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## Glia–Neuron Interactions in Nervous System Function and Development

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Nervous systems are generally composed of two cell types—neurons and glia. Early studies of neurons revealed that these cells can conduct electrical currents, immediately implying that they have roles in the relay of information throughout the nervous system. Roles for glia have, until recently, remained obscure. The importance of glia in regulating neuronal survival had been long recognized. However, this trophic support function has hampered attempts to address additional, more active functions of these cells in the nervous system. In this chapter, recent efforts to reveal some of these additional functions are described. Evidence supporting a role for glia in synaptic development and activity is presented, as well as experiments suggesting glial guidance of neuronal migration and process outgrowth. Roles for glia in influencing the electrical activity of neurons are also discussed. Finally, an exciting system is described for studying glial cells in the nematode *C. elegans*, in which recent studies suggest that glia are not required for neuronal viability. © 2005, Elsevier Inc.

### I. Introduction

Glia were described as components of the spinal cord nearly 160 years ago by the German pathologist Rudolph Virchow (1846). Virchow and others (e.g., Cajal, 1913) elaborated on these initial studies to show that glial matter

pervade the nervous systems of vertebrates. Although glia were recognized as the predominant cell type in the vertebrate brain, early experimentation aimed at elucidating their functions was unsuccessful. Part of the problem was noted by Santiago Ramón y Cajal in his classic volume *Histology of the Nervous System* (Cajal, 1911). Cajal stated: “What is the function of glial cells in neural centers? The answer is still not known, and the problem is even more serious because it may remain unsolved for many years to come until physiologists find direct methods to attack it. Neuronal function was clarified by the phenomena of conduction ... But how can the physiology of glia be clarified if they cannot be manipulated?” Even today, Cajal’s dilemma reflects the central problem in understanding glial function: what is the readout for glial activities?

There are three broad possibilities concerning the roles of glia in the nervous system: (1) they may have no role, (2) they may have a role that is completely independent of the neurons with which they physically associate, or (3) they might function in concert with neurons to perform nervous system tasks. Although recent studies have begun to hint at intimate functional connections between glia and neurons, it is somewhat surprising that a hundred years after Cajal’s writings, we still lack clear-cut evidence to distinguish among the possibilities described above. Indeed, given our current state of understanding, it is still very possible that glia perform both neuron-dependent and neuron-independent functions in the nervous system. Nonetheless, because of the spatial proximity of glial cells to neurons, as seen most clearly with the myelin-forming glia, it has been a central assumption in the field that glia must function, at least in part, to regulate neuronal parameters. How can this hypothesis be addressed experimentally? One approach would be to examine neurons *in vitro* or *in vivo* in the presence or absence of glia and compare their properties and development. Although a completely reasonable approach, this simple strategy has, in many cases, failed because neurons usually died when cultured without glia or in mutants lacking glia (e.g., Hosoya *et al.*, 1995; Jones *et al.*, 1995; Ullian *et al.*, 2001). Thus, although it is clear that glia provide survival capacity to neurons, this very property often makes it impossible to study roles for glia in regulating neuronal function.

How to proceed, then? A number of strategies to overcome neuronal death upon glial removal have recently been employed. Substances that promote neuronal survival, some of which are of glial origin, have been added to neuronal preparations, allowing neurons to be cultured without the physical presence of glia (e.g., Meyer-Franke *et al.*, 1995; Ullian *et al.*, 2001). These studies have yielded important information; however, significant caveats remain. For example, might survival factors function in other capacities to alter neuronal physiology? Might dissociation of primary nervous tissue to its cellular components affect critical neuronal properties?

Glial function has also been perturbed more subtly so that neuronal death will not result. For example, chemicals that specifically inhibit glial proteins

without affecting neuronal survival have been used to explore glial effects on neuronal parameters (e.g., McBean, 1994; Robitaille, 1998). It should also be possible, in principle, to generate glia harboring mutations that affect neuronal function but not survival.

