord, and basal poles resting on the exterior basal lamina. The neural tube, peripheral nerves, and epidermis are bordered by a continuous basal lamina that separates them as a single tissue from the rest of the body (Ruppert, 1997). Several types of glia are described by Lacalli and Kelly (2002) in the anterior neural tissue of amphioxus larvae. The ependymoglia are characterized by prominent filament bundles, similar to vertebrate radial glia. A second glial group was large, and mostly devoid of cytoplasmic structures, these being chiefly "golgi, mitochondria, and scattered vesicles." A third class, "axial glia," they suggest to be related to oligodendrocytes, but with a primary role in axon guidance. The cephalochordate amphioxus retains the gene *repo* in its genome, albeit its expression has not been reported yet. The gene is lost in the urochordates and craniates (Holland et al., 2008). Although basal among chordates, the living cephalochordates have undergone extensive glial diversification, and hence are already far from the evolutionary origin(s) of glia in deuterostomes.

Urochordata. Under current phylogenies, this group, which includes the tunicates, is the sister taxon to the vertebrate line (including hagfish and lampreys). While the larvae are actively swimming "tadpole" forms, the

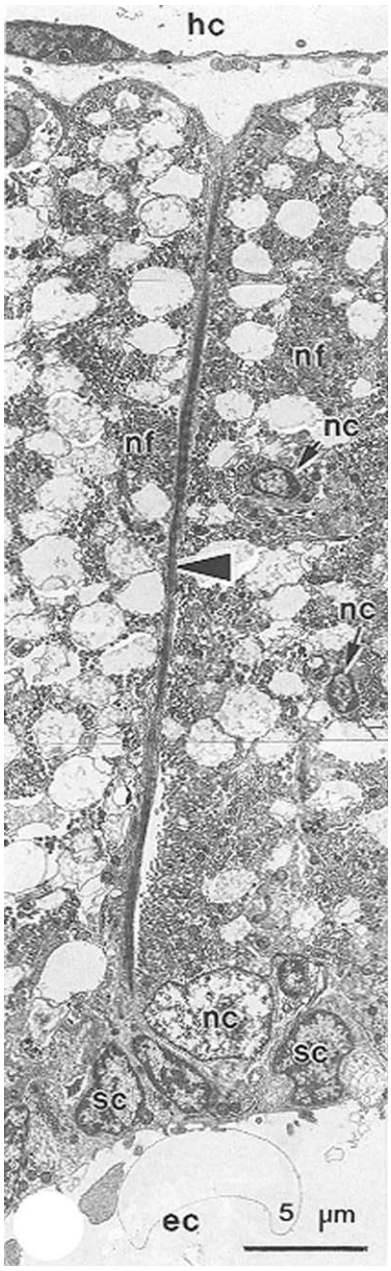

Fig. 2. Echinoderm glia-like supporting cell. Electron micrograph of a section through a sea urchin (*Mespilia*) radial nerve cord showing a fibril-filled support cell extending from the apical to the basal surface of the neuroepithelium. ec, epineural canal; hc, hemocoel; nc, nerve cell; nf nerve fibers. From Cavey and Märkel (1994) with permission.

adults are all sessile, with nervous system features reduced owing to their sedentary habit. The CNS of the adult consists of a single cerebral ganglion buried in mesenchyme and surrounded by a connective tissue sheath (Bullock and Horridge, 1965). The ganglion has the standard invertebrate structure of an outer cell body cortex or rind and an inner fibrous medulla. Bullock and Horridge (1965) suggested that a few nuclei in the core of the ganglion are probably glial, but a subsequent detailed electron microscopic study by Koyama and Kusunoki (1993) found only putative hemocytes and no glial cells. Similarly, in the peripheral nerves, no Schwann cells were found, although the nerves were consistently wrapped by collagenous sheaths (Lane, 1972). The larval nervous system, which for *Ciona* contains only ca. 330 neurons (Meinertzhagen et al., 2004), originates from a neural plate that rolls up into a neural tube. This results in the neural tube being lined with ciliated nonneuronal "ependymal" cells, one of the glial classes in vertebrates. Metamorphosis entails major rearrangements and apoptosis of larval neural tissue (Meinertzhagen et al., 2004). The fate of the ependymal cells seems unclear.

Echinodermata. The adult forms of these organisms have three semiseparate nervous subsystems, the "aboral" (or "apical"), the "ectoneural" (sensory and interneuronal), and the "hyponeural" (motor) arranged in a circumoral ring, usually with five radial cords (Cobb, 1995). Neurons are either intraepithelial or scattered along nerve tracts. The fine structure of the nervous system of the most basal class, the Crinoida, has not been well studied. In more advanced classes, there are differences of definition regarding the glial situation. Some investigators have concluded that glial cells are absent altogether (Cobb, 1989). However, the neuropil of the aboral nerve plexus of asteroids is wrapped by slender granule-containing cell processes possibly elaborations of the epidermal supporting cells (Chia and Koss, 1994). Basal processes of epidermal support cells run alongside, and may be hard to distinguish from, axons of the basiepithelial plexus of the ectoneural system in ophiuroids (Byrne, 1994). Asteroids (Chia and Koss, 1994) and holothuroideans (sea cucumbers) (Mashanov et al., 2006, 2009) have elongate nonneural cells ("Stützzellen"), resembling the radial glia of vertebrates, characterized by bundles of intermediate filaments (Bullock and Horridge, 1965; p. 1525). Like radial glia, the cell bodies are located in the apical region, of both the ectoneural and hyponeural neuroepithelia. Their elongate basal processes span the distance from the apical region to the lumen of the epineural or hyponeural canals. In echinoids, Cavey and Märkel (1994) describe similar filament-rich "support cells" of the ectoneural neuroepithelium of the radial nerves (echinoids lack a hyponeural system) (Fig. 2). A homolog of the molecular marker *gcm* (*spgcm*) is present in the sea urchin, albeit not in the nervous system. It controls the development of mesodermal pigment cells (Ransick and Davidson, 2006). Given the likelihood that what might be identified as "glia" in echinoderms lacks the all-neuron-encompassing forms that typify glia in more advanced taxa, it appears that although rather slow, complex, well-functioning nervous systems can be constructed without well-developed glia. The situation was aptly summed up by Radojcic and Pentreath (1979): "Should an as yet undetermined glial function be demonstrated for these cells they will be the most primitive glia amongst invertebrates. . ." The larval nervous system appears to be completely replaced at metamorphosis (Elia et al., 2009), and its glial situation remains to be determined.

Hemichordata. This second branch of the Ambulacraria includes enterpneusts ("acorn worms") and ptero-

branchs. The nervous system is simple, being characterized by Bullock and Horridge (1965) as "There is no central nervous system proper. . ." The most concentrated region is that of the collar nerve cord, which is a submerged epidermal strip. As is characteristic of other basal taxa, the nervous system consists mainly of a basiepidermal plexus (that is, nerve cells and processes are confined to the deeper part of the epidermis, adjacent to the basement membrane). In the "collar nerve cord," the epidermal thickening containing the nervous elements separates from the surface and enters the collar coelom for the length of the collar. In passing reference to "glia," Benito and Pardos (1997) write "Beneath the epidermal cells and among their bases, a neural layer comprised of [sic] neuronal cells, glial cells, and nerve fibers occurs." The evidence presented is an electron micrograph of a submesothelial bundle of unmyelinated axons with a tenuous envelopment by a mesothelial (/epithelial) cell, a cell that presumably performs other functions in the epithelium in which it resides. In general, the neurons of the hemichordates seem to be without dedicated companion glia.

Lower Ecdysozoa

Cycloneuralia. As with the Deuterostomia, understanding of the phylogenetic relationships among different branches of extant ecdysozoans is currently in flux. Advanced taxa (especially the Arthropoda) uniformly have well-developed glia by morphological, developmental and molecular criteria. The molecular markers *repo* and *gcm* from *Drosophila* are more likely to be reliable glial markers in ecdysozoans than nonecdysozoans, although there are few studies available. The gene for the vertebrate marker GFAP is missing in *Drosphila* (Doherty et al., 2009), and intermediate filaments are generally absent from the Arthropoda (Roots, 1978, but see Allodi and Taffarel, 1999), yet GFAP immunoreactivity has been reported in crabs (Florim da Silva et al., 2004) as has S100 immunoreactivity (Allodi et al., 2006). Glutamine synthetase immunoreactivity has also been reported in glia of decapods (Allodi et al., 2006; Linser et al., 1997) so these "glial" markers would be worth seeking in more basal ecdysozoan taxa. Candidates for reflecting "primitive" conditions in this group are:

Onychophora. These lower Panarthropoda show well-developed glia, but lack a blood–brain barrier (Lane and Campiglia, 1987). They also apparently lack the gap junctions and tight junctions that are typical of a lot of glia (albeit their epithelia possess apical *zonulae adherentes*), but possess septate junctions in some tissues (Lane et al., 1994). While basal among the Panarthropoda, they appear to represent a stage well after the origin of glia in the Ecdysozoa.

