Ancestral roles of glia suggested by the nervous system of *Caenorhabditis elegans*

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The nematode Caenorhabditis elegans has a simple nervous system with glia restricted primarily to sensory organs. Some of the activities that would be provided by glia in the mammalian nervous system are either absent or provided by non-glial cell types in C. elegans, with only a select set of mammalian glial activities being similarly provided by specialized glial cells in this animal. These observations suggest that ancestral roles of glia may be to modulate neuronal morphology and neuronal sensitivity in sensory organs.

**Keywords:** *C. elegans*, invertebrate glia

INTRODUCTION

Glia have recently been shown to regulate neuronal survival, differentiation, migration, morphogenesis, synapse formation, and synaptic transmission (Allen and Barres, 2005; Freeman, 2006; Ma *et al.*, 2005), suggesting that at least some glial activities may be fundamentally required for nervous system function. A priori, one might expect to find three classes of glial activities: (1) those that help regulate but are not essential for nervous system function; (2) those that are required for nervous system function but can be supplied by any cell type; and (3) those that are required for nervous system function and, for some reason, must be provided by specialized glia. If such classes exist, they may shed light on the evolutionary origins of glia, suggesting, respectively, activities that arose late in nervous system evolution, activities that were present early in other cells and were later subsumed by glia, and finally activities that represent an ancient role for glia. The existence of these classes may be elucidated by considering glial activities in the mammalian nervous system and asking whether each is provided, and, if so, by what cells, in an evolutionarily distant nervous system.

The nematode *Caenorhabditis elegans* possesses a nervous system composed of only 302 neurons, yet it is able to move gracefully, to detect and integrate complex information from the environment and decide how to respond, to eat, to mate, and even, in some form, to learn. Thus, whatever activities constitute the core requirements for nervous system development and function are present in some fashion in this simple organism.

Information flows into the *C. elegans* nervous system primarily from an array of sensory neurons each bearing a cilium, or modified cilia, loaded with sensory receptors specific to that neuron, and usually presented at or near the outer surface of the animal (Ward *et al.*, 1975; White *et al.*, 1976; Hall and Russell, 1991). Together, these neurons sense soluble chemicals (taste), volatile chemicals and social pheromones (smell), mechanical force (touch), osmotic force, oxygen concentration, and temperature (Bargmann and Kaplan, 1998; Gray *et al.*, 2004; Cheung *et al.*, 2005). Information from the sensory neurons is transmitted along dendrites to a dense arrangement of axons and interneurons in the head called the nerve ring, where it is integrated, and output is sent to motor neurons and other efferents (Ware *et al.*, 1975). Axons and dendrites of the motor neurons are arranged into a major fascicle called the ventral nerve cord and a minor fascicle called the dorsal nerve cord (White *et al.*, 1986). Thus, roughly speaking, the anatomy of the *C. elegans* nervous system approximates that of mammals: an array of sensory neurons similar to the peripheral nervous system relays information to the nerve ring, or brain, where it is processed and then transmitted along a nerve cord similar to the spinal cord (Fig. 1).