Although these approaches have proven quite informative, it is yet unclear how relevant these studies are to the functioning of the nervous system *in vivo*. Glial alterations leading to obvious organismal consequences have been described. Demyelinating diseases, such as multiple sclerosis, Dejerine-Sottas syndrome, or Guillain-Barre syndrome, that result from alteration of glial-derived myelin, severely affect organismal motor and sensory behaviors (Franklin, 2002; Newswanger and Warren, 2004; Plante-Bordeneuve and Said, 2002). However, in these diseases, neuronal death often occurs. As in other areas of biological inquiry, theories regarding glial function will ultimately be tested by generating animals harboring specific glial deficits that do not affect neuronal survival and looking for behavioral and/or developmental abnormalities.

A third approach to circumvent the effects of glia on neuronal survival has been to search for a natural setting in which glia are not required for neuronal survival. Recent studies have demonstrated that such a setting exists in the nematode *Caenorhabditis elegans* (T. Bacaj and S. Shaham, unpublished results; Perens and Shaham, 2005). Furthermore, anecdotal reports as well as more comprehensive recent studies (T. Bacaj and S. Shaham, unpublished results) suggest that glial deficits in *C. elegans* have clear behavioral and developmental consequences. These observations, combined with the facility of genetic studies in *C. elegans*, suggest that this organism may provide an exciting new system in which to decipher both the roles of glia in the nervous system and the molecular effectors of these roles.

Insights into the roles of glia–neuron interactions in nervous system function are examined in this chapter. It is not the purpose of this chapter to provide a comprehensive review of all aspects of glia–neuron interactions; rather, the intention is to point out some of the salient and novel functions that have recently been attributed to glia in the control of neuronal function and development. In the following sections, highlights of recent progress are presented, beginning with insights into the roles of glia in neuronal development and regulation of synaptic and conductive activities of neurons. Discussions of more indirect roles for glia as an energy source for neurons and as regulators of neuronal cell survival follow. The chapter concludes with a description of new studies on glial function in *C. elegans*.

## II. Defining Neurons and Glia

Before embarking on a discussion of glia–neuron interactions, it is important to define each cell type. This is no small matter, since valid comparisons of glia–neuron interactions across different species rest on the assumption that

the cell types under study are fundamentally similar. Neurons are, in some sense, easier to define than glia. Although these cells come in myriad shapes and sizes, they share a number of basic properties. Neurons conduct fast currents and connect to other neurons, or to terminal cells (such as muscles or gland cells), by synapses or gap junctions. They also extend processes. The molecular mechanisms controlling these basic properties are generally conserved in neurons of different organisms; however, some widely used functional and molecular markers are probably not appropriate neuronal identifiers. For example, while the action potential and its associated voltage-gated sodium channel are hallmarks of neurons in vertebrates, neither exists in neurons of the nematode *C. elegans* (Bargmann, 1998; Goodman *et al.*, 1998). However, *C. elegans* clearly possesses cells that elaborate processes, connect by synapses and gap junctions, and conduct fast currents (Goodman *et al.*, 1998; Lockery and Goodman, 1998; White *et al.*, 1986). Cell shape criteria can also lead to confusion. For example, the neuroepithelial cells housed in vertebrate taste buds are not usually classified as neurons, primarily for morphological reasons. However, these cells possess sensory receptors (for detection of taste substances) and synapse onto neurons (Barlow, 2003), suggesting that they must share basic neuronal properties.

Vertebrate glia are generally classified according to morphological and molecular criteria. In vertebrates, glia of the peripheral nervous system (PNS) are termed Schwann cells. These extend processes that ensheath or myelinate axons but can also ensheath synapses between neurons. Glia of the central nervous system (CNS) generally fall into three categories: oligodendrocytes, which myelinate CNS axons; astrocytes, which extend many processes that contact both blood vessels and neurons; and microglia, cells thought to be of mesodermal origin that are hypothesized to function in an immune capacity in the CNS (Peters *et al.*, 1991). Microglia may, thus, not be truly glial cells. A popular marker for vertebrate glia is the glial fibrillary acidic protein (GFAP), an intermediate filament protein found in some but not all glia (Eng *et al.*, 1970, 1971).