Scalidophora (Priapula, Loricifera, Kinorhyncha). This group of three mostly marine phyla of minute organisms is unified morphologically by possession of spiny sensory scalida borne on an extensible proboscis, the "introvert." They share having a sparse mostly intraepidermal nervous system that includes a ventral nerve cord as a midline epidermal structure. The cord is medullary (neurite core with a cortex of somata) and quasisegmental. Somata are located in a ventral column on each side (Bullock and Horridge, 1965). In the priapulids (Rehkämper et al., 1989) and the loriciferans (Kristensen, 1991), modified fiber-bundle-containing epidermal cells, described as "tanycyte-like glial cells" (elongate radial-glia-like ependymal cells especially noted in fish and amphibians, e.g. Roots, 1978) are present in the ganglia, running through the nervous system to attach to muscles nearby. There does not seem to be much glial development beyond these, and glia appears not to have been described in the kinorhynchs.

Nematoida. Nematoida will be discussed elsewhere in this issue. Much molecular and genetic work has been possible with this taxon. Its glial cells wrap axons and neuronal somata and produce tropospongium in the latter (Chitwood and Chitwood, 1950; Bird, 1971, cited in Radojcic and Pentreath, 1979; Bullock and Horridge, 1965; p. 615). It seems to have well-developed, nonbasal glia, but relatively few in numbers. In *C. elegans*, most of these glial cells are dedicated to sensory cell support, thus much of the nervous system must manage without glia. It might thus serve as a model for early functions of basal glial (Heiman and Shaham, 2007).

Lower Lophotrochozoa: Trochozoa

The classification of the Lophotrochozoa, too, is in constant flux as more molecular markers are added to their database (e.g. Halanych, 2004). Traditionally, it is split into two main branches the Trochozoa (having trochophore larvae) and the Lophophorata (having a ciliated lophophore as a feeding structure). Advanced taxa (Mollusca; Annelida) uniformly have well-developed glia by morphological criteria (Coggeshall, 1965, 1967; Golding, 1992; Fernández et al., 1992). GFAP immunoreactive labeling has been reported in gastropods, cephalopods and leeches (Cardone and Roots, 1990; dosSantos et al., 2005), as has glutamine synthetase (GS) labeling in *Aplysia* (N.D. Norenberg and B.I. Roots cited in Roots, 1981). GS expression has been reported in annelid nervous system, but not yet localized (Niva et al., 2008). S100-like immunoreactivity has been reported in annelid neurons but not glia (Endo and Endo, 1988). Basal clades occur in both groups. Among the Trochozoa, these include:

Sipuncula. (Reviewed by Rice, 1993.) This taxon of worm-like creatures has a standard brain and ventral nerve cord (VNC) akin to that found in annelids. It includes many neuronal cells types and has well-developed neuropils. Somata are clumped, unlike the scattered cells typical of lower forms. There is typically a well-developed connective tissue sheath, surrounded by peritoneal cells, around the VNC and nerves and forming a capsule around the brain. Inside of the VNC, the sheath is continuous with a network of fiber-containing glial cells that pervade the cord, albeit less profuse than in annelids and arthropods. Banded glial cell tonofilaments have been noted in nerves exiting the brain. Glial

cells are distinguished from neurons in having denser nuclei and large cytoplasmic granules. Thus, this group seems well along on the path of developed glia, and distant from the ancestral condition.

Nemertea. This enigmatic taxon of unsegmented vermiform "ribbon worms" has defied attempts to conclusively locate it phylogenetically. It has recently been included with the Brachiozoa (Brachiopoda + Phoronida) in the trochozoan clade "Kryptrochozoa" (e.g. Hejnol, 2010). Its nervous system is not particularly complex, and in the more basal members it is submerged in the epidermis. However, the prevalence of unipolar neurons as compared with the multipolar ones of the platyhelminths may indicate a more advanced design (Bullock and Horridge, 1965; p. 583). The brain and nerve cords have connective tissue capsules (neurilemma), and the last-cited authors refer to a "limiting layer" of glia separating the nerve cell body rind from the fibrous coat. Cells containing distinctive pigment granules are found in association with extracellular matrix, epidermis and gastrodermis. Similar granule-containing cells identified as "glia-like" make close contact with neurons as well. Together they form a system that has been suggested as similar to the "glio-interstitial" system of annelids and molluscs (Turbeville, 1991). GFAP labeling has been reported in the nemertian *Lineus gesserensis* (Salnikova and Golubev, 2003, cited in Biserova et al., 2010). At this juncture, there seems not to be enough information available on nemertian "glia" and phylogeny to position it in the evolutionary scheme for invertebrate glia. Nevertheless, the evidence for quasi-glial cells involved in other-than-nervous-system support suggests a route through which more specialized nerve-only glial cells of more advanced taxa might have arisen.

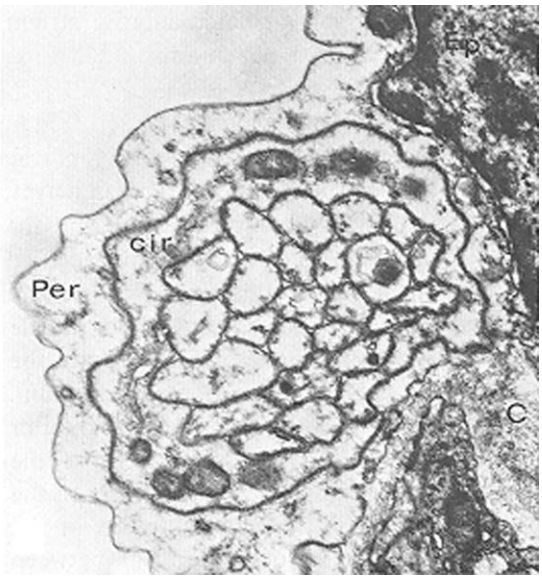

Fig. 3. Bryozoan nerve bundle. Electron micrograph of transverse section through the mixed nerve in the tentacle sheath from *Electra pilosa* showing partial investment by the peritoneal layer (Per) and a "circumadjacent element of the nerve bundle," possibly an oddly shaped giant axon. C, collagen. From Lutaud (1977) with permission.

Lower Lophotrochozoa: Lophophorata

This clade also is in flux, but from time to time, it has included ectoprocts (Bryozoa), entoprocts, brachiopods, and phoronids. These are sedentary animals feeding by means of a ciliated sheet or tentacle-like "lophophore" and having a simple, probably reduced, nervous system.

Bryozoa. In this group, a ganglion lies in a basiepithelial position between the epidermis and somatopleure (not in the coelom). Glial tissue has rarely been described. A ganglionic sheath consisting of a single layer of flattened cells was mentioned by Bullock and Horridge (1965; p. 633) and described as "a thin stratified envelope separating the nervous tissue from the peritoneal lining" by Lutaud (1977). Mention of "investing glial cells" as one of three cellular morphotypes in the small (fewer than 50 cells) cerebral ganglion of a gymnolaemate species is made by Lutaud (1977), and she states as well that "the possibility of a neuroglial sheath has to be considered in the case of the great mixed peripheral nerves in the tentacle sheath." However, Gruhl and Bartolomaeus (2008), in a careful electron microscopic examination of the neuroepithelial organization of the cerebral ganglion of a different class (Phylactolaemata) looked for and failed to find glial cells. The published electron micrographs of the ganglion as well as of the peripheral nerves show bundles of naked axons without sheaths, or surrounded by peritoneal or epithelial cells. One curious cell investing the tentacle sheath nerve of a gymnolaemate has the cytological appearance of a neuron, and may be an oddly-shaped giant axon rather than a "primitive glial cell" (Lutaud, 1977; Fig. 3). The evidence for glia in bryozoans seems tenuous at best and clearly needs more extensive study (also see Mukai et al., 1997).

Phoronida. Phoronida are worm-like tubicolous filter feeders possessing a true nerve net and usually included among the lophophorates on morphological grounds (but see Hejnol et al., 2009; Hejnol, 2010). According to Bullock and Horridge (1965), their nervous system is the most superficial among lophophorates. All nerve cells are intraepithelial and this does not appear to be a secondarily-derived simplicity. They have three types of nerve cells in addition to sensory cells. A giant fiber is surrounded by a thick sheath of concentric laminae, fibrillae, and flattened nuclei. Temereva and Malakhov (2009) in an electron microscopic study of *Phoronopsis* described a nervous system composed of three cellular layers: first, a layer of nerve processes surrounded by glia; second, a layer of glial perikarya; and finally a layer of neuron cell bodies overarched by epidermis. In the neural plexus of the integument, the glia-like cells contain "numerous electron-dense granules 150–300 nm in diameter" that were characteristic of the cells, and their processes "run between the nerve fibers" of the plexus. For a supposedly primitive intraepithelial nervous system, this is a surprising level of glial development. Such a placement within the epithelium raises problems for scenarios of original glial origin. The place-

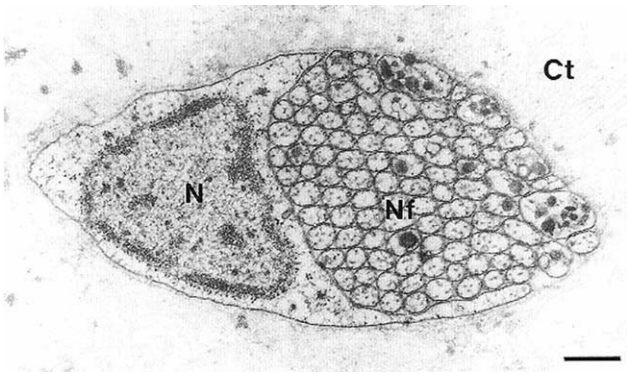

Fig. 4. Brachiopod nerve bundle. Electron micrograph of transverse section through a bundle of unmyelinated nerve fibers (Nf) of *Calloria inconspicua* partially enveloped by an accessory cell (N) and embedded in connective tissue (Ct). Scale 0.5 μm. From James (1997) with permission.

ment would reduce the attractiveness of the hypothesis that isolation from supporting-cell contact experienced by a central nervous system dissociated from epidermal interactions is a necessary glia-inducing condition.

Brachiopoda. Classed with the phoronids in the Brachiozoa (Cavalier-Smith, 1995), this ancient, once highly successful group of lophophore-bearing organisms inhabits bivalved shells resembling those of clams and oysters. Parts of its nervous system are basiepithelial, and those nerve bundles have no sheaths or other glial associations. However, subepithelial nerve bundles are partially surrounded by glial cells having prominent nuclei and containing rough ER, electron opaque granules, free ribosomes, and membrane-bound vesicles (James, 1997; Fig. 4). This "glial" involvement seems to be restricted to investing axon bundles. Details on the ultra structure of the ganglia were not given.

Entoprocta. This is a small taxon of microscopic, often colonial, organisms with a trochophore larva and spiral cleavage. A review of the entoproct microanatomy by Nielsen and Jespersen (1997) describes briefly the presence of a pair of ganglia connected by a commissure, but with no mention of glia, nor are such cells evident in the electron micrograph they present.

Cycliophora. This recent addition to the lophophore-bearing phyla (classed as a poloyzoan by Hejnol et al., 2009) is commensile on the mouth parts of lobsters. Surprisingly for its microscopic size, it possesses a large brain. Funch and Kristensen (1997) examined the brain of an attached dwarf male and describe it as containing two clusters of 12–15 nuclei each connected by a large commissural neuropil ensheathed by three or four glial cells. Elsewhere, glial cells were uncommon.

Lower Lophotrochozoa: Platyzoa

The animals of this group have traditionally been classified as among the more basal bilaterians and hence are key in determining whether glias in diverse taxa are homologous. It is in this group that a compact nervous system seems to have evolved first. Currently linked to the Lophotrochozoa as one of the spiralian clades (e.g. Hejnol et al., 2009), the group includes the taxa Rotifera (Gnathifera) and Platyhelminthes. The latter group, too, has been reorganized according to recent molecular phylogenetics (Ruiz-Trillo et al., 2004). The Platyhelminthes have had the Acoelomorpha separated out (Fig 1). (Egger et al., 2009), leaving them comprising the Catenulida and the Rhabditophora. However, assigning evolutionary relations within these remaining taxa is still difficult. Considered to represent the early architecture of bilaterian nervous systems, theirs includes, beneath the outer layer of epidermal cells and separated by a well-defined basement membrane, a subepithelial plexus and central nervous system, then a muscular layer with a submuscular plexus beneath (Bullock and Horridge, 1965; Lacalli, 1982; Reuter and Gustafsson, 1995). An infraepithelial plexus is present in the basal Catenulida (and some more advanced taxa: Reuter and Gustafsson, 1995). Platyhelminths have a compact internal anteriorly-located brain arranged with a cortex of neuron somata outside of a medulla of neurites, the appearance of which has been linked to the appearance of glia (Bullock and Horridge, 1965). Several submuscular nerve cords proceed outward from the brain and are cross-connected by commissures in a grid termed an "orthogon." Cell bodies are typically scattered along the cord, often at the intersections of the grid. The latter authors indicate, however, that there is still an open question as to what nervous system features are most "primitive."

Rotifera (Gnathifera). Glial cells appear to be absent in this taxon (Clément, 1977 cited by Clément and Wurdak, 1991), albeit a well-developed nervous system is present. The cells surrounding nerves and ganglia are either epithelial or muscular. Presence of a neurilemma is unresolved (Bullock and Horridge, 1965; p. 601).

Platyhelminthes. While different taxa present differing situations with respect to glia-like cells, it is not clear how the pattern relates to the emergence of glia as opposed to its loss, nor will it until the phylogenetic analysis for the group stabilizes. Morphologically identifiable glia has not been described in platyhelminth taxa currently considered most basal, the Catenulida (Littlewood and Bray, 2000; Reuter and Gustafsson, 1995) and Macrostomida (Morris et al., 2004; Reuter and Gustafsson, 1995). It is missing in Rhabdocoels as well (Golubev, 1988).

Reports of glia-like cells occur for the polyclads, flatworms placed basal to triclads in the phylogenomic study by Hejnol et al. (2009). A layer of cells is found surrounding axons in the ganglia and nerve cords, including in some cases, multiple layers and invaginations into neuron somata. Unlike triclads, a sheath is present around the outside of the CNS. Glia-like cells are found in both the plexus and the CNS (Koopowitz, 1989). Although reported absent in one study of triclad planaria (*Procotyla fluviatilis*, Lentz, 1967), Bullock and Horridge (1965; p. 547) summarized the work of several authors indicating as "doubtless glia" a class of "Type G cells" occurring in rows in fiber tracts and applied to the inside of the capsule and along the peripheral trunk,

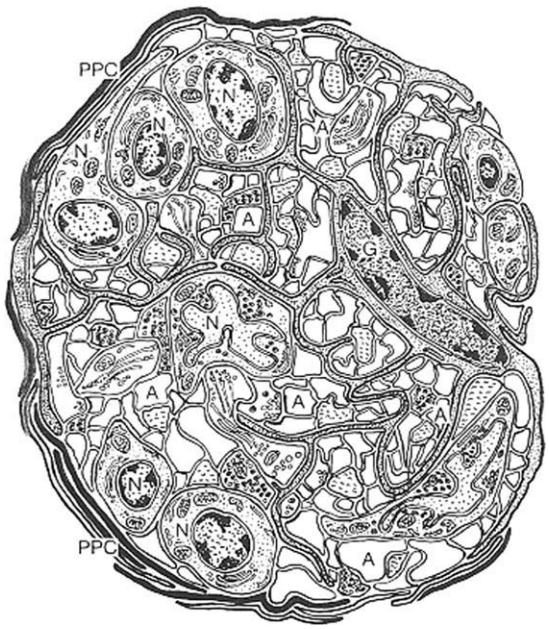

Fig. 5. Planarian (Turbellaria) ventral nerve cord. Diagram of key ultrastructural features. A, axon; G, glial cell; N, neuron; PPC, processes of parenchyma cells. From Golubev (1988) with permission.

and a class of "Type E" supporting cells. Golubev (1988) described glia-like cells in triclads as "randomly scattered in the nerve cords among the numerous nerve fibers and neuron bodies." Morita and Best (1966) describe "accessory cells" that they found indistinguishable from glia in the planarian *Dugesia*, albeit clear images were not presented. A universal character of platyhelminth "glia" appears to be the thin band of cytoplasm surrounding the nucleus, as compared with neurons (Böckerman et al., 1994; Golubev, 1988). The sometimes numerous branches insinuated among axons are described as having typically electron lucent cytoplasm with, in contrast to the vesicle-filled neurons, relatively few organelles (Golubev, 1988; Morita and Best, 1966; Reuter and Gustafsson, 1995), although, in some cases microtubules, endoplasmic reticulum and ribosomes are reported. Golubev (1988) reported that those branches seen reaching the surface of nerve cords form "thin plates," but not a sheath *per se* (Fig. 5). However, he distinguished planarian "glia" from that of vertebrates and more highly organized invertebrates by its "thin cytoplasmic structure": organelles are few and scattered, and cytoplasm often appears empty, an observation confirmed by Böckerman et al. (1994). Morita and Best (1976; cited in Golubev, 1988) observed glycogen granules and vacuoles containing engulfed neuronal parts in "glial" cytoplasm, although this has been challenged by Golubev (1988). A homolog of the glial marker *gcm* is expressed in the planarian *Dugesia* but not in the neural line (Umesono and Agata, 2009). Immunoreactivity to the vertebrate glial marker *S100* is reported in the nervous system of a triclad (Kerschbaum and Hermann cited as a personal communication in Reuter and Gustafsson, 1995). Three homologs of the *Drosophila* gene *orthodenticle*, a homeobox family involved in brain specification in vertebrates and insects, were found in *Dugesia* brain, suggesting that parts of the neural, if not the glial development machinery are held in common across widely divergent taxa (Umesono and Agata, 2009).

Surprisingly, well-developed glial cells of several distinguishable types have been described recently in highly derived but parasitic (and hence supposedly degenerate) Cestoda. Four types were identified by Biserova et al. (2010): multilamellar light-cytoplasm cells of the main trunks, fibroblast-like cells secreting ECM, "sandwich" cells wrapping neuropil with alternating cellular and ECM layers, and even cells forming myelin-like structures. Included were dark and light cytoplasmic varieties and cells showing S100B-like immunoreactivity.

Gastrotricha. This taxon is currently thought to be closely associated with the Platyhelminthes and hence relatively basal. Multipolar spindle-shaped glial cells of perhaps two types are reported in restricted locations: wrapping neurons anterior to the commissure and bounding the dorsal commissure. They are described as containing few internal membranes, some ribosomes, and a mitochondrion but in general have not been well studied (Teuchert, 1977 cited in Ruppert, 1991). The gastrotrich nervous system seems to reflect a state with glia well started but not fully elaborated.

Lower Bilateria: Acoelomorpha

This group of interstitial (sediment-dwelling) flatworm-like animals, while being shuffled from one taxon to another, has long been considered to be at or near the base of present-day bilaterians (e.g. Hejnol et al., 2009) albeit not without uncertainties (e.g. Egger et al., 2009). It has a well-developed bilateral nervous system with a frontal organ and brain, as well as longitudinal nerve cords linked by numerous commissures. However, it also possesses several features that support its claim to a basal bilaterian position, such as a noncompact brain and neuropil, absence of a capsule, and exiting nerve fibers that enter an irregular network of nerves lacking a linear orthogonal organization of commissures and connectives, and thus resembling the nerve nets of cnidarians and ctenophores (Bedini and Lanfranchi, 1991; Ramachandra et al., 2002). An electron microscopic study by Bedini and Lanfranchi (1991) presented an image of a "rare cell type" identified as presumptively glial, with "very few organelles" and some "attenuated cytoplasmic projections extend[ing] into surrounding nervous tissue" (images not shown). They considered these similar to glia described in planarians. Bery et al. (2010) examined a juvenile acoel, *Symsagittifera roscoffensis*, and showed images of electron-dense cells with irregularly-shaped "glial-like" thick "lamellated" processes insinuated into neurite regions of the medullary neuropil. They also reported glial-like electron dense processes forming partial sheaths around and within the nerve cords. While having the appearance of dark glial cells, and indeed

arguably approaching in form that of the "glia" of more derived phyla, the authors point out that ultrastructural evidence alone is not sufficient for certain identification. They gave no further cytological descriptions.

What does phylogeny tell us?

While simple in principle, the analysis of the origin of glia based on a phylogenetic survey produces a somewhat confusing picture. This is partly due to the current uncertainty about deep phylogenetic relationships among basal clades. The current favorites as basal bilaterians are the Acoelomorphs. These primitive animals at least possess glia-like cells, apparently in a supporting role for neurons (Bery et al., 2010), albeit distinguishing glia from neuron based on ultrastructure alone has some intrinsic uncertainties. Many of the studies of basal taxa reviewed here have not focused on a thorough and rigorous examination of purported glia in their material (Table 1). If indeed the cells so identified in acoels are early glial cells, they offer the possibility that all glial cells are related, originating at the very start of the bliaterian line. However, other "basal" bilaterians apparently lack glia. This pattern of possessing or not possessing glia is depicted in Figure 1, placed in relation to a currently proposed phylogeny (Hejnol et al., 2009). Absence of glia is represented by blue color; presence of well-developed glia by red, and intermediate development by green. With glia showing up in the acoels, yet missing from some of the more "advanced" taxa, it would seem that we either must imagine that glia arose multiple times, or that it arose fewer times, e.g. once in the acoels, and then was lost several times. To shed further light on which scenarios are likely, we turn next to the developmental picture.

Developmental Origins

In this approach, we examine where glial cells first appear in development, and how they associate with neurons in the most basal taxa for which the information is available. The idea is that the same sequence may provide a model for where glia arose. As described earlier, most of what has been termed "neuroglia"—in vertebrates, insects, and annelids—is derived from ectoderm. This, broadly, is its undoubted evolutionary origin across taxa, as it is for neurons.

Central nervous systems of most advanced bilaterians are internal or at least subepidermal, requiring a developmental step for removing neuronal precursor cells (NPCs) from the ectoderm and internalizing them. As summarized by Meyer and Seaver (2009), this occurs through neurulation in vertebrates, while in arthropods, there is ingression (inward migration) of neuroblasts out of the epithelium, followed in some by ectodermal overgrowth of neuroectoderm. In molluscs, inward migration of individual or small groups of NPCs populates future ganglia. In leech, a set of ectodermal stem cells termed "teloblasts" give rise to the nervous system and in polychaete annelids ingression is the primary mode. This internalization step may tend to obscure the evolutionary origin of the glial cells that can derive from the same precursor cells.

In the vertebrate CNS, most glia originates from portions of the developing neural tube (the exception being olfactory ensheathing glia: Ramon-Cueto and Avila, 1998), while in the PNS, the Schwann cells originate from neural crest (e.g. Klämbt, 2009). In *Drosophila*, a set of neuroglioblasts of mesectodermal origin arrayed along the midline and distinct from the neuroectoderm, gives rise to a series of glial progeny distinct from all other glial cells in not expressing the transcription factors *gcm* and *repo*. These glial cells provide guidance factors for axons running in the two commissural tracts linking the hemicords on either side of the midline. They end up ensheathing the mature axons in these tracts. At the lateral edges of the neuropil, the axon tracts that run in an anteriorposterior direction in the ventral nerve cord are ensheathed by a second subset of cells, the longitudinal or interface glia. As with vertebrates, there is a separate source for some *Drosophila* PNS glia. Cells born in the periphery follow ingrowing sensory axons to differentiate into glia along peripheral nerves (von Hilchen et al., 2008). The evolutionary equivalent of this pattern might be that epithelial cells in contact with neurons in a basiepithelial plexus might follow the neurons as they become internalized in more advanced taxa. Another possibly relevant feature of developing nervous systems is that in many cases, glial cells migrate along nerve fibers that have already found the proper paths. Thus, glial cells are followers of neurons. With exceptions, many neurons can find their targets even in the absence of glia. Thus, with a defective *gcm* gene, development of some *Drosophila* axons is still normal until the stage where fasciculations normally formed by glia would take shape (Hidalgo and Booth, 2000).

In platyhelminths, nervous system development has some significant differences from the arthropod and vertebrate patterns, beginning which the basal pattern of spiral cleavage of oocytes, shared with the Lophotrochozoa (Hejnol, 2010). The nervous system, too, exhibits both similarities and differences (Hartenstein and Ehlers, 2000; Younossi-Hartenstein and Hartenstein, 2000;Younossi-Hartenstein et al., 2000, 2001). Younossi-Hartenstein et al. (2000, 2001) noted that the nervous systems of some turbellarians continued to develop by the outgrowth of axons following along "pioneer" neurons, which represented a small fraction of the ultimate total of outgrowing axons. The axons initially grew along the surface of the brain and then leaving the brain, traveled in contact with myoblasts. They suggested that the myoblasts might be used for guidance cues in this phase, providing an early version of a function that becomes glial in some more advanced groups.

What does development tell us?

Developmentally, glia, indeed neurons as well, derive from ectoderm. This intimate linkage might even lead one

to entertain the possibility that glial cells are of neuronal origin. The glia-like investment of axon tracts by a giant axon described by Lutaud (1977; Fig. 3) in gymnolaemate bryozoans is intriguing in this context, as is the apparent inversion of the cytological marker scheme in turbellarians, with neurons having many organelles and "glia" having few. An intraepithelial location of the central nervous system has been mostly preserved in many of the groups considered "basal" (Bullock and Horridge, 1965) (albeit there is a bit of circularity to this argument). Such nervous systems occur in Echinodermata, Scalidophora (Loricifera, Priapulida), Bryozoa, Brachiopoda, and possibly Gastrotricha or originate that way embryonically, maintaining connections into adulthood. Thus neurons evolved in more or less close association with epidermal cells. However, the region of the ectoderm that gives rise to them developmentally varies. Glia of the CNS originates from the same or near-by regions of ectodermally-derived precursor cells that give rise to CNS neurons. Both neurons and undoubtedly the glia of the CNS support the central-processing role of the CNS. The PNS includes sensory neurons having a peripheral origin, and glial cells of peripheral derivation follow their in-growing axons. Glial functions are doubtless geared to the needs of long axonal processes (e.g. Nave, 2010), as are those of central glia serving long-distance tracts. Glia accompanying motor supplies of central origin have yet another potential role at the motor endings. The adaptational demands on glia of different origins serving different classes of neuronal function are likely reflected in different evolutionary trajectories. In more advanced taxa, these demands end up producing cortex glia, neuropil glia, Schwann cells and teloglia, as well as the surrounding sheath glia. These selective pressures undoubtedly begin at a basal phase in the evolution of the nervous system.

Retrodicting Evolution: Ancestral State Inferences

Extrapolating backward ("retrodicting" in current terminology) an inferred evolutionary progression of character development to the presumed most basal taxon in which "glia" might have arisen employs a comparative approach among different lines to deduce common principles in the progression of the innovation and to reconstruct a common ancestry. We first need to examine the proposed functions of extant glia and determine which are capable of promoting the emergence of the first glia. Then we need to work the cases of known early glia backwards, comparing across taxa to identify commonalities that might be plesiomorphic (ancestral) from which an ancestral state might be reconstructed. A "best guess," based on the phylogeny of extant glia haves and have-nots, is that we will end up near the origin of each of the major bilaterian groups, but it is not clear how close to the last common bilaterian ancestor this will be.

Shared functional characters

Function is what provides the evolutionary drive for new cell types and new morphologies, including glia. Glial cells in diverse taxa are thought to perform similar functions (Barres, 2008; Bullock and Horridge, 1965). The classes of function glia are thought to serve in vertebrates were outlined by Kuffler (1967) in his Ferrier Lecture and further articulated by Pentreath (1989). Classical views include structural support (protection against nerve deformation); electrical/chemical isolation; nutritive support (metabolic interaction); various roles in development including in replacement, regeneration and growth; and trophic support (production of signaling substances for growth, development, and maintenance) to which he added homeostasis (maintenance of external neuronal environment; permeability barriers including blood–brain barrier), modulation of neuronal activity and myelin formation. Since then, phagocytosis (removal of apoptotic cellular debris), uptake or release of neurotransmitters, circuit regulation, provision of neuronal stem cells, and mediators of inflammatory responses in the nervous system have been added to the list (Huxtable et al., 2010; Kreigstein and Alvarez-Buylla, 2009; Pawate and Bhat, 2008; Pentreath, 1989). This list of possible functions is formidable, albeit the experimental data to back up any give function is less so, especially outside of a small number of "model" taxa. However, the list of potential catalytic roles is shorter. For an effective homeostasis function, meaning control of the external environment of neurons, glial cells must almost completely surround the neurons, or they lose control of the extracellular medium through diffusion. Since no advantage will accrue until such envelopment is extensive, it seems unlikely that this function, operating via extracellular pathways, was instrumental in the emergence of early glia. The same is true of nutritive/metabolic support albeit this function has been proposed for the forms of glia found in some flatworms (Littlewood and Bray, 2000). Indeed the glycogen granules reported in flatworms by Morita and Best (1976; cited in Golubev, 1988) would be evidence for such a function in a basal group, albeit Golubev (1988) challenged the observation, and it seems not to have been confirmed subsequently. It has also been suggested that in many "higher" taxa, neurons do not turn over at the high rate allowed by neoblasts in flatworms, so the need for trophic support might be lessened in the latter case (*anon*. personal communication). The initial viability of a neonate mammal with a lethal genetic defect of the nervous system that does not manifest itself until later in life provides a possible model. As in the case of homeostatic functions, the supply of nutrients, metabolites (e.g. oxygen), transmitters and trophic signaling molecules by cells initially not in close contact with neurons faces diffusional dilution problems without some sheath to limit escape. Electrical isolation has similar constraints. Laming et al. (2000) suggest partial separation by early glia would "prevent cross-talk," but again effective prevention depends on almost complete ensheathment. It requires special conditions beyond

just proximity for intrinsically weak ephaptic interactions to be effective (Kamermans and Fahrenfort, 2004; Krnjevic, 1986). To be sure, glial sheaths, partitions, and compartments play important roles in isolation, support, and neurite guidance in complex nervous systems. However, a sheath is unlikely to spring into being suddenly, and intermediate forms will have little obvious advantage for such purposes. Such a sheath is likely to arise after an ancestral sheath has been created for other purposes. It should be noted that already in presumptively basal polyclad flatworms, a sheath and even tropospongium seems to have been achieved (Koopowitz, 1989). There remains the possibility in basal glia for gap junctions to enable metabolic cooperation, ionic coupling, trophic control and developmental signaling between neurons and glia (Green, 1989). Gap junctions were evolved early in metazoan history, but while present among glial cells, and among neurons, they are rare between glia and neurons (further discussed by Green, 1989, but see Fróes and Campos De Carvalho, 1998; Martin et al., 1986), presumably in part because if placed near active zones, they would shunt the electrical signaling essential to neuronal operation. Other possible generative functions remain more likely for "protoglia" that either start at a distance from a neuronal target or adjacent to it as a coincidence of birth:

Byproduct removal. Removal of transmitters or "waste" products might be roles more easily attained by cells located at a distance from the nerve-cell sources. Such capabilities have been demonstrated in more advanced taxa, specifically arthropods and molluscs (e.g. Morgan et al., 1999; Pentreath, 1989). Diffusion to the external medium or removal by fluid flow from ciliated epithelia or the circulatory system would be the basal means of control, and could be increasingly augmented even by distant protoglial cells. Such mechanisms can respond to rising extracellular concentrations and thus help control them within an evolutionarily decreasing distance as a "point" sink. However, given an open circulatory system typical of most basal invertebrates, and reasonable diffusional access to the outside of a nerve cord provided it is not too large, this too seems of limited potential. Perhaps as animals evolved larger size and larger nervous systems, this might become a spur for innovation. A similar argument can be made for electrical insulation. A small amount of glial separation between axons will have a negligible effect on their ephaptic interaction, which interaction is better reduced, as has been shown for bundles of unmyelinated vertebrate axons, by shuffling fibers among bundles. Formation of myelin is a more extreme example of the same principle. It must have required an already-close relation between neuron and surrounding glia to get started. This leaves us with a somewhat shorter list to investigate:

Structural support. This is a commonly-ascribed role for glia (e.g. Pentreath, 1989; Radojcic and Pentreath, 1979; Roots and Laming, 1998). The supposed need for such support derives from the movements of the animal that might deform and injure nerve (Pentreath, 1989). Evidence for this function lies in the extensive system of intercellular adhesive structures found linking glial cells (gap junctions, septate junctions, tight junctions), and sometimes between glia and neurons (Lane, 1981). Perhaps more importantly is the frequent occurrence of concentrations of intermediate filaments, and some times microfibrils or microtubules, in glial cells (leading to the glial marker GFAP - glial fibrillary acid protein) that are presumed to lend strength and stretch-resistance to neighboring neurons as well as to the glial cell itself (reviewed by Fuchs and Weber, 1994). This function has been primarily supported for annelids and molluscs, with their lack of skeletal elements, and contrasts with the relative lack of such need in exoskeleton-possessing arthropods (Radojcic and Pentreath, 1979). Glial cells in some platyhelminths have been observed to contain microtubules, a sign of early evolution of a structural support function (Golubev, 1988). It might be argued that most modern-day, and likely ancestral, basal bilaterians, although soft-bodied, do not, behave in violent ways that might derive an advantage from such support. Some supposedly glialess basal rhabdocoel flatworms, in fact, are predatory, endowed with a rapid attack that enables them to capture mosquito larvae and daphnids (Wrona and Koopowitz, 1998). The major innervation of the enteropneust (hemichordate) proboscis is used for burrowing apparently without the benefit of glial investment. To be sure, an animal evolving a more active life style would derive an advantage from evolving supportive protection for the nervous system. The presence of connective tissue matrix limiting extensibility of neural structures in a soft-bodied animal seems more likely to be of adaptive value. However, it is hard to escape assigning a support function to the radial-glial-like "support" cells of echinoderm neuroepithelia, with their prominent fiber bundles, and such cells might thus serve as models for the form and function a basal glial cell might take (Fig. 2).

Phagocytosis. When considering the evolutionary origin of glia, a primary constraint is that all intermediate stages in its creation must serve some function. In the more basal taxa, the roles rudimentary glia can play are limited. One such role that can be performed by individual cells acting on their own is phagocytosis. Among vertebrates, microglia are CNS macrophages programmed for engulfing foreign pathogens as well as the products of apoptosis (e.g. Barres, 2008). In invertebrates generally, many or all glial cells have been thought capable of similar function (e.g. Pentreath, 1989). Evidence adduced for this includes the large numbers of vacuoles and lysosomes that characterize much glial cytoplasm. In *Drosophila*, the ability of subperineurial glia to engulf apoptotic neurons in the absence of macrophages has been demonstrated (Sonnenfeld and Jacobs, 1995), and phagocytic glia of the antennal lobe (ensheathing glia) have been shown to express the gene *Draper*, of the engulfment-signaling pathway, while nonphagocytic glia of the same region do not (Doherty et al., 2009). However, apoptosis is not a feature of all nervous systems (e.g. Williams and Herrup, 1988), and whether it is in

ancestral bilaterians is not clear. Further, macrophages *are* present as part of a normal complement of cell types, so it would not seem to *require* evolution of a new phagocytic cell type (vertebrate microglia presumably arrived so equipped). The presumed phagocytic vacuoles reported by Morita and Best (1976; cited in Golubev, 1988) in a planarian, if real, may be an evolutionarily early example of this function. However, such vacuoles have not been reported in the literature reviewed here of other basal taxa, and in general the paucity of internal inclusions in platyhelminth glia has been remarked upon (Reuter and Gustafsson, 1995).

Development: Neuron growth, migration, and axon guidance. Glia are active participants in nervous system development and repair (e.g. Stork et al., 2010). A key function ascribed to vertebrate and invertebrate glia alike, in particular that of insects, is regulating neuronal migration and growth (Edenfeld et al., 2005; Oland and Tolbert, 2003). Could such a role be germinal for the innovation of a new cell type, the glia? Glia produces chemical signals used in axonal guidance, thus shaping the formation of neural circuits (Hidalgo and Booth, 2000; Lemke, 2001). As with vertebrates (e.g. radial glia of the brain), interference with glial cells in developing *Drosophila*, in particular the midline glia, can lead to defects in axonal outgrowth (Lemke, 2001). Glia modulates stem cell proliferation in vertebrates (Ebens et al., 1993). Glia shapes *Drosophila* neural development by providing guidance signals for growth cones, diffusible and contact-mediated, attractive and repulsive; by providing appropriate substrates for migration; by providing trophic interactions that regulate neuronal survival and by engaging in axonal pruning at metamorphosis (Parker and Auld, 2006). However, the outgrowth of pioneer axons in vertebrates and insects is also guided by chemical signals produced by the neuroepithelium, the epidermis and the neural cell bodies (Hartenstein, 1993). The evidence that glial cells are not essential for guidance reduces the strength of the argument that guidance might have instigated the evolutionary origin of glia. The outgrowth of pioneer axons in platyhelminths in the absence of glia was noted above. The "nerve nets" of cnidaria have some organization, including collection of axons into tracts or nerves, but no glia to assist. Thus, whatever roles glia has come to play in guiding development in advanced organisms the acquisition of targets for outgrowing neurons did not originally depend on glial signaling. An example of a more refined case might be the echinoid basiepithelial plexus which despite a nerve-net-like appearance, seems to have precise targeting between local control centers with perhaps only palisade-like epithelial supporting cells (as opposed to ensheathing glia) demarcating nerve tracts (Bullock, 1965; Bullock and Horridge, 1965 p. 1532). However, the opportunities for more precise localization, and more complex connectivity would likely be improved by evolving a cell type specializing in providing guidance, which type might come from preexisting glia or from a nonglial precursor.

Circuit modulation, inflammation, and neurogenesis. Increasingly in recent years, it has come to be realized that glia are capable of much more than a supporting role in the operation of the nervous system (Roots, 1978). Evidence is mounting that they participate in the active modulation of neural networks (Huxtable et al., 2010), assume active roles in inflammatory reponses (Pawate and Bhat, 2008), and even serve as the source of stem cells in neurogenesis (Kriegstein and Alvarez-Buylla, 2009). Neurons able to modify their behavior by monitoring the state of adjacent nonneuronal (e.g. epidermal) cells, especially those in broad communication through epithelial junctions with adjacent regions of a primitive organism might realize a selective advantage thereby that would promote evolution of a new-cell type. Roles in inflammatory responses and producing new neurons require migratory capabilities that indeed may be a key property enabling small numbers of sparsely distributed basal glia to still perform valuable services. Such initial roles might evolve into a broader commitment and diversification. Although communication, control, and migration may be among the more recently appreciated functions for adult glia, it does not mean that they are the more recently evolved.

What can we infer from basal glia form and function?

Heiman and Shaham (2007) point out that roles glial cells serve might be classed into three categories: (1) those for which a specialized cell type is essential to nervous system functioning; (2) those that are required for function but can be supplied by another cell type; and (3) those that are helpful but optional or redundant. The first category, being an impediment to further nervous system evolution, would likely be evolved first, followed in sequence by the other two, which are successively more permissive. However, applied to *current* glial roles in complex nervous systems this could overlook the fact that new roles or changing priorities created in the course of glial evolution may make a given role more (or less) essential (e.g. the role of "guide post glia" in developing grasshopper embryos is apparently absent in *Drosophila*: *e.g*. Edenfeld et al., 2005). Thus, we will gain better insight if we start with as basal a group as possible.

Two general observations about the relationship between neurons and glia that have functional consequences of evolutionary relevance are: first, the morphological observation that glia surround neurons rather than either the other way around or in a side-by-side coexistence. Why would this be? Second, the relationship may change drastically between functions during development and those in a more stable (albeit still dynamic) adult state. In development and repair, migration toward distant targets is a characteristic of glial precursors, where neurons often differentiate earlier and instead elongate their axons to reach distant targets. Fasciculation-based guidance of out-growing axons is one point where these two aspects come together, but much axonal outgrowth and pathfinding appears not to

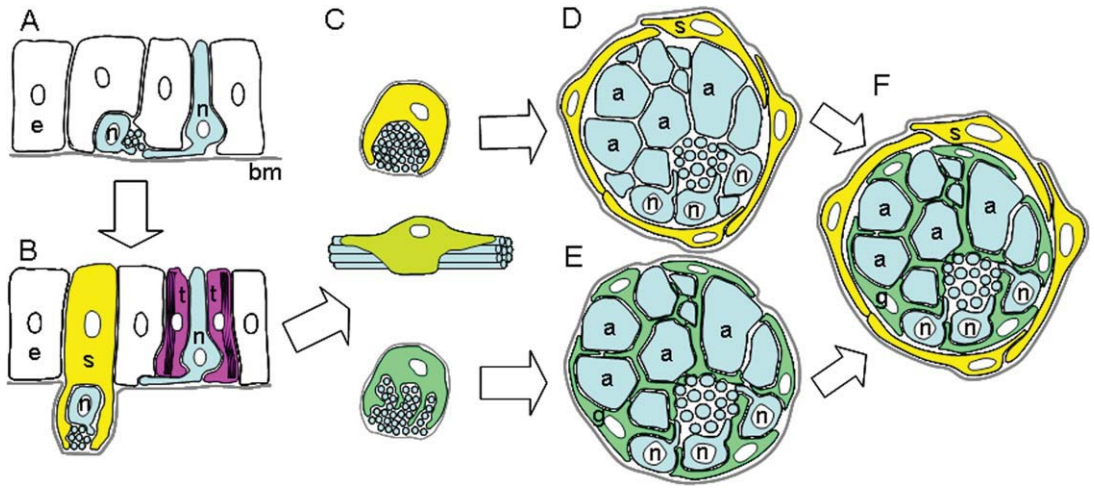

Fig. 6. Schematic for some possible paths in evolutionary emergence of glia. (**A**) Most basal state (e.g. as represented in Cnidaria), with neurons (n) either basiepithelial or penetrating between epithelial cells (e) to the outside as sensory cells. (**B**) Two possible origins: on the left an epithelial cell maintains a partial sheath around neural elements that descend to subepithelial positions; alternatively (right), supporting cells reinforced with microfilaments or microtubules come to lie alongside neurons to provide physical support, assist in axonal bundling and perhaps axon guidance. (**C**) Internalized neuronal elements are accompanied by sparse, perhaps migratory, glial cells that partially or fully ensheath axon bundles, with (lower schematic cross section) or without (upper section) cytoplasmic penetration between axons. (**D**) Elements of the internal nervous system (a = axons; n = neuron somata) come to be fully surrounded by sheath cells (s; yellow). (**E**) Alternatively, space between elements of the internal nervous system come to be invaded by sheet-like interstitial glial cells ("g"; green). (**F**) Sheath cells from stage D invade the spaces between neuronal elements to generate interstitial or "neuropil" glia; alternatively interstitial glial cells from stage E expand around the outside of the neural elements to provide ensheathment. Grey line in all drawings represents the basement membrane that surrounds neural tissue and segregates it (as well as epithelial tissue) from other tissue types. From www.pbrc.hawaii.edu/~danh/GliaEvolution/with permission.

be glia-dependent, or if it is, to depend on contact but not envelopment (Edenfeld et al., 2005).

Epithelial ensheathment. One commonality among taxa lacking glia or possessing it in a rudimentary state is the possession of basiepithelial nervous systems. From this, a glial origin from epidermal supporting cells is a reasonable assumption (Fig. 6A). The somata of sensory cells and even "ganglion" cells in cnidarians are surrounded by cells inferred to be epitheliomuscular in nature (Lentz and Barrnett, 1965). Epithelia, especially the epidermis, in general are structured to provide strong mechanical protection against external damage, and an intraepithelial location is an intrinsically well-protected environment in which cells specialized for support and protection are not in as high demand. An exception is for epidermal sensory cells, which indeed tend to have their own specialized "supporting" or "sheath" cells, which indeed represent a form of early glia. As nervous systems evolve to take up deeper positions in the body, simple forms of partial ensheathment may occur as in taxa like the Brachiopoda with a seeming afterthought of a cytoplasmic sheet from an epithelial cell being thrown around a nerve bundle (Fig. 4 and 6B "s"). While too poorly formed to offer much isolation or environmental protection, it might come to assist in removing waste products, while offering a reasonable amount of support, and it may serve to segregate axons from adjacent tissue for targeting or other purposes, however minimally. This could be the evolutionary bridge to more extensive contact and a diversification of roles.

Epithelial support. An alternative model, again remembering the close relationship with epidermal cells in basal nervous systems, is of the tanycytic-like or radial-glia-like cells of scalidophorans and echinoderms (Fig. 2). Their fibrous content indicates a support function which, although it may not originally have been of high priority for the nervous tissue near which it occurs, still the proximity could evolve into a more intimate supporting role as an organism becomes more active and needs increasing amounts of dedicated support.

Trophic support. The platyhelminths have diversified from simple nervous systems to rather complex ones quite successfully, some possessing and some not possessing glia-like cell types, and so provide within one closely related group cases useful for comparisons. Assuming for the moment that the absence of glia is a plesiomorphic (i.e. ancestral) platyhelminth character, several cases of documented glia in more advanced taxa are instructive. One commonality in this glia is the relative paucity of internal organelles, unlike the glia of more "advanced" taxa (Table 1). In addition, unlike the cases just mentioned from basal deuterostomes or trochozoans, there seems to be a more intimate relation of the basal platyhelminth glia with the neurons. It insinuates processes among the neurites, but has not in all cases formed a supportive or protective sheath around the CNS (Fig. 5). A function like trophic support seems to fit this picture better than mechanical support or bundling, although how such intimate contact might have started is harder to fathom.

These scenarios have suggested three functions that might provide independent pathways for the evolution of glia, and indeed any or all of these might have been operative to produce the large variety of invertebrate glias we see today.

Glial Loss

Next we inquire whether some taxa lacking glia might have lost it, and if so, why? This topic brings us full circle, and helps avoid one potential problem: with our present state of knowledge, we often still can only guess at what characters are plesiomorphic and what apomorphic. Major controversies have raged among taxonomists and evolutionary biologists over such issues. In the present context, for example, Morris et al. (2007) opine that "the absence of morphologically detectable glial cells in basal flatworms... should also be taken as a primitive trait." Such a conclusion can only be made in the context of many other characters (including molecular analyses) converging on a given phylogeny. There is no *a priori* way to know that the glia was not lost. However, the absence of glia in an extant organism, whether attained by never having had the cell type in the ancestral line or because of no longer needing the cell type, is still indicative of the lack of need. The significance of the "lack of need" is greater for cases of loss since in general it is easier to lose a complex trait than to gain it. Thus independent of the reason, nervous systems without glia are functioning perfectly well for the possessor's purposes, and a comparison with nervous systems possessing glia is instructive.

Candidates for glial loss

Which taxa might be candidates for secondary loss? As mentioned, it is hard to avoid the answer that all taxa without glia might be. However, we would expect this to be accompanied by a change in lifestyle such as (but not limited to) "regression" that renders glia superfluous. We might also expect such a loss to be impeded by the tendency of glia to take on different functions and once started, to evolve into a number of different cell types. Removing one factor promoting glial existence may not eliminate all of the subsequently-evolved roles it plays. Nevertheless, in the case of the Bilateria, the Acoela, which are currently placed at the base of the bilaterian phylogeny (Hejnol et al., 2009), have purported "glia" as well as more neuronal cell types than more derived platyhelminths (e.g. Reuter and Gustafsson, 1995). This might mean that all of the "lower" platyhelminths, in particular the catinulids, macrostomatids and even rhabdocoels, have lost the character. Given their active life style and other factors that might promote glia (see below), it is a little hard to accept. Aside from the Platyzoa (flatworms and their relatives), there are taxa apparently missing glia in two of the three major bilaterian branches: hemichordates and adult urochordates among the Deuterostomia, and bryozoa and entoprocts among the Lophotrochozoa (Fig. 1).

Nervous system size

Size might contribute to selective pressures toward glia formation, and hence reduction in size might promote its loss. In vertebrates, evidence for the greater importance of glia in trophic support of longer axons is deduced from the preferential degeneration of such axons in disease conditions affecting the glial sheath (Nave, 2010). Several of the taxa apparently lacking glia (Rotifera, Kinorhyncha, Bryozoa) tend to be small—in the millimeter range in size. Of the smallest bilaterians, some nematodes (1 mm) and rotifers (50–1000 μm: Clément and Wurdak, 1991) have few or no glial cells (and few neurons as well). However, other equally small basal organisms such as the priapulids (Rehkämper et al., 1989) and the loriciferans (Kristensen, 1991) possess recognizable forms of glia. Copepods of 80–200 μm length have perfectly good glia resembling that of other crustaceans (J. Kong and D. Hartline, unpublished). Tardigrades of <1 mm length still possess glial cells, albeit comprising, both by number and volume, a small fraction of the nervous system total (Greven and Kuhlman, 1972 cited in Dewel et al., 1993). On the other hand, medusozoans (jellyfish) with nerve nets extending for meters and individual neurons at least several times the size of those of glia-invested small organisms, manage very well without glia (Bullock and Horridge, 1965, p. 468; Mackie, 2003). As a further example, the nematode, *Ascaris*, has the same body plan as tiny *C. elegans* but is over 100 times larger. Overall, it does not seem that size itself is a determining factor.

Nervous system compactness

The transition to glia has been linked to the evolution of more compact, more structured, nervous systems including discrete peripheral nerves, nerve cords, and ganglia (Bullock and Horridge, 1965). As neurons come into closer physical association, it would appear that new needs arise, requiring evolution of new mechanisms for satisfying them. Closer spacing might impede diffusional access to needed nutrients and ions from the blood, and interfere with removing waste products. Were this the primary causal factor, one might predict that glia-less nervous systems might exhibit a less-compact structure. However rotifers, lacking glia, still have modestly compact nervous systems (Bullock and Horridge, 1965). Studies of ion penetration through extensive narrow extracellular paths between glial cells suggest that they are not a major impediment to diffusion in invertebrates of modest size, at least (centimeters) (Kuffler, 1967).

Mechanical support, trophic support, and nervous system complexity

These three factors have also been linked to possible glial roles. Certainly, the more "advanced" and complex the nervous system, the more differentiated and diverse the glia appear to be. Thus, among the platyhelminths, the triclads, with more complicated nervous systems than the more basal rhabdocoels, possess glia, while the latter do not (Golubev, 1988). This also appears to hold comparing the more complex nervous systems and well-developed glia of the supposedly basal cephalochordates (Lacalli and Kelly, 2002) with the lack or poor development of glia in the simpler nervous systems of more derived hemichordates. These factors would be modified when an organism exchanges an active life for a sessile one, leaving the dispersal task to its larvae or its gametes. It might also leave behind the potential damage to soft body parts, including nerves, that a more active life entails, and hence its needs for supporting structures (or those supporting roles might be taken up by the armor the animal must evolve to enable such a lifestyle). Also, with a less complicated life (presumably), need for a complex neural computer to deal with it would be reduced as well, and their needs for precise and complicated developmental information might reasonably be expected to increase. Were support or complexity key factors, one would expect that secondarily simplified nervous systems might have lost their glia. The urochordates might be such an example, with their sessile adult phase apparently lacking glia (Koyama and Kusunoki, 1993) but their larval phase possessing ependymal cells (Meinertzhagen et al., 2004). Indeed, this could represent a prime case of active glial loss during development and might spur the search for other similar cases. On the other hand, for platyhelminths turned parasite with a sedentary adult phase, the well-developed glia of a cestode seems to falsify this hypothesis (Biserova et al., 2010). Lower platyhelminths lacking glia are usually held to have better-developed nervous systems than the basal acoelomorphs (Bullock and Horridge, 1965, but note Reuter and Gustafsson, 1995), yet the former lack and the latter possess glia, and as mentioned above, some of the latter are active predators (Wrona and Koopowitz, 1998). Barnacles have somewhat reduced nervous systems in the adult compared with the actively swimming cypris larva, yet they, too, have well developed glia (Walker, 1992). While support and complexity still have potential for relevance, the evidence so far is equivocal.

DISCUSSION: WHEN, WHERE, AND WHY GLIA?

When?

Forces for convergent evolution

The answer to the question posed at the beginning of this review "Where did glia come from," still has the answer "We don't know," but the issues behind the question are perhaps clearer. The pattern of the "haves" and "have nots" of glia supports the idea that evolving glia is a necessity for a complex nervous system, even one as simple as that of a parasitic cestode (Biserova et al., 2010). This complicates the picture because it means that the pressure for parallel evolution of glia is strong, and it should be expected that lines lacking glia will independently evolve offshoots capitalizing on its advantages, to build a more capable nervous system. Also, it seems clear that once the initial step has been taken of originating a glial class of cells, evolutionary pressures heap on an ever-increasing load of demands for parallel evolution of sophisticated roles that were not present in the original glia. Combine this with 550 My of evolution, without an overt fossil record, and we have a herculean task of solving the glial evolution mystery.

Evidence of independent evolution

It is possibile that glial origin is independent in two or more stem groups based on the observation that the most basal platyhelminths lack glia, and that the rest of the Bilateria (including the more advanced platyhelminth taxa, and setting aside for the moment the acoelomorphs) are thought to arise from ancestors of similar organization. Most current phylogenies place the Platyzoa in a spiralian clade, distinct form the Trochozoa, Ecdysozoa, and Deuterostomia (Hejnol et al., 2009). Assessing this situation, Reuter and Gustafsson (1995) concluded "The observation of 'glial' cells and wrappings of neurons and axons in scattered flatworm taxa indicates that glial cells in Platyhelminthes may have originated independently from the glial cells in Eubilateria." The fact that basal members of each of the Deuterostomia (Echinodermata), Ecdysozoa (Scalidophora), and Lophotrochozoa (Bryozoa) lack or have rather poorly-developed glia supports this hypothesis and extends it to the other major bilaterian branches. However, too little is still known about deep phylogenies and the evolution of glia to rule out glial loss in seemingly "primitive" taxa (Porter and Crandall, 2003).

The distribution of glia-specific transcription factors, albeit sketchy, seems to support the lack of homology. For the particular case of the Deuterostomia versus Protostomia, Klämbt (2009) points out that despite similar morphological types, the mechanism for inducing gliogenesis is significantly different, the transcription factor *gcm* being used in *Drosophila* but the unrelated *olig2* (oligodendrocyte transcription factor 2) being used for example in vertebrate oligodendrocytes (and the homolog of *gcm* is not highly expressed in vertebrate nervous systems). *Gcm* is also found in a highly conserved form in a planarian (Umesono and Agata, 2009), and an echinoderm (Ransick and Davidson, 2006), but again, not in the nervous system. While conservation across deep phylogenies is less certain than across shallow ones, nevertheless the conservation of genes and regulatory networks in the evolution and development of metazoan body plan has become an article of faith (e.g. Meireles-

Filho and Stark, 2009; Morris et al., 2007) that suggests a lack of glial homology in these two lines at least. Similar studies are needed in the Spiralia. In contrast, the distribution of genes involved in nervous system development confirms, as would be hoped, that the neuronal specification and developmental machinery, if not the glial development machinery, is indeed held in common across widely divergent taxa (Umesono and Agata, 2009; Younossi-Hartenstein et al., 2000 Zhu et al., 2008). However, this just pushes the molecular issues up a level. Homologs of glial markers like *repo* and *gcm* likely exist in the nervous systems of other ecdysozoan clades while perhaps being nonneural in others still more basal. Tracing preglial markers transitioning phylogenetically into glial markers should shed light on glial evolution in the Ecdysozoa, and in time, in Lophotrochozoa, as the genetic basis of neurogliogenesis becomes better understood in that taxon.

Why?

Complexity

The correlation between glial elaboration and the complexity of the nervous system has been noted repeatedly (Bundgaard and Abbott, 2008), and was discussed above in relation to glial loss. Among other things the increasing ratio of glial cell numbers to neuronal numbers in "higher" nervous systems has been pointed to—estimated at 50:50 in vertebrates but only 10:90 in "invertebrates" (by which is usually meant *Drosophila*). It is unclear, however, whether a strict numerical accounting is any better an index of glial importance than is an assessment of mass, which is closer to equal. One point to note in platyhelminth glia is the relative lack of cytoplasmic inclusions. This indeed contrasts sharply with the situation for neurons, which are typically filled with vesicles of various kinds (Reuter and Gufstafsson, 1995). It suggests a lack of the usual nutritive (glycogen for energy), supporting (filaments), phagocytic (phagosomes) and synthetic (ribosomes) functions. There remains the close association with neurons, albeit not in terms of a complete sheath (Fig. 5). Further, the observation of glia as "scattered" along the nerve cords suggests a function that can be maintained with little direct contact. This raises the possibility, combined with the migratory propensities of glia in advanced groups, that early glia were in fact mobile and provided a mobility-related function.

Where?

Whether glial cells arose once or more than once in evolution, there is still the question of from what source or sources they did evolve. Reviewing the evidence, four likely sources for glia might be identified: (1) epidermal cells from a basiepithelial phase in nervous system evolution; (2) epithelial support cells; (3) phagocytes; (4) developmental guide cells (Fig. 6 diagrams some hypothetical stages).

Basiepithelial internalization

The close proximity of epidermal cells to nerve fibers of the commonly-found "primitive" basiepithelial nerve plexus or surrounding nerves supplying sensory innervation, offers a natural setting in which nonneural tissue occurs in close proximity to neural tissue (Fig. 6A). The migration of ectodermally-derived peripheral glial precursors along ingrowing sensory axons in *Drosophila* reinforces this possibility. The envelopment of nerve tracts by such cells that are also functioning in other capacities (e.g. epidermal barriers), as occurs in the ectoneural system of ophiuroids (Byrne, 1994), suggests a possible model for the primitive association of nonneural with neuronal cells (Fig. 6B "s"). The hemichordates offer a similar example (Benito and Pardos, 1997; see above). An internalized compact nervous system has fewer such opportunities since much of the nervous environment is made up of other neurons (a possible but perhaps rather unlikely pre-glial cell type, but see Fig. 3) or extracellular matrix such as basement membrane or collagen, but opportunities should exist for carrying such "supporting cells" along, perhaps initially migratory, as the neural elements descend to deeper layers (Fig. 6C). Again, evidence for such a mechanism can be noted in the nerve ring of hemichordates and the observation that brachiopod nerves are only wrapped by auxiliary cells when they leave their basiepithelial environment to travel subepithelially in the body cavity.

Origin from supporting cells

Another potential model for early glial precursors is presented by the echinoderms, with their fiber-filled supporting cells (Fig. 6B "t"). These cells do not seem to support neural structures directly, but they do pervade the nervous systems in their path between epidermis and underlying muscle. In so doing, they divide the nerves passing though the epidermal plexus into groups, if not bundles, thus perhaps representing a basal form of the bundling of axons seen more clearly in more advanced stages of glial evolution. The precise utility of such bundling is not clear, but its importance in some aspects of axon guidance during development might serve as a model. A relatively randomly-targeted nerve net as is the appearance presented by cnidarians, should become more efficient and sophisticated when axons become more specific in the signals that guide their outgrowth and connection to targets. It might be noted that partial envelopment of nerve bundles in hemichordates and of ganglia in turbellarians occurs as well, even though the degree of envelopment seems inadequate for tightly controlling the environment within the bundle. Such bundling may help organize developing and regenerating axons with respect to their destination.

Origin from sheath cells

The cases just touched on represent fairly tenuous connections between neurons and quasi-glial cells. One might push this speculation a bit further by noting that several cases of more basal "glial" associations with the nervous system have to do with external envelopments or sheaths rather than the intricately interlaced pervasive elaborations among neurites seen at more advanced levels (Fig. 6D). This seems to be the case for epithelial cells that invest internalizing nervous system, as just described. It can also be noted in the descriptions of bryozoans (Bullock and Horridge, 1965; p. 633) and brachiopods (James, 1997). A general ensheathment of nerve trunks and ganglia, evolved for whatever evolutionary purpose, might make a good "launching pad" for elaboration of more intimate associations with interior neurons (Fig. 6F). Nevertheless, the "neuropil" glia forms found in platyhelminths still lacking a sheath stand as a reminder that there are alternative possibilities (Fig. 6E).

WHICH ASPECTS OF MAMMALIAN GLIAL CELL BIOLOGY MIGHT BE MOST EFFICIENTLY STUDIED IN INVERTEBRATES? WHICH ASPECTS MIGHT NOT?

Comparative studies in biology are almost universally illuminating. This is because all biological systems obey the same basic rules of evolution, so they come to their present state through principles held in common. A trait such as a particular function of supporting cells that evolves convergently suggests a mandated constraint that is being satisfied, however obscure that necessity might be. At the same time, the *differences* in detail of that convergently-evolved trait suggest what features are more *permissive*. Thus, the occurrence of a complex variety of similar forms and functions for "glia" in distantly-related bilaterian taxa (e.g. vertebrates, arthropods, and molluscs) including physical support, nutrition, trophic support, and electrical isolation where most of these functions were missing in the last common ancestor provides fertile ground for the application of comparative studies. Then, too, invertebrates are known for the diversity of their glial types, which frustrates a comprehensive classification scheme (Radojcic and Pentreath, 1979). However, mapping the evolutionary divergence will bring understanding to this diversity and benefit understanding of all glia.

A second lesson might be taken from nondrosophilid nonnematode invertebrate glia: model species are far from providing all of the significant answers. The phylogenetic tree shown in Fig. 1 could not have been produced without a broad sampling of nonmodel taxa, yet such a tree is key for understanding the course of evolution, including that of glia. The main hope for answering basic questions such as the origin of glia rests on establishing a stable phylogeny of the Bilateria, which will only happen if more nonmodel organisms are investigated with genomic and developmental approaches. Increasingly, it is being realized that some of the more basal metazoans share more genes with vertebrates than do more "advanced" nematodes and flies (Putnam et al., 2007), giving more reason to investigate a variety of nonmodel organisms. A glial specialization that has particular potential for providing insight into vertebrates including mammalian neurobiology is the evolution of myelinating glia. This has occurred in both annelids (oligochaetes) and crustaceans (decapod shrimp), but not in any of the 13 or so "official" model organisms (Roots, 2008). This advanced function could not have evolved in the most basal condition, but had to wait for the evolution of multilayer sheathing of the sort found in most advanced taxa. From this foundation, mechanisms of membrane elaboration and compaction, and shunt blockage could evolve in turn (Hartline, 2008).

A third feature that should not be overlooked is Krogh's (1929) Principle: when one has a basic question needing an answer, look for the best system in which to pursue it. Certain invertebrates possess glia that offers unique opportunities for studies. Among the nondrosophilid, nonnematode groups, an outstanding example of this was provided almost 50 years ago by the work in Steve Kuffler's lab on glial cells of leech (Kuffler and Potter, 1964). These proved to be exceptionally large, and thus relatively easy to penetrate with microelectrodes for study. They also proved to be large, spanning the entire distance of 1 mm or so between adjacent ganglia in a ventral chain, and the same cells occurred in the same positions from individual to individual—i.e., they were "reidentifiable," just as are the neurons of the leech nerve cord (Kuffler, 1967). The studies of Kuffler and his colleagues helped galvanize the interest in glial cells and showed that they were amenable to study with physiological techniques.

WHAT ARE THE MOST IMPORTANT CONTROVERSIES RELEVANT TO YOUR PARTICULAR TOPIC?

Most of the controversy derives from the current flurry of shaking the phylogenetic tree of life that occurs with each new analysis. Each new tree contradicts the last one, and they all contradict the trees based on morphology or physiology alone. But molecular phylogenies, while seeming more objective than taxonomists' "judgements," are far from error free. If there is a 99% chance of two clades being related, one in 100 such pairs will on average be in error, and the error could be pivotal. It is not so much that there are burning controversies in the field as it is that there is a serious lack of new data, albeit progress is being made slowly. Still, except for a couple of genetically-tractable "model systems" very little is known about the molecular architecture of glial function in invertebrate groups. We cannot even answer the question of how many times glia evolved based on molecular data, given the small number of taxa surveyed to date.

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