Unlike in mammals, most of the surface area of the *C. elegans* nervous system does not come in contact with specialized cells that could constitute dedicated glia. The exceptions are select synapses of the nerve ring as well as the sensory synapses at the ciliated dendritic endings of sensory neurons, all of which form close associations with specialized support cells (Fig. 1; see also Shaham, 2006). The nerve ring is ensheathed by four glial cells named the CEP sheath cells after their separate association with four cephalic (CEP) neurons. The CEP sheath cells not only ensheathe the nerve ring but are interwoven with it, making intimate contacts with several synapses where information processing is likely to occur (Fig. 1). But the most abundant glial cells of *C. elegans* are the ones that associate with dendritic endings, forming the cellular boundaries that define discrete sensory organs, or sensilla. Of the 60 ciliated sensory neurons, 54 are arranged into 24 discrete sensilla, 20 sensilla in the head and four posteriorly (Ward *et al.*, 1975). Males have nearly double that number, with four additional sensory neurons in the head and 46 additional sensory neurons in the tail that are organized into 23 sensilla used for mating, particularly for physically sensing the vulva of a mating partner (Sulston *et al.*, 1980; Liu and Sternberg, 1995). Sensilla contain as few as one or as many as
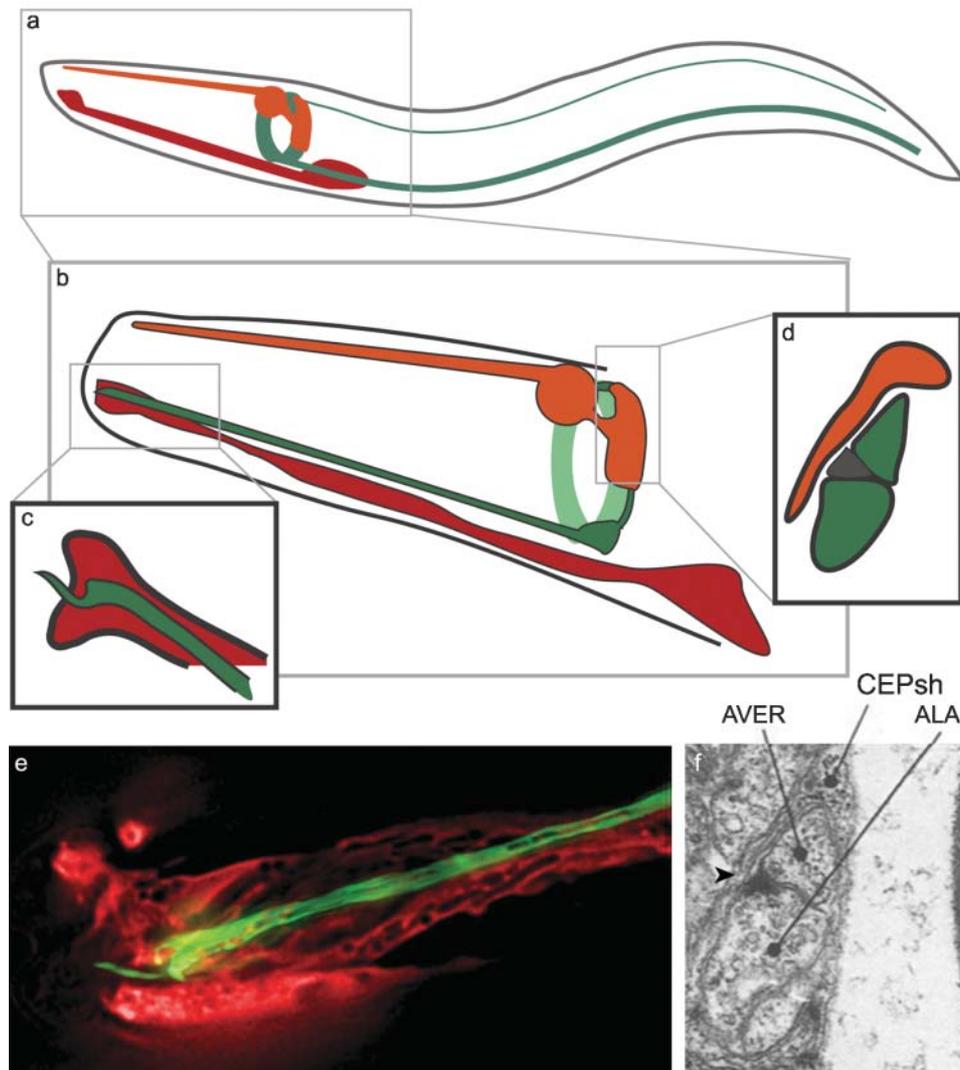
Fig. 1. Schematic of the *C. elegans* nervous system. (a) Major neuron tracts in green and two representative glia, an amphid sheath glial cell (red) and a CEP sheath glial cell (orange). (b) Each of these glia extends a process to the nose as part of a sensillum, while the CEP sheath glial cell additionally extends fine processes into the nerve ring, or brain. (c and e) The amphid sheath glial cell (red), completely ensheathes the ciliated sensory ending of a dendrite (green), shown in a fluorescence micrograph of an animal expressing *vap-1* promoter::RFP and *gcy-5* promoter::GFP transgenes.. The region of the glial cell adjacent to the dendrite is densely populated with secretory vesicles, visible as fluorescence exclusions. (d and f) The CEP sheath glial cell (orange, CEPsh) extends fine processes into the nerve ring that make intimate contact with synapses, shown in an electron micrograph (reprinted with permission from White *et al.*, 1986) in which the synapse appears in cross-section as a triangular density (dark gray) between two neurites (green, AVER and ALA neurons) closely apposed to a thin glial process (arrowhead).

12 neurons, but each sensillum has a dedicated pair of specialized glial cells called the sheath and socket cells (in the male tail, most sensilla contain a single glial cell called the structural cell). The sheath cell extends a process that contacts and ensheathes the dendritic endings of its associated sensory neurons, forming adherens-like junctions with the neurons, and surrounding them in a tube-like channel (Fig. 1). In some sheath cells, vesicles filled with an electron-dense matrix have been observed, apparently depositing into this channel a substance that bathes the dendritic endings (Perkins *et al.*, 1986). The socket cell extends a process that terminates distal to the sheath channel and also forms a tube-like channel, continuous with the sheath channel on one side and with the outer hypodermal layer of the animal on the other side. Most sensory cilia extend through the channels of both sheath and socket cells

to reach the surface of the animal where they receive environmental input (Ward *et al.*, 1975).

Thus, the bulk of the *C. elegans* nervous system functions either without the support of specialized glia or else depends on glial-like activities provided by non-glial cells, while a distinct class of synapses – intriguingly, those most likely to play a role in learning and adaptation, behaviors that require modulation of synaptic gain – makes close contacts with glia, hinting at the existence of a specialized, and perhaps ancient, glial activity.

Activities of mammalian glia not observed in the *C. elegans* nervous system

One activity of mammalian glia – the use of myelin to electrically insulate axons for long-range conduction of

action potentials – has no known correlate in the *C. elegans* nervous system, where the distance from nose to tail at adulthood is only about 1 mm. Other activities of mammalian glia are more difficult to rule out in *C. elegans*, but one in particular is noteworthy for not having been observed: the dynamic regulation of neuronal differentiation and survival, provided by glia in the mammalian nervous system, is apparently absent in *C. elegans*.

In the human brain, neurons are generated by neural stem cells (NSCs) that resemble astrocytes and give rise to terminally-differentiated neurons and glia (Merkle and Alvarez-Buylla, 2006). Co-culturing mammalian NSCs with astrocytes increases neurogenesis, showing that mammalian glia are not only lineally related to neurons but also regulate neuron production (Lim and Alvarez-Buylla, 1999; Song *et al.*, 2002). Mammalian glia also produce neurotrophic factors that regulate neuronal survival (Lemke, 2001). Similarly, in *C. elegans*, a collection of precursor cells gives rise both to neurons and glia (Sulston *et al.*, 1983); however, unlike the dynamic regulation observed in the mammalian nervous system, in *C. elegans* neuronal and glial fates are largely determined when the cell is born.

For example, glial identity in *C. elegans* is established in part by intracellular stabilization of the zinc-finger transcription factor LIN-26, which is required in glial daughters to prevent their adopting neuron-like fates, and which is symmetrically distributed during cell division and then destroyed in the daughters specified to become neurons (Labouesse *et al.*, 1996). Likewise, neuronal identity also appears to be encoded by a cell-intrinsic program. Following dissociation of *C. elegans* embryos to a single-cell suspension and plating in culture medium for several days, undifferentiated cells specified to become neurons adopt neuronal morphologies and express neuronal subtype-specific markers in the absence of any cell-cell contact (Christensen *et al.*, 2002). Despite sharing a common culture medium, at least some neuron subtypes are generated at approximately the frequencies seen during *in vivo* development (Colosimo *et al.*, 2004), indicating their fates are not determined by specific cell-cell contacts or diffusible signals.

Survival, like differentiation, appears intrinsically programmed in *C. elegans* neurons. Unlike mammalian neurons, which undergo programmed cell death or exhibit signs of degeneration if the associated glia are damaged or removed, *C. elegans* neurons survive for the life of the animal even if glia are deleted by genetic means or through laser ablation (Hedgecock *et al.*, 1985; Vowels and Thomas, 1994; T. Bacaj and S. Shaham, unpublished). Moreover, the neurons show no outward signs of degeneration, retaining full-length axons and expressing appropriate subtype-specific markers. Importantly, this observation allows for the *in vivo* analysis of defects in neuronal function following withdrawal of glia without confounding effects of cell death and degeneration, an experiment difficult to conduct in a nervous system like that of mammals, where glia dynamically regulate neuronal survival.

Activities of mammalian glia provided by both glial and non-glial cells in *C. elegans*

During early development in mammals, glia help shape the nervous system. For example, neuronal migration along radial glia establishes cortical layers (Hatten, 1999). Elsewhere in the mammalian nervous system, axon pathfinding is guided by expression of Netrin and Slit proteins at the midline by neuroepithelial cells that express radial glial markers and in the visual system by astrocyte precursors and other glial-like cells (Colamarino and Tessier-Lavigne, 1995; Lemke, 2001). In the developing mammalian brain, synapse formation has recently been shown to be stimulated by factors secreted by astrocytes (Ullian *et al.*, 2004; Christopherson *et al.*, 2005). Mammalian glia also play a distinct role in the mature nervous system in the re-uptake and recycling of synaptic neurotransmitters, especially glutamate (Hertz and Zielke, 2004). In the *C. elegans* nervous system, however, each of these activities is often provided by non-glial cells.

In the developing *C. elegans* embryo, for example, neuroblast migration first occurs immediately following gastrulation to close the cleft left behind by the ingression of endodermal precursor cells (Sulston *et al.*, 1983; George *et al.*, 1998) and, dramatically, later in embryogenesis as certain neurons undertake long-range migrations to occupy their proper positions. Neuroblast migration after gastrulation requires the ephrin receptor VAB-1 and its ephrin ligand VAB-2 which are expressed by complementary sets of neurons (George *et al.*, 1998). Later, the long-range posterior migration of the CAN neurons requires the repulsive Slit-family ligand SLT-1, expressed in an anterior cap of hypodermal cells as well as several socket glial cells in anterior sensilla and some head muscle cells (Hao *et al.*, 2001). The migration of ALM neurons is also guided by SLT-1 as well as the attractive netrin UNC-6, which is expressed by a collection of neurons along the length of the developing embryo and by several sheath glial cells in anterior sensilla (Wadsworth *et al.*, 1996; Hao *et al.*, 2001; Watari-Goshima *et al.*, 2007). Thus, one major activity of mammalian glia, the expression of conserved guidance molecules that direct neuronal migration, is provided in *C. elegans* by multiple cell types, including glia, as well as neurons, hypodermal cells and muscle.

In addition to their roles in neuroblast migration, VAB-2, SLT-1, and UNC-6 are also required for axon guidance as circumferential cues at the nerve ring and as dorsal-ventral cues at the midline (Blelloch *et al.*, 1999; Wadsworth, 2002). Intriguingly, the CEP sheath glia that encircle the nerve ring express UNC-6 and, in addition to an early role in guiding neuron migration, are likely to play a later role in guiding axons to establish nerve ring size and position (Wadsworth *et al.*, 1996). However, anatomy dictates that axon guidance at the midline is provided by non-glial cells as all glia reside in sensory structures in the head and tail. In the case of Netrin-mediated guidance, for example, UNC-6 is expressed early by several epidermoblasts along the midline and later in a dynamic pattern by several putative guidepost neurons (Wadsworth *et al.*, 1996). Ablation of UNC-6-expressing neurons causes axon misrouting: for example, ablation of the AVG neuron in the ventral nerve cord causes other axons to fail to fasciculate into a single tract; ablation of the PVQ neuron in the lumbar ganglion causes other axons there to fail to reach the ventral nerve cord; and ablation of the guidepost neuron PVT in the preanal ganglion causes axons that normally enter the nerve cord in commisures at that point to enter instead in a dispersed, disorganized fashion

(Wadsworth *et al.*, 1996; Ren *et al.*, 1999). Thus, axons in the *C. elegans* nerve ring, like axons in the mammalian brain, may depend partly on glial signals for their organization, while, in contrast, axons at the *C. elegans* midline and elsewhere receive guidance primarily from non-glial participants, demonstrating that axon guidance need not be provided by specialized glia.

Likewise, synapse formation in much of the periphery of the *C. elegans* nervous system takes place in an environment devoid of glia. The specification of a set of synapses that assemble between the HSN neuron and its postsynaptic partners has been examined in detail. In this case, assembly of presynaptic components in the HSN neuron does not depend on the presence of any of its postsynaptic partners, the VC4 and VC5 neurons or a vulval muscle cell (Shen and Bargmann, 2003). Rather, synapse assembly depends on the presence of particular vulval epithelial cells that act as guideposts. These guidepost epithelia express the Immunoglobulin (Ig) domain-containing protein SYG-1 which is required for synapse assembly (Shen and Bargmann, 2003). SYG-1 physically interacts with SYG-2, an Ig-domain protein expressed by the neurons (Shen *et al.*, 2004). The presence of SYG-2 on the guidepost epithelia results in localization of neuronal SYG-1 at that site, which in turn leads to local accumulation of synaptic vesicles and other components (Shen *et al.*, 2004). SYG-1 and SYG-2 resemble proteins required in *Drosophila* for myoblast fusion, a process that also requires precise localization of specialized vesicles, and a homologous module may act in neurons during development of mammalian olfactory glomeruli, a process requiring precise neuron-neuron pairing (Strunkelnberg *et al.*, 2001; Serizawa *et al.*, 2006). Thus, in at least one example, synapse assembly in *C. elegans* is specified by a conserved cell-cell interaction module that provides signaling between a neuron and an epithelial cell, demonstrating that synapse formation cues need not be provided by specialized glia.

The absence of glia at many of the synapses in *C. elegans* also raises the question of how neurotransmitter reuptake and recycling is accomplished. Serotonergic neurons express MOD-5, the only serotonin reuptake transporter in *C. elegans*, and presumably are able to reclaim the neurotransmitter they themselves release (Ranganathan *et al.*, 2001). Similarly the dopamine transporter DAT-1 is expressed exclusively by dopaminergic neurons (Nass *et al.*, 2005). Likewise, with the exception of two sets of inhibitory motor neurons, most GABAergic neurons in *C. elegans* express the GABA transporter SNF-11; however, the transporter is additionally expressed by several classes of muscle cells that may perform post-synaptic neurotransmitter reuptake (Mullen *et al.*, 2006). The multiple glutamate transporters encoded in the *C. elegans* genome have only begun to come under investigation, but a recent comprehensive study of their expression and function indicates that non-neuronal cells may perform glutamate reuptake: one glutamate transporter in particular is expressed primarily in hypodermal and muscle cells and mutants lacking this transporter exhibit defects in neurotransmission (I. Mano, S. Straud and M. Driscoll, personal communication). The reuptake of glutamate by non-neuronal cells in *C. elegans* is an intriguing parallel to the reuptake of glutamate by astrocytic glia at the mammalian synapse.

Activities of mammalian glia that are provided by specialized glia in *C. elegans*

In the mammalian brain, glia surround synapses, creating confined environments in which neurotransmission takes place. Mammalian glia can also shape synapse morphology. For example, ephrin signaling by synaptic astrocytes influences the structure of dendritic spines (Nishida and Okabe, 2007; Murai *et al.*, 2003). Finally, signaling between glia and neurons at the vertebrate synapse has recently been shown to modulate synapse strength, implicating glia in learning and memory (Rousse and Robitaille, 2006; Serrano *et al.*, 2006).

Like perisynaptic glia in mammals, the most obvious function of *C. elegans* glia is to provide a sealed environment around the synapse, in this case the sensory synapse, where the receptor-bearing cilia of sensory neurons reside. For example, in the largest sense organ, the amphid, the ciliated endings of eight sensory dendrites sit within a sealed tube-like channel formed by the sheath and socket glia. Serial section electron microscopy revealed that the socket glial cell forms a cuticle-lined pore at the surface of the animal; this pore is fastened via adherens-like junctions to a channel in the sheath glial cell that is itself fastened via adherens-like junctions to the sensory dendrites (Ward *et al.*, 1975). This arrangement creates a sealed environment around the sensory synapse into which signals from the environment freely diffuse; the environment around the sensory cilia may also be regulated by the sheath glial cell which is densely laden with secretory vesicles in that region. Thus, like some mammalian glia, *C. elegans* glia are positioned to control the microenvironment at specific synapses.

Likewise, as in the mammalian nervous system, glial morphogenesis in *C. elegans* is coupled to neuronal function and structure. For example, sense organs of the male tail require the zona-pellucida-domain protein RAM-5, expressed by glia, for function (Yu *et al.*, 2000). Mutants lacking RAM-5 display morphologically normal sensory neurons but morphologically aberrant glia; as a result the sense organs are non-functional (Yu *et al.*, 2000). Similarly, the Patched-related protein DAF-6, expressed by both sheath and socket glia of the amphid, is required for formation of the tube-like channel of the glia (Perens and Shaham, 2005). Mutants lacking DAF-6 display sensory neurons with grossly normal morphologies, but with cilia misrouted into blind pockets within the sheath glial cell and unable to access the environment; as a result the animals are unable to sense specific environmental signals (Perens and Shaham, 2005). These examples demonstrate that proper glial morphology in *C. elegans* is necessary for neuronal function. Importantly, as in mammals, glial morphogenesis in *C. elegans* is coordinated with morphogenesis of neurons: during entry into the dauer larval stage, a specialized developmental program triggered by starvation, the sheath glial cell undergoes a dramatic expansion in width that is precisely matched by an accompanying increase in surface area of the flattened wing-like sensory cilium of the AWC neuron (Albert and Riddle, 1983).

Reciprocal signaling between neurons and glia likely underlies the precise coordination between these two cell types in *C. elegans*. Indeed, mutants that fail to develop cilia due to a cell-autonomous defect in sensory neurons show alterations in the physiology of the sheath glial cell, including

Table 1. A restricted set of activities are shared between mammalian and *C. elegans* glia