The morphological and molecular markers described above do not account for all vertebrate cells that have been termed glia, however. For example, olfactory ensheathing cells are neither oligodendrocytes nor astrocytes by morphology, yet they express GFAP and are intimately associated with olfactory neurons. Similar observations hold for Müller glia in the retina, Bergmann glia in the cerebellum, and support cells of the inner hair cells. Furthermore, GFAP expression fails to mark some cells considered glial in nature, and GFAP is not expressed in astrocytes and radial glia of some vertebrates (Dahl *et al.*, 1985).

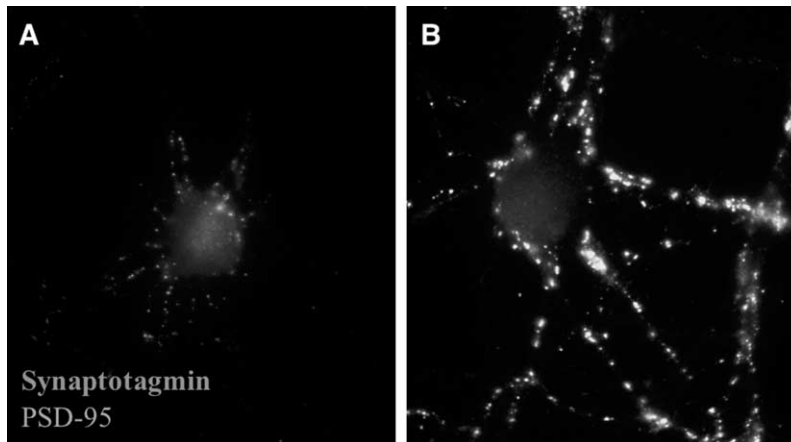
All glia, however, meet three criteria, which do not also apply to cells of non-glial nature. First, glia are always physically associated with neurons. Second, glia are not neurons themselves; they generally do not transmit

fast currents or form presynaptic structures (although neuronal synapses onto glia have been documented). Third, glia and neurons are lineally related. Recent studies on the nature of stem cells in the vertebrate brain have revealed that glia and neurons often arise from common ectodermally derived precursor cells such as radial glial cells (Alvarez-Buylla *et al.*, 2002; Doetsch, 2003). In the PNS, many glia and neurons are derived from the neural crest—a developmentally discrete ectodermal cell population originating near the neural tube in early vertebrate development (Le Douarin and Dupin, 2003; Le Douarin *et al.*, 1991). In the fruit fly *Drosophila melanogaster*, glia and neurons also arise from common precursor cells (Jones, 2001). Thus, kinship between glia and neurons is an important aspect of glial identity. In the following sections of this chapter, all references to glia and neurons, regardless of organismal origin, conform to the definitions elaborated in this section.

### III. Glial Roles in Synaptogenesis

Ultrastructural studies of the vertebrate CNS have shown that glial processes, usually those associated with astrocytes, can be found adjacent to, or ensheathing, synaptic connections between neurons (Peters *et al.*, 1991; Spacek, 1985; Ventura and Harris, 1999; Wolff, 1976). In the periphery, synaptic Schwann cells envelop most neuromuscular junctions (Herrera *et al.*, 2000; Hirata *et al.*, 1997; Kelly and Zacks, 1969). These observations have led to the hypothesis that glia may play important roles in synaptogenesis and synaptic function.

A number of recent observations have provided evidence that glia in the CNS can promote synaptogenesis. Purified cultured postnatal rat retinal ganglion cells (RGCs) that have been separated from their glial components can be kept alive in culture using a number of survival factors, including brain-derived neurotrophic factor (BDNF) and ciliary neurotrophic factor (CNTF) (Meyer-Franke *et al.*, 1995). These cultured RGCs normally form functional synapses only inefficiently, as assessed by electrophysiological criteria and by localization of pre- and postsynaptic proteins. However, when these cells are co-cultured with glia from the RGC target region, synaptic efficacy is dramatically enhanced (Fig. 1) (Pfrieger and Barres, 1997). Specifically, the frequency of spontaneous postsynaptic currents in such cultures is increased 70-fold, and current amplitudes are increased 5-fold. In addition, a larger number of synapses can be visualized in such cultures (Nagler *et al.*, 2001; Ullian *et al.*, 2001). Incubation of RGCs with glia-conditioned medium reproduced the effects seen in the co-culture experiments, suggesting that a soluble factor or factors were required for the increase in synapse number and efficacy. Biochemical purification



**Figure 1** Glia promote synaptogenesis. (A) Retinal ganglion cells (RGCs) cultured in the absence of glia, stained using anti-synaptotagmin (a pre-synaptic marker; red) and anti-PSD-95 (a postsynaptic marker; green). Note few yellow puncta. (B) RGCs cultured in the presence of glia. Note large increase in yellow puncta. (Image courtesy of Erik Ullian and Ben Barres.) (See Color Insert.)

approaches suggested that one relevant component of the glia-conditioned medium was cholesterol bound to the apoE lipid-carrying protein (Mauch *et al.*, 2001). It is unclear whether cholesterol exerts specific roles in this setting, or whether it plays a more general role. For example, cholesterol could be a limiting component of synaptic vesicle membranes. Interestingly, the epsilon4 allele of apoE has been implicated in susceptibility to Alzheimer's disease, in which reduction in synaptic efficacy is observed (Myers and Goate, 2001). Thus, glia may underlie some aspects of this disease.

A second glia-derived component that is sufficient to induce the formation of postsynaptically silent RGC synapses of normal morphology has also been recently identified (Ullian *et al.*, 2004a). The secreted protein, thrombospondin, a large, extracellular matrix component best known for its roles in clotting, may serve to stabilize physical interactions between neurons at the synapse. The fact that thrombospondin-induced synapses are postsynaptically silent suggests that yet another glial component must allow for activation of synapses stabilized by this protein.

A number of *in vitro* studies also suggest that Schwann cells can promote synaptogenesis. For example, Ullian *et al.* (2004b) showed that Schwann cells induce the formation of glutamatergic synapses between cultured spinal motor neurons. Furthermore, Schwann cell-conditioned medium induced synapse formation between cultured *Xenopus laevis* motor neurons and muscle cells (Peng *et al.*, 2003). Selective ablation of perisynaptic Schwann cells *in vivo* using antibody-driven complement-induced lysis revealed that

growth and addition of synapses significantly decreased, and existing synapses often retracted (Reddy *et al.*, 2003). Thus, Schwann cells seem to be important for maintaining synapses *in vivo*, although whether the effects observed in this study were secondary to a general deterioration of neuronal health is not clear.

#### IV. Glial Modulation of Synaptic Activity

The modulation of synaptic activity is hypothesized to be an essential component of nervous system versatility. The reigning hypothesis suggests that alterations in synaptic efficacy, as manifested by the ability of a post-synaptic cell to respond to a presynaptic cell, are essential for complex phenomena such as learning and memory. A number of recent observations suggest that glia may play an important role in regulating synaptic efficacy. Such a function at the *Xenopus* neuromuscular junction has been described. In *Xenopus*, as in other vertebrates, the synapse between a motor neuron and a muscle fiber is associated with a perisynaptic Schwann cell (Couteaux and Pecot-Dechavassine, 1974). High-frequency stimulation of the motor neuron leads to a decrease in muscle fiber activity, as measured by decreases in the end-plate potential following neurotransmitter release (Colomar and Robitaille, 2004; Robitaille, 1998). This phenomenon is often termed long-term depression (LTD), and similar phenomena in CNS neurons have been suggested to play essential roles in memory acquisition (Zucker and Regehr, 2002). Release of  $\text{Ca}^{2+}$  from intracellular stores within the perisynaptic Schwann cell is also observed during high-frequency presynaptic stimulation (Jahromi *et al.*, 1992; Reist and Smith, 1992; Rochon *et al.*, 2001). This observation shows that the perisynaptic Schwann cell can somehow monