The Glia of *Caenorhabditis elegans*

GRIGORIOS OIKONOMOU AND SHAI SHAHAM*
Laboratory of Developmental Genetics, The Rockefeller University, New York, New York

ABSTRACT
Glia have been, in many ways, the proverbial elephant in the room. Although glia are as numerous as neurons in vertebrate nervous systems, technical and other concerns had left research on these cells languishing, whereas research on neurons marched on. Importantly, model systems to study glia had lagged considerably behind. A concerted effort in recent years to develop the canonical invertebrate model animals, *Drosophila melanogaster* and *Caenorhabditis elegans*, as settings to understand glial roles in nervous system development and function has begun to bear fruit. In this review, we summarize our current understanding of glia and their roles in the nervous system of the nematode *C. elegans*. The recent studies we describe highlight the similarities and differences between *C. elegans* and vertebrate glia, and focus on novel insights that are likely to have general relevance to all nervous systems.   ©2010 Wiley-Liss, Inc.

INTRODUCTION

Animal nervous systems promote a spectacular range of functions, from simple reflexive escape behaviors, to complex cognitive tasks. The diversity of animal behavior is executed through signals speeding down neurons connected in complex ways, and tasked with sensing stimuli, analyzing sensory input, and generating responses. Other complex multicellular assemblies, such as the immune system, are generally composed of multiple cell types, each providing important contributions towards the activity of the system as a whole. It would be, therefore, surprising if nervous systems comprised only one relevant cell type. Nature seems to agree. Neurons have also been assigned dedicated partners: the glia.

Whether cells functioning as glia exist in cnidarians such as hydra, whose nervous systems are simple nerve nets, is not yet clear. However, as organisms increase in complexity, an explosive expansion in glia number and morphological complexity is evident. A recent study, for example, suggests that the human brain consists of roughly equal numbers of neuronal and glial cells (Azevedo et al., 2009). Furthermore, each human astrocyte can contact thousands of neurons (Oberheim et al., 2009; Spacek, 1985; Ventura and Harris, 1999). These observations suggest that nervous systems benefit greatly from having an additional cellular component. Indeed, in vertebrates, there are few if any aspects of neuron development and function in which glia have not been implicated. Neuronal differentiation, survival, proliferation, migration, morphogenesis, circuit formation, synaptogenesis, establishment and maintenance of ionic balance, and neurotransmission all seem to involve glia (Barres, 2008).

Although an ultimate understanding of nervous systems must eventually explain the detailed action of the human brain, a basic understanding of neurons and glia at the level of cell biology and simple circuitry is more easily achieved by studying relevant model systems. Despite the differences between invertebrate model systems and humans, these systems have been invaluable in deciphering important aspects of human biology. Studies in *Drosophila*, for example, have led to major insights into animal development and human innate immunity, whereas research in the nematode *Caenorhabditis elegans* has provided a deep understanding of the underpinnings of cell polarity, cell death, and RNA interference processes in humans. There is every reason, therefore, to expect similar discoveries in the nervous system.

Progress towards understanding the glia of *Drosophila* is described elsewhere in this issue; here we summarize our understanding of glia development and function in *C. elegans*.

THE ANATOMY OF *C. elegans* GLIA

The nervous system of *C. elegans* consists of 302 neurons, 50 glial cells derived from neuronal/epithelial progenitors, and six glial cells that are mesodermally derived. The nervous system is essentially identical anatomically between individuals, even at the level of the positions of individual synapses, and has been reconstructed in its entirety using electron microscopy (Hall and Russell, 1991; Ward et al., 1975; White et al., 1986). Sensory cues are detected by specialized sensory neurons, most of which reside in the head of the animal. Information from sensory neurons is relayed to a central processing neuropil called the nerve ring, which encircles the pharynx and which can be thought of as the animal's brain. The nerve ring is densely packed
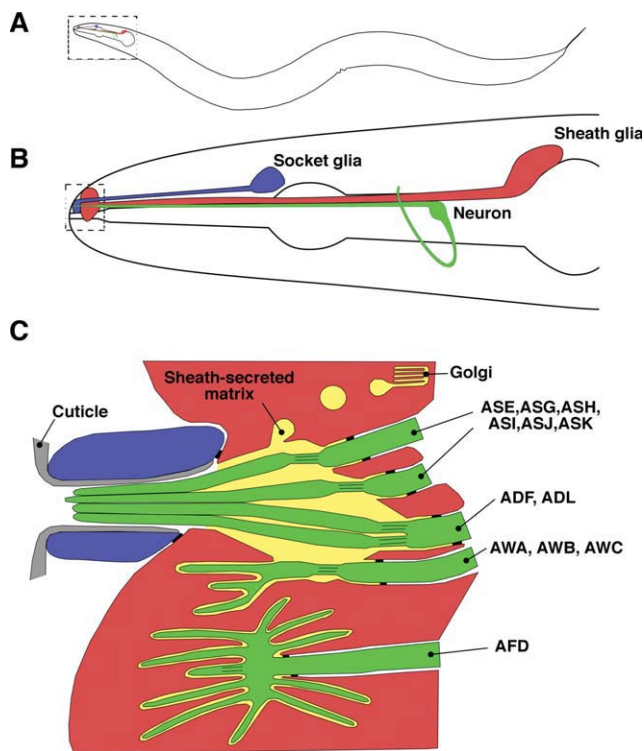
Fig. 1. The amphid sensory organ is a model system for neuron–glia interactions. **A**: Schematic of an adult *C. elegans* hermaphrodite. The amphids are located in the head. **B**: Each amphid consists of 12 neurons (in green; only one is drawn here for simplicity) and two glial cells, the sheath (AMsh, in red) and the socket (in blue). **C**: Processes from the neurons and glia come together at the tip of the nose. The glial cells align to form a tube, the amphid channel, through which some amphid neuron dendrites extend sensory cilia that adopt distinct morphologies: single cilia (neurons ASE, ASG, ASH, ASI, ASJ, and ASK), double cilia (neurons ADF and ADL), wing cilia (neurons AWA, AWB, and AWC) and the finger cilia of AFD. Adherens junctions between the dendrites and the sheath glia, as well as between the sheath and socket glia establish a niche for the sensory cilia. This niche is filled with matrix material secreted by the sheath glia; thus, the site where the nervous system of the animal meets the environment is under the direct control of glia. Adapted from Perkins et al., 1986.

with *en passant* synapses between sensory neurons and interneurons, and between interneurons. Some interneurons connect to motor neurons that control the musculature of the head and body of the animal (Ware et al., 1975). Glial processes are found at neuronal junctions at every level: at sensory receptive endings, at neuron-neuron synapses, and at neuromuscular junctions, raising the intriguing notion that glia in *C. elegans* may be primarily involved in intercellular information transfer, an idea that is supported by several lines of evidence (see following sections).

Glia at Sensory Receptive Sites

Glial functions at sensory receptive endings of *C. elegans* have been primarily examined in the context of the amphid, a bilateral sensory organ located in the head of the animal (Fig. 1A), and consisting of twelve sensory neurons and two glial cells, also referred to as sheath

and socket cells (Ward et al., 1975). All cells of the amphid extend processes to the tip of the nose (Fig. 1B). At the tip, the sheath and socket glia come together to form a tube known as the amphid channel (Fig. 1C). The amphid channel contains a material called matrix, at least some components of which are secreted by the sheath glia (Perens and Shaham, 2005).

Of the 12 neurons comprising the amphid (Fig. 1C), eight extend non-motile, microtubule-based sensory cilia that travel the length of the amphid channel to become exposed to the environment. These specialized receptive endings are sites of localization for receptors that recognize chemical and mechanosensory stimuli (Bargmann, 2006). The receptive endings of three other amphid sensory neurons enter the amphid channel, but then divert and expand elaborately branched cilia into blind pockets within the sheath glial cell where the detection of volatile chemicals takes place. The remaining amphid sensory neuron, which senses temperature, is also embedded within the sheath glia, but its dendritic terminus does not track through the amphid channel.

In addition to seven classes of hermaphrodite sensory organs, all of which have an architecture resembling that of the amphid, *C. elegans* males possess four more classes of sensilla, located in the tail, that facilitate mating with hermaphrodites (Sulston et al., 1980). Although all of these male-specific sensilla include glial cells, studies of glia in the male have focused on the ray sensilla. Each ray consists of two sensory neurons that extend dendrites through a channel formed by a single glial cell known as the structural cell.

In all sensory organs, a key role for the sheath glia seems to be the formation of an isolated extracellular compartment in which to house neuronal sensory receptive endings. Such an arrangement has been conserved in sensory organs of other invertebrates and vertebrates.

Synaptic Glia

All glia in *C. elegans*, including the sheath glia of the cephalic sensilla (CEPsh glia), are associated with sensory organs (Fig. 2A,B). However, the four CEPsh glia are unique in that they also extend sheet-like processes that wrap around the nerve ring (Fig. 2A). Anatomically, these sheets may serve to isolate the nerve ring in the same way the blood-brain barrier isolates neurons in vertebrate brains. Consistent with this idea, the ancestral vertebrate has been postulated to have had a glial blood-brain barrier, with the endothelial barrier arising only later in evolution (Bundgaard and Abbott, 2008). However, no functional evidence supporting a barrier role for CEPsh glia has been described so far.

The sheet-like processes of the CEPsh glia also send processes that penetrate deep within the nerve ring and contact a small number of synapses (Fig. 2C), an architecture reminiscent of the fine processes of vertebrate astrocytes that surround synapses. As with vertebrate astrocytes (Bushong et al., 2002; Halassa et al., 2007; Ogata and Kosaka, 2002), CEPsh glia processes are
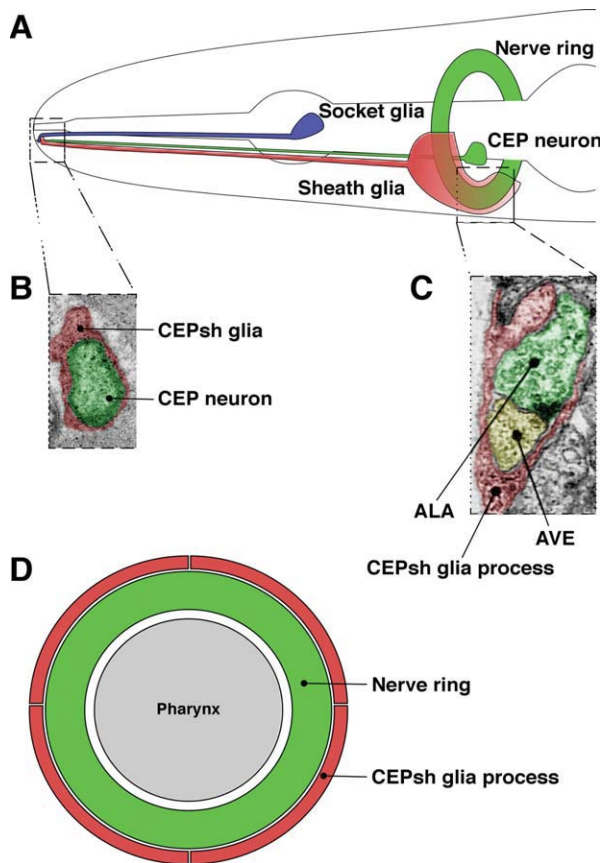
Fig. 2. The cephalic sheath glia (CEPsh glia) physically interact with both the sensory receptive endings of the CEP neurons, and with the nerve ring. **A**: The CEP sensillum displays the same basic architecture as the amphid. A thin anterior process from the CEPsh glia runs parallel to the CEP neuron, whereas a sheet-like posterior process wraps around the "brain" of the animal. **B**: The anterior process establishes a channel similar to, but smaller than the amphid channel. **C**: Thin projections from the posterior process enter the nerve ring and can ensheath synapses, similar to astrocytes of vertebrate systems. Adapted from White et al., 1986. **D**: Schematic of a cross-section through the nerve ring. Each animal has four CEPsh glia that wrap around the nerve ring without overlapping with each other. Thus, CEPsh glia establish independent domains, in a fashion reminiscent of astrocytic domains.

organized in sharply defined, non-overlapping domains with respect to the neuronal processes they contact (Fig. 2D), suggesting that tiling is a fundamental property of synapse-associated glial cells. The anatomy of CEPsh glia suggests the hypothesis that in evolution, glia first appeared in the periphery in association with sensory receptive endings, and were then co-opted by the central nervous system to ensheath axons and neuron-neuron synapses (Heiman and Shaham, 2007).

Glia at Neuromuscular Junctions

C. elegans possesses three pairs of GLR cells (dorsal, ventral, and lateral) that are arranged in a six-fold symmetrical fashion around the pharynx, just posterior to the nerve ring (White et al., 1986). From each GLR cell body emanates an anteriorly directed sheet-like process that wraps
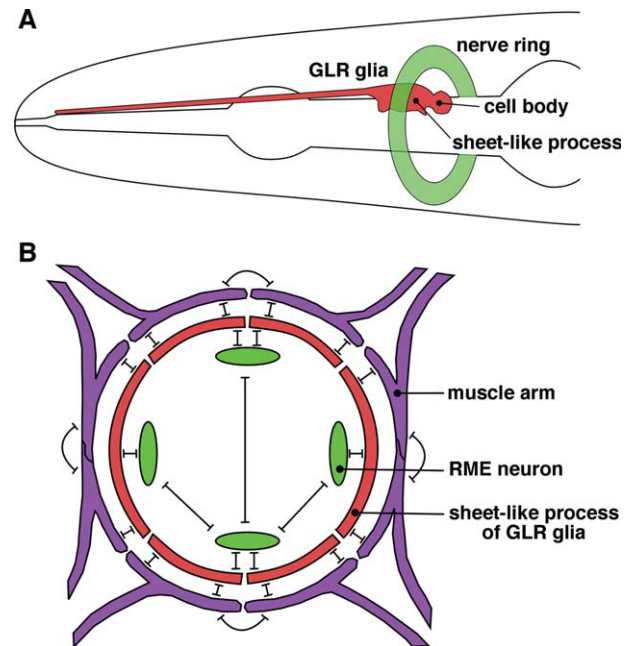
Fig. 3. The GLR glia establish gap junctions that bridge neurons with muscles. **A**: Each animal has six GLR glia. Only one is depicted here. The cell body lies posterior to the nerve ring. An anterior, sheet-like process lines the inside of the nerve ring and then tapers to a thinner process. **B**: The sheet-like processes of the GLR glia make gap junctions to head muscle cells and the RME motor neurons that innervate them. Adapted from White et al., 1986.

around the inside aspect of the nerve ring (Fig. 3A). Here, GLR cells make gap junctions to head muscle cells, as well as to the RME motor neurons that innervate these muscles (Fig. 3B). More anteriorly, the sheet-like processes narrow into thin extensions (Fig. 3A). Unlike other glial cells, GLR cells express the *C. elegans* homolog of myoD (Krause et al., 1994) and are of mesodermal origin. Although GLR glia are situated in an ideal position to affect neuron-muscle communication, their functions have not been studied. It is intriguing to speculate that they may serve functions analogous (but probably not homologous) to those of perisynaptic Schwann cells. It is striking that only *C. elegans* head muscles form partnerships with glia. These muscles mediate fine motor behaviors that are less stereotypical than the undulations produced by body wall muscles, perhaps explaining the need for glial companionship.

SPECIFICATION OF GLIA IN *C. elegans*

An important regulator of glia determination in *C. elegans* is the zinc-finger transcription factor LIN-26. Mutations in the *lin-26* gene were initially reported to transform epithelial (hypodermal) cells into neurons (Ferguson et al., 1987). Later, Labouesse et al. (1996) realized that *lin-26* is also important for glial specification: mutations in *lin-26* lead either to glial cell death or, more rarely, to glial transformation into neurons (Labouesse et al., 1996). The function of *lin-26* is reminiscent of the function of the glial-cells-missing (*gcm*)

gene in *Drosophila* (Hosoya et al., 1995; Jones et al., 1995; Vincent et al., 1996). GCM protein contains a Zn-finger domain, and *gcm* mutations transform glia into neurons. However, LIN-26 and GCM proteins are not obviously homologous.

More recent studies of CEPsh glia development demonstrate similarities with aspects of mammalian glial differentiation. *hlh-17*, the *C. elegans* gene most closely related to the mammalian oligodendrocyte differentiation gene Olig2, is specifically expressed in CEPsh glia (McMiller and Johnson, 2005; Yoshimura et al., 2008). *hlh-17* expression in and differentiation of the two dorsal CEPsh glial cells depends on the Paired-domain of the transcriptional regulator VAB-3. Expression of *hlh-17* in ventral CEPsh glia depends on the homeodomain of VAB-3, as well as on its downstream target, the MLS-2 transcriptional regulator. Strikingly, VAB-3 is the *C. elegans* protein most similar to vertebrate Pax6 and Pax7 transcription factors, which regulate ventral and dorsal expression, respectively, of Olig2 in the developing spinal cord. Likewise, MLS-2 belongs to the Nkx/Hmx superfamily of transcriptional regulators and is distantly related to Nkx6, which promotes Olig2 expression in the ventral pMN region of the developing vertebrate neural tube. Thus, despite the evolutionary distance between nematodes and vertebrates, it seems that aspects of glia specification are related.

GLIA AND NERVOUS SYSTEM MORPHOGENESIS
Sensory Channel Development

Sheath glia

The amphid sensory channel allows sensory neurons to communicate with the outside environment. The sheath glial cell contributes the posterior end of this channel, and a number of recent studies have provided insight into the mechanism by which the sheath glia channel is formed. These studies were initially enabled by the identification of DAF-6 (Riddle et al., 1981), a Patched-related protein that acts within glia to regulate amphid channel shape (Herman, 1987; Perens and Shaham, 2005). In animals carrying mutations in the *daf-6* gene, the sheath glia channel is bloated and malformed. Furthermore, sensory cilia appear trapped within the channel and are unable to reach the outside environment. *daf-6* mutants are defective in behaviors that depend on the direct contact of sensory neurons with the animal's surroundings (Albert et al., 1981; Perkins et al., 1986), suggesting that channel integrity is required for amphid functionality (Perens and Shaham, 2005).

DAF-6 lines the amphid channel and also localizes to apical surfaces of other luminal structures in *C. elegans*. Furthermore, animals mutant for both *daf-6* and *che-14*, a Dispatched homolog that is also important for amphid morphogenesis (Michaux et al., 2000), display defects in many tubular systems. These results suggest that the machinery for channel formation overlaps, at least to some extent, with that controlling tube formation, rais-
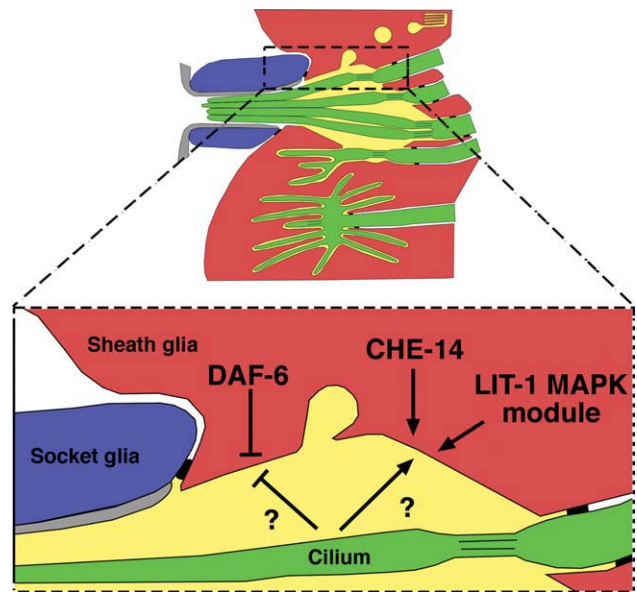
Fig. 4. The size of the amphid glia channel is regulated by two opposing forces. DAF-6/Patched-related acts within the glia to reduce channel girth, whereas CHE-14/Dispatched and the LIT-1/Nemo-like kinase MAPK module act (also within glia) to increase channel cross-section. Unidentified neuronal signals are required for the correct localization of DAF-6 and LIT-1, as well as normal channel morphogenesis.

ing the possibility that glial ensheathment of neurons in other animals may also be related to tubulogenesis.

The amphid channel lining fits snuggly around the neuronal cilia it ensheaths, suggesting the possibility that a neuronal signal may be involved in controlling channel diameter and shape. Interestingly, the shape of the sheath glia channel and localization of DAF-6 along the length of this channel depends on the presence of neuronal sensory cilia. In animals carrying mutations that stunt or block cilia development, DAF-6 protein is no longer localized to the channel surface and accumulates instead in a punctate structure, which may be the sheath/socket junction. In addition, the channel acquires an abnormal expanded morphology (Perens and Shaham, 2005).

Both *daf-6* and neuronal signals seem to limit the size of the surrounding glial channel (G. Oikonomou, Y. Lu and S. Shaham, unpublished observations), suggesting the possibility that a counteracting mechanism exists to promote channel expansion. To identify components of such a pathway, we used random mutagenesis to screen for *daf-6* mutant animals possessing patent amphid channels. We have shown that one such suppressed strain harbors a mutation in the gene *lit-1*, a NEMO/MAP kinase homolog (G. Oikonomou, E. Perens, Y. Lu and S. Shaham, unpublished observations). Strikingly, some *lit-1* single mutants have channels that are either narrower than wild-type channels, failing to accommodate all amphid sensory cilia, or that fail to form altogether. *lit-1* is expressed in many glial cells and the LIT-1 protein localizes to the luminal membranes of the amphid channel. This localization also depends on the presence of cilia and seems to be important for *lit-1* function. It seems, there-

fore, that two opposing forces, one promoting lumen expansion, the other limiting lumen growth, act in balance to form the properly proportioned glial channel of wild-type animals (Fig. 4, G. Oikonomou, E. Perens, Y. Lu and S. Shaham, unpublished observations).

Socket glia

Socket glia have been less intensively explored than their sheath glia counterparts, however, studies of the *C. elegans alr-1* gene support an important role for these glia in amphid function. *alr-1* (Tucker et al., 2005) encodes the *C. elegans* homolog of the Paired class homeobox transcription factor Aristaless (Galliot et al., 1999), first characterized in *Drosophila* (Schneitz et al., 1993). In humans, mutations of the Aristaless homolog ARX cause a range of neurological defects (Strømme et al., 2002). In *C. elegans*, *alr-1* mutants hatch with no detectable abnormalities, but progressively lose amphid functionality as the animal grows. Ultrastructural studies revealed that in older *alr-1* mutants, the tight junctions between sheath and socket glia cannot be detected, while matrix material and sensory cilia can now be found throughout the tip of the head (Tucker et al., 2005).

The *alr-1* mutant defects reflect the importance of a precise and stable connection between the sheath and socket glial channels for sensory organ function. Embryonic ablation studies of socket glia progenitor cells reveal that long-range signaling may be involved in directing this connection. Specifically, Sulston et al. (Sulston et al., 1980) used a laser microbeam to ablate progenitor cells of specific sensory organ socket glia and found that the cognate neuron and sheath glia now associated with socket glia of a different sensory organ. This observation suggests an active attraction between socket glia and neurons/sheath glia. The molecular basis of this attractive signal has not yet been determined.

The Role of Glia in Neurite Extension and Nerve Ring Structure

The sensory neurons of the amphid extend dendrites over a length of about 100 microns towards the tip of the nose. These dendrites fasciculate into stereotyped bundles that run in parallel and in close proximity to the process of the amphid sheath glial cell. Recently, aspects of the mechanism underlying amphid process extension and the coordination of glia and neuron process lengths have been described (Heiman and Shaham, 2009).

During embryogenesis, amphid sensory neurons are born near the tip of the developing head (Sulston et al., 1983). There they extrude a short projection that is attached to the tip of the nose. Posterior migration of the neuronal cell bodies then results in dendrite extension—a mechanism that has been coined retrograde extension (Heiman and Shaham, 2009). The tip anchor is composed of at least two proteins (Heiman and Shaham, 2009): the zona pellucida (ZP) domain protein DYF-7, which is secreted by the neurons, and DEX-1, a
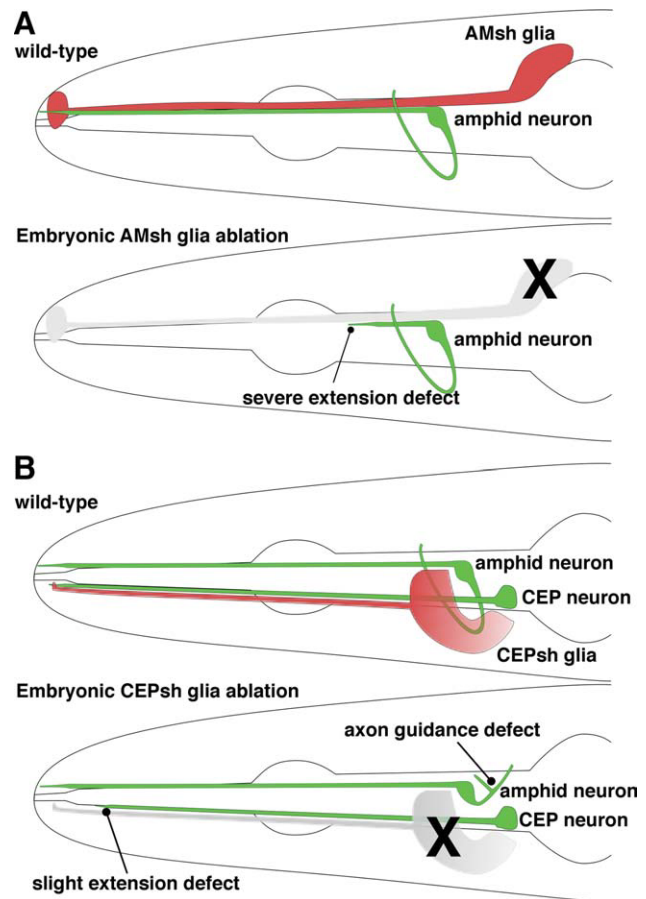
Fig. 5.   Embryonic ablation of glia results in dendrite extension and axonal guidance defects. **A**: Ablation of the amphid sheath glia during embryogenesis results in amphid neurons with dendrites that are much shorter than normal, reminiscent of *dex-1* and *dyf-7* mutants (see text). **B**: Ablation of the CEPsh glia results in slightly shorter CEP dendrites, and axon guidance defects.

zonadhesin domain containing protein secreted by nonneuronal neighboring cells. DEX-1 and DYF-7 are similar to α and β tectorins, proteins that compose the tectorial membrane, an extracellular matrix that anchors hair cell cilia in the inner ear (Legan et al., 1997). Like the tectorins and other ZP domain proteins, DYF-7 can multimerize, and its multimeric state seems to be required for proper dendrite anchoring. These parallels suggest a common, evolutionarily conserved module used in sensory organ development.

dyf-7 and *dex-1* mutants are defective not only in the lengths of their amphid sensory dendrites, but also in the length of the sheath glia process, suggesting that the morphogenesis of neurons and glia in this sensory organ is tightly linked (Heiman and Shaham, 2009). Although it is not yet clear whether DEX-1 is expressed in the amphid sheath glia, other observations suggest a direct role for glia in amphid dendrite extension. First, ablation of the amphid sheath glia during embryogenesis results in short dendrites (Fig. 5A), similar to the ones seen in *dyf-7* and *dex-1* animals (T. Bacaj and S. Shaham, unpublished observations). A similar result is obtained in the

cephalic sensory organ where ablation of the CEPsh glia results in dendrite extension defects of the CEP neurons (Fig. 5B) (Yoshimura et al., 2008). Second, expression of either *dex-1* or *dyf-7* cDNA by the amphid glia can rescue the dendrite extension defect of each respective mutant, suggesting that glia are positioned to establish or modify the extracellular matrix required for dendritic tip anchoring. Third, a study of amphid sheath glia gene expression using gene microarrays revealed that of the 298 glia-enriched gene transcripts, 159 encoded secreted/transmembrane proteins, including a number of proteins predicted to contain ZP domains that could, perhaps, interact with DYF-7 and DEX-1 (Bacaj et al., 2008).

The gene *ram-5* also encodes a ZP domain protein and is expressed in many glial cells, including the structural cell of the ray sensilla in the male tail. Interestingly, in *ram-5* mutants, the overall architecture of the ray sensillum is grossly malformed due to defects in the shape of the glial structural cell (Yu et al., 2000), supporting the notion that ZP domain proteins secreted by both neurons and glia play important roles in shaping sensory organs.

Glia in *C. elegans* also play important roles in organizing and directing axon outgrowth. In addition to affecting the lengths of CEP neuron dendrites, embryonic ablation of CEPsh glia precursor cells has profound effects on nerve ring structure and axon guidance (Yoshimura et al., 2008). In 20% of animals in which two of the four CEPsh glial cells are eliminated, the nerve ring is not formed, and instead neuronal processes are splayed over the entire head region of the animal. In the remaining animals, a distinct nerve ring is formed, however neuronal axons within the ring are disorganized (Fig. 5B), with individual axons often displaying extensive guidance and branching defects. At least part of the axon guidance defect is due to the lack of UNC-6/Netrin secretion from the ventral CEPsh glia, which normally guides axons within the nerve ring (Wadsworth et al., 1996; Yoshimura et al., 2008), consistent with the observation that UNC-6/Netrin is required for proper guidance of the axon of the nerve ring interneuron RIA (Colón-Ramos et al., 2007). Thus, glia play key roles in both dendrite and axon extension in *C. elegans*.

Glia and Synaptogenesis

In vertebrates, glia seem to play an important role in synaptogenesis by secreting proteins that establish postsynaptic competence (Christopherson et al., 2005; Eroglu et al., 2009). In *C. elegans*, non-neuronal epithelial cells have been shown to play direct roles in synaptogenesis (Shen and Bargmann, 2003; Shen et al., 2004), suggesting that glia may also have synaptogenic roles in this animal. Studies of CEPsh glia, which envelop the synapse-rich nerve ring, suggest the possibility that these glia might participate in synapse formation. Colón-Ramos et al. (2007) demonstrated that synapse formation between the AIY and RIA neurons requires activity of the netrin receptor *unc-40*/DCC in AIY (Colón-Ramos et al., 2007), raising the possibility that

glial UNC-6/Netrin might direct RIA-AIY synaptogenesis. This idea was supported by the demonstration that *unc-6* mutants exhibit defects similar to *unc-40* in RIA-AIY synapse formation. Although UNC-6/Netrin may play a direct role in the formation of this synapse, EM serial reconstruction of the synaptic site in adult animals suggests that the CEPsh glial process does not closely abut this synapse (White et al., 1986), raising the possibility that UNC-6 may affect synaptogenesis indirectly, by affecting the fine-tuning of axon guidance. Higher resolution studies of the early development of this synapse are likely to shed more light on the role of glia in establishing this synaptic contact.

Phagocytosis

Vertebrate and *Drosophila* glia engulf neuronal cell corpses and neurite debris during development and following neuronal injury (Aldskogius and Kozlova, 1998; Logan and Freeman, 2007). Glia in *C. elegans* are also able to phagocytose dying cells during embryogenesis (Sulston et al., 1983). Whether injury to glia-associated neurons promotes glial phagocytic activity and whether phagocytosis could contribute to regulation of synapse formation, as has recently been shown in *Drosophila* (Fuentes-Medel et al., 2009), remains to be determined.

The Roles of Glia in Nervous System Function

Glial control of sensory neuron function

C. elegans responds to a variety of stimuli including temperature, taste, smell, touch, high osmolarity, oxygen, UV light, and pheromones (Bargmann, 2006). Many of these responses are well characterized, robust, and quantifiable, and begin with the detection of the stimulus by specific sensory neurons of the amphid. The amphid, therefore, provides a suitable setting for investigating the role of glia in sensory neuron function.

To determine whether amphid sheath glia are required for sensory neuron function, these glia were ablated (Bacaj et al., 2008) in larvae in which amphid morphogenesis had been completed. Animals lacking glia were then subjected to a battery of behavioral assays to test the functions of different amphid neurons. Morphological studies were also performed to examine effects of glia on neuronal receptive ending shapes. The results of these studies suggest that amphid neurons can be classified into three categories with respect to their interactions with the sheath glial cell. Upon glia removal, three of the four glia-embedded neurons (AWA, AWC, and AFD) display involution of their receptive endings (Fig. 6), accompanied by defects in the behaviors controlled by these neurons. A similar result can be elicited by transiently blocking the secretory pathway in glia (A. Singhvi and S. Shaham, unpublished). These results demonstrate that amphid sheath glia are continuously required to maintain sensory neuron receptive ending morphology of a subset of sensory neurons.
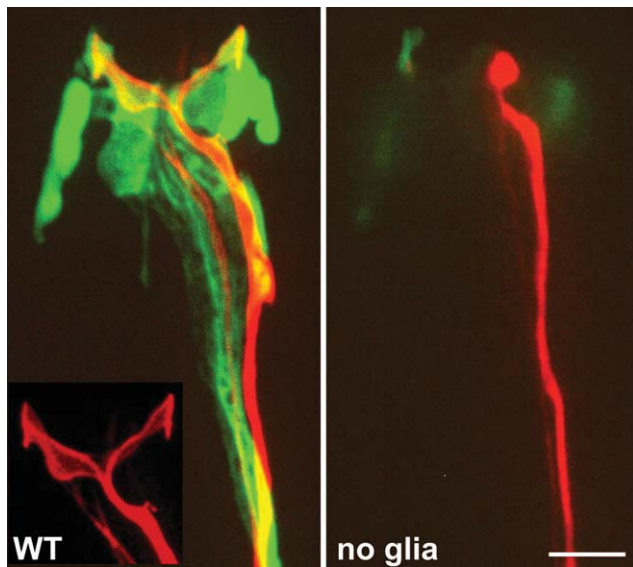
Fig. 6.   Ablation of the amphid sheath glia in adult animals can result in severe disruption of the morphology of neuronal receptive endings. Left panel, unablated amphid. Glia, green; AWC neuron, red. Right panel, glia-ablated amphid. Note loss of extended sensory receptive ending of AWC neuron. Scale bar 5 μm. Adapted from Bacaj et al., 2008.

The remaining glia embedded neuron, AWB, makes up the second class of amphid neurons. AWB receptive ending morphology is less disrupted by glia ablation, and AWB seems to maintain its function. The third class of neurons comprises the channel neurons. Although these neurons retain their normal morphology after glia ablation, and properly localize ciliary components and odorant receptors, they are, nonetheless, unable to promote normal behavior. Calcium transients normally induced in one of these neurons, ASH, in response to a stimulus of high osmolarity are absent in glia-ablated animals. These results demonstrate that the maintenance of neuronal structure cannot be the sole function of amphid sheath glia.

A first step towards deciphering the mechanism by which glia promote channel neuron activity was provided by analysis of the gene *fig-1*. *fig-1* is expressed specifically in amphid sheath glia and is predicted to encode a secreted protein containing two thrombospondin TSP1 domains, multiple C6 domains of unknown function, and a putative EGF-like type II motif. Mutations in *fig-1* stunt the aversive response of *C. elegans* to 1-Octanol, a behavior mediated by amphid channel neurons. *fig-1* mutants also display a curious defect in the ability of channel neurons to fill with the lipophilic dye DiI: wild-type animals soaked in DiI take up the dye through the amphid channel, resulting in labeling of some, but not all, channel sensory neurons. Despite possessing amphid channels of normal morphology, amphid sensory neurons in *fig-1* mutants are unable to take up dye. A similar defect is seen in animals in which amphid sheath glia are ablated. The basis of this defect is not yet known; however, the observation raises the possibility that glial FIG-1 protein may affect aspects of neuronal membrane architecture.

TSP1 and EGF-like type II domains are also found in the vertebrate protein thrombospondin, which is secreted by astrocytes, and which promotes synaptogenesis by acting on postsynaptic membranes (Christopherson et al., 2005; Eroglu et al., 2009), raising the possibility that FIG-1 may share common functions with thrombospondins. Many similarities exist between the "sensory synapses" at which environmental signals are detected and processed by sensory neurons, and neuron-neuron synapses. These similarities have been recently reviewed (Shaham, 2010), and raise the interesting possibility that sensory synapses may serve as useful models to understand glial functions at neuron-neuron synapses.

Glia have been postulated to control ion homeostasis in synaptic regions, and studies of the *C. elegans* amphid may support this notion. *C. elegans* can detect and avoid solutions of low pH, and a recent study suggests that ion channels of the DEG/ENaC variety may play a role in this behavior. Mutations in the gene DEG-1, which is expressed in the amphid channel neuron ASK, result in defects in low pH avoidance (Wang et al., 2008), raising the possibility that this channel is involved in some aspect of the response. Mutations in another DEG/ENaC channel, *acd-1*, do not result in acid-avoidance defects; however, *acd-1* mutations enhance the avoidance defects of *deg-1* mutants. Surprisingly, *acd-1* is expressed and functions in amphid sheath glia. Although the precise means by which glial *acd-1* modulates acid avoidance is not clear, one possibility is that it regulates the ionic balance in the environment of the neurons. Alternatively, *acd-1* may have a more specific role in the detection of low pH; indeed ACD-1 protein exhibits pH-dependent activity *in vitro* that correlates with *in vivo* function.

DEG/ENaC channels are expressed in vertebrate glia (Berdiev et al., 2003; Brockway et al., 2002; Golestaneh et al., 2000; Hitomi et al., 2009) and have been implicated in synaptic plasticity (Voglis and Tavernarakis, 2008; Wemmie et al., 2002), further supporting the notion that glia enveloping sensory neuron receptive endings and those supporting neuronal synapses may have overlapping functions.

Glia and sensory neuron plasticity

To survive in a changing environment, animals need to respond appropriately to harsh conditions. Under stress (e.g., lack of food or increased temperature), *C. elegans* larvae can enter an alternative developmental stage called dauer. Dauer animals are resistant to treatments that kill nondauer animals and can survive up to a year without feeding. Upon establishment of more favorable conditions, animals exit the dauer state and resume development to become fertile adults. The integrity of amphid sheath glia is required for dauer entry, as demonstrated by amphid sheath glia ablation studies (Vowels and Thomas, 1994), and by analysis of *daf-6* mutants, which were originally isolated as defective in dauer formation (Riddle et al., 1981). More recently, *daf-6* and *che-14* have been impli-
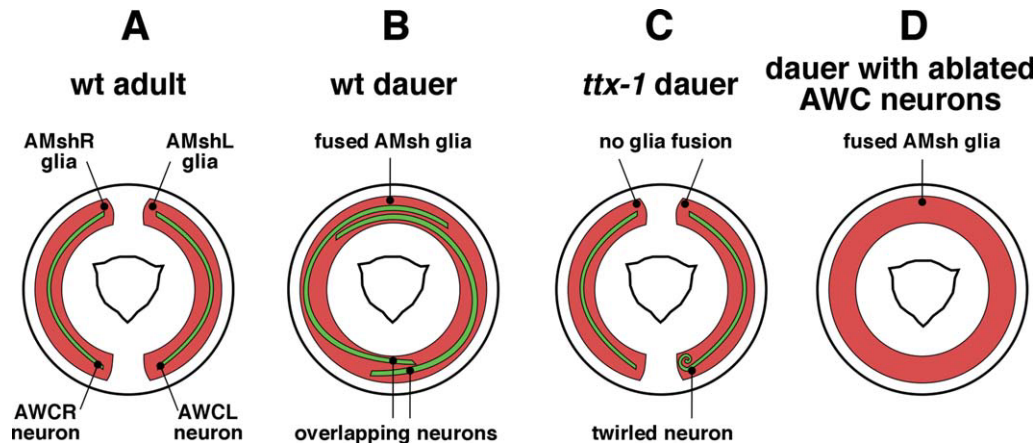
Fig. 7. Glia are required for sensory receptive ending remodeling. **A**: Diagram of a cross-section at the tip of the head. The wing-like processes of the bilateral AWC neurons are ensheathed by the bilateral amphid sheath glia (L and R for left and right respectively). **B**: In dauer animals, the glia expand and fuse to accommodate the expansion of the neuronal wing processes. **C**: If glia lack *ttx-1* they fail to expand and fuse, resulting in lack of neuronal remodeling. **D**: Conversely, glia remodeling takes place independently of neurons.

cated in the dauer stress response in a novel context. Serotonin is involved in the regulation of dauer: animals that produce no serotonin are more likely to enter dauer (Sze and Ruvkun, 2003), and less likely to exit dauer under favorable conditions (Moussaif and Sze, 2009). Mutations in *daf-6* or *che-14* result in transcriptional upregulation of the serotonin biosynthetic enzyme gene encoding Tryptophan Hydroxylase in the amphid neurons (Moussaif and Sze, 2009), suggesting a possible link between glia and serotonin signaling in neurons, although this link may be indirect. The physiological importance and mechanism underlying the regulation of neuronal serotonin levels by glia remain to be elucidated.

The goal of dauer animals is to escape or outlast harsh environmental conditions and to steer towards better pastures. At least two adaptations of the sensory machinery of dauers may promote such behavior. First, upon dauer entry, the repertoire of odorant receptors expressed by individual sensory neurons in the amphid sensory organ is altered (Peckol et al., 2001), perhaps sensitizing the animal to hints of improvement in the environment. Second, some amphid neurons undergo extensive morphological remodeling upon dauer entry (Albert and Riddle, 1983; Mukhopadhyay et al., 2008), none more striking than the AWC neuron, whose glia-embedded wing-like receptive ending greatly expands in dauer animals. This expansion results in extensive overlap of the receptive endings of the left and right AWC neurons. Although the precise significance of this remodeling is unknown (one hypothesis is that it may increase detection sensitivity towards attractive food cues), it is clear that glia play an important role in reshaping AWC (Fig. 7A,B).

Accompanying the remodeling of the AWC neuron is a concomitant remodeling of the amphid sheath glia, which accommodates the large expansion in AWC neuron receptive ending surface area (Fig. 7B). Glial remodeling consists of expansion of the glial processes around the AWC neurons, as well as fusion of the two glia to create a continuous glial torus in the animal's nose. Mutations in the Otx/Otd transcription factor gene *ttx-1* prevent glia expansion and fusion, and rescue studies suggest that *ttx-1* normally functions within amphid sheath glia (C. Procko, Y. Lu, and S. Shaham, submitted). Remarkably, remodeling of AWC neurons in animals carrying a *ttx-1* mutation is defective, and AWC receptive endings fail to overlap in the head of the animal as they would normally, suggesting that glia guide AWC remodeling (Fig. 7C). In support of this hypothesis, the glial processes will expand and fuse in animals in which AWC neurons have been ablated (C. Procko, Y. Lu, and S. Shaham, submitted) (Fig. 7D).

Amphid sheath glia fusion during dauer remodeling requires the fusogen AFF-1 as well as the TTX-1 target gene *ver-1*, which is related to VEGF receptors. Intriguingly, *ver-1* expression is induced in amphid sheath glia not only in dauers, but also in non-dauer animals in response to high ambient temperature, a known stimulus for dauer entry (Golden and Riddle, 1984). Temperature-dependent induction of *ver-1* does not require sensory neuron function, suggesting that amphid sheath glia are able to detect temperature directly. The mechanism of temperature sensation in these glial cells is not known.

The ability of amphid sensory organ glia to sense an environmental cue and to promote morphological changes in sensory neurons is strikingly reminiscent of the ability of astrocytes to detect presynaptically-released neurotransmitters and to modulate dendritic spine architecture (Jahromi et al., 1992; Murai et al., 2003; Reist and Smith, 1992), and is consistent with the seemingly conserved aspects of FIG-1 and ACD-1 functions.

Glial control of synaptic function

Most synapses in the *C. elegans* nerve ring are not ensheathed by the CEPsh glia. However, a very small

C. elegans   Vertebrates
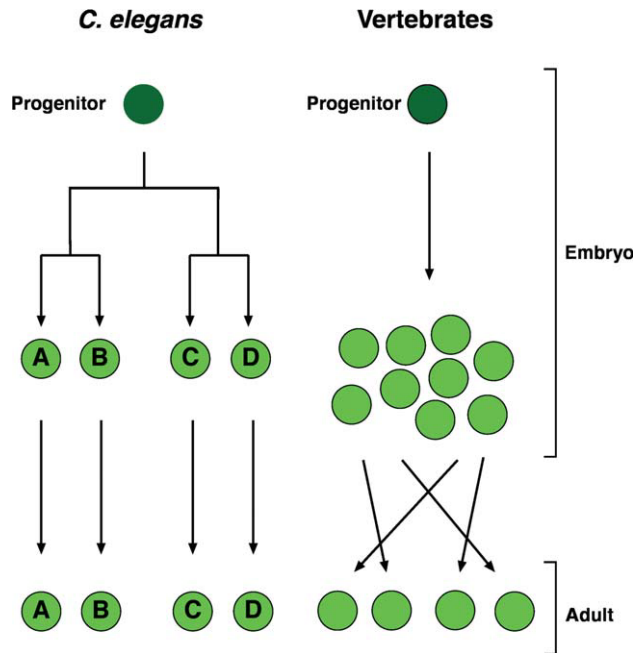
Fig. 8. Neuronal development in vertebrates and *C. elegans*. In vertebrates, neurons are born in excess and then undergo a selection process that is hypothesized to include competition for glia-derived survival factors. In *C. elegans*, neurons, like all cells of the soma, are born in a predetermined fashion, and in predetermined numbers. Their survival does not depend on signals from other cells, including glia. Therefore, in contrast to vertebrates, *C. elegans* glia can be ablated without neuronal demise, and the role of glia in neuronal function can be probed *in vivo*.

COMPARING GLIA IN *C. elegans* WITH GLIA IN OTHER ANIMALS

Until recently, *C. elegans* researchers tended to use terms such as "support" or "glia-like" cells when referring to *C. elegans* glia, a sign of caution over whether these intriguing cells indeed resemble vertebrate glia. The studies summarized in this review have uncovered similarities between glia in *C. elegans* and those of other animals on several levels: morphology, development, anatomy, and function, establishing *C. elegans* as an exciting system in which to study neuron-glia interactions. Nonetheless, differences between glia in *C. elegans* and other organisms are also apparent. These differences point towards *C. elegans* as a reductionist setting for studying glial function, and reveal where the power of the system is best exploited. Two main differences are apparent:

Myelin

Axonal myelination by glia has arisen independently several times during animal evolution, in response to the need for either higher speeds or longer distances of electrical conduction (Hartline and Colman, 2007). *C. elegans* does not exhibit axonal myelination, probably because neuronal process length is short (less than 1 mm) and current loss, therefore, negligible.

Trophic support

In contrast to neurons in other model systems, *C. elegans* neurons can survive without trophic support from glia. This difference likely stems from differences in modes of development between nematodes and other metazoans. In *Drosophila* and vertebrates, neurons are born in excess, and the final neuronal complement of the organism is determined by competition and pruning. Culling of cells in such a setting requires an extracellular signal that communicates to individual cells whether they are in excess, and glia provide components of this signal (Fig. 8). By contrast, the number of *C. elegans* neurons is predetermined by lineage, is identical between all *C. elegans* individuals, and is not governed by cell interactions. Therefore, with no competition for growth signals, it is not surprising that glia have no influence on the survival or demise of neuronal cells (Fig. 8). Indeed, to date, none of the programmed cell death that takes place in the soma of *C. elegans* has been shown to occur in response to an extracellular cue. The lack of trophic support of neurons by glia provides an obvious and powerful technical advantage: the opportunity to dissociate such support functions from regulatory functions, a problem that has plagued analysis of glia-neuron interactions in other model systems. Many of the studies described here have taken advantage of this unique *in vivo* arena to study glial influences on neuronal development and function.

number of synapses are tightly and reproducibly associated with glial processes, suggesting that glia may play important modulatory roles at these sites. Postembryonic ablation studies of CEPsh glia demonstrate that even after establishment of nerve ring architecture, glia are required for maintenance of aspects of normal neuronal morphology (M. Katz and S. Shaham, unpublished). Furthermore, animals in which CEPsh glia are ablated after nerve ring formation exhibit a set of stereotypical locomotory defects. These defects can be strongly suppressed by ablation of the ALA neuron (M. Katz and S. Shaham, unpublished). Strikingly, the synapse between ALA and its postsynaptic neuron AVE is one of the few synapses tightly associated with a CEPsh glial process (White et al., 1986) (Fig. 2D). Further analysis of this synapse promises to be exciting for two reasons. First, it will allow exploration of roles for glia in synaptic function in an organism with facile genetics and excellent infrastructure for gene discovery. Second, it will allow the study of activity at a single synaptic site using animal behavior as a direct readout. Correlation of single synapse physiology and animal behavior remains essentially impossible in vertebrates or *Drosophila*, thus *C. elegans* provides a powerful setting for testing a variety of hypotheses regarding the role of synaptic modulation on animal behavior and learning.

CONCLUDING REMARKS

Glia are one of the great mysteries in neuroscience today. Although the possibility that glia actively participate in the nervous system was acknowledged over 100 years ago (Cajal, 1911), progress in exploring possible roles played by these cells has been slow. A number of reasons are likely to have contributed to this state of affairs. Unlike glia, the output of neuronal function can be readily assayed by electrophysiology, thus attracting the interest of neuroscientists long before the revolution ushered in by molecular biology. Furthermore, the dependence of neurons on glial trophic factors has hindered progress in understanding how glia influence neuronal activity because manipulation of glia often leads to the demise of their associated neurons.

Only recently has it become apparent that the popular invertebrate model systems, *Drosophila* and *C. elegans*, have glia that are developmentally, morphologically, and functionally homologous or analogous to the glia of vertebrate systems. *C. elegans* offers a unique arena in which to explore glia since the trophic dependence of neurons on glia is absent in this animal. The powerful tools of genetics and genomics, combined with the complete anatomical reconstruction of the nervous system (Hall and Russell, 1991; Ward et al., 1975; White et al., 1986) and with the ability to perform studies *in vivo* at single cell and single synapse resolution all suggest that exploration of glia in this organism is likely to provide important insight into the roles and functions of these enigmatic cells.

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