Activity of mammalian glia	Known to be provided in *C. elegans* by
Neurotrophic activities	
Promoting neuronal differentiation	Neurons (cell-autonomous)
Promoting neuronal survival	Neurons (cell-autonomous)
Guidance and positioning cues	
Guiding neuronal migration	Neurons (non-cell-autonomous), glia, hypodermal cells, muscle
Axon pathfinding	Neurons (non-cell-autonomous), glia, epidermoblasts, possibly others
Synapse positioning	Epithelial cells
Facilitating/modulating neurotransmission	
Myelination	Absent
Neurotransmitter reuptake	Neurons, muscle, hypodermal cells
Dendrite morphogenesis at the synapse	Glia
Neuron-glia signaling	Glia

an accumulation of secretory vesicles and relocalization of DAF-6 protein, demonstrating that the glial cell 'knows' the state of the sensory cilia (Perens and Shaham, 2005; Perkins *et al.*, 1986). DAF-6 is a polytopic transmembrane protein localized to the lumenal surface of the tube-like channel surrounding the sensory cilia and is related to the Hedgehog-receptor Patched, suggesting a mechanism by which it might receive intercellular signals. Other intercellular signaling proteins expressed by *C. elegans* glia include the Dispatched-related protein CHE-14, the Warthog-family protein WRT-6 which shares features with Hedgehog, and the vascular-endothelial growth factor (VEGF) receptor homolog VER-1 (Popovici *et al.*, 2002; Michaux *et al.*, 2000; Aspock *et al.*, 1999). Conversely, neurons are sensitive to the state of the glia: ablation of the amphid sheath glial cell results in specific functional defects in the associated sensory neurons (T. Bacaj and S. Shaham, unpublished). The function of the CEP sheath glial cell surrounding the synapses of the nerve ring has not been characterized. Identifying the mechanisms and consequences of neuron-glia signaling in *C. elegans* is currently a major goal for the field, but the evidence so far suggests that, like mammalian glia, *C. elegans* glia participate in two-way conversations with their associated neurons, and that these exchanges are important for nervous system function.

CONCLUSIONS AND THOUGHTS ON ANCESTRAL FUNCTIONS OF GLIA

In summary, *C. elegans* presents a nervous system in which glia are not ubiquitous, as in the mammalian nervous system, but are mainly restricted to sensory synapses at which they play a critical role (Table 1). Some activities of mammalian glia are not present in *C. elegans*. For example, myelination is not needed because conduction distances are relatively small, and neurotrophic factors are not needed because the developmental lineage is fixed. Other activities of mammalian glia are required, such as guidance of cell migration, axon pathfinding, synapse positioning, and neurotransmitter reuptake, but can be provided in *C. elegans* by non-glial cell types, including neurons, epithelia, and muscle. Instead of fulfilling these roles, most of the glia that are present in *C. elegans* control the structure and environment surrounding sensory dendrites and appear to participate in reciprocal signaling with those neurons. The restricted association of *C.*

elegans glia with synapses likely to be involved in adaption and learning hints at an ancestral role for glia in regulating synapse strength.

Given that most activities of mammalian glia can be provided by other cell types – at least in a simple nervous system – one wonders why *C. elegans* has glia at all and how such a cell type arose. The observations presented here suggest one highly speculative view of glial origins. As the lineal relationship of neurons and glia is conserved in *C. elegans*, these two cell types may have arisen from a common ancestral cell. A modern model for such an ancestral cell may exist in the neuroepithelia of mammalian sensory organs, such as the ciliated hair cells of the inner ear or the ciliated sensory cells of the taste bud. The unique relationship of glia with ciliated sensory neurons in *C. elegans* is consistent with this notion. Intriguingly, the only glia in *C. elegans* that associate with non-sensory neurons are the CEP sheath glia, which envelop the nerve ring; however, these cells also serve as the sheath glia for the CEP sensory neurons and it is through a distinct set of processes that they separately associate with the nerve ring. This arrangement suggests that the sensory organ glia may have acquired the ability to associate with non-sensory synapses. As nervous systems grew larger and more diverse, glia may have acquired additional functions, including myelination and the ability to regulate neuronal differentiation and survival. Meanwhile, as glia acquired the ability to interact with more synapses and spread further into the central nervous system, they may have subsumed activities previously provided by other cell types. This highly speculative view implies that the ancestral functions of glia – perhaps the ability to shape synaptic environments and modulate neuronal inputs – are those that originated in the glia of sensory organs.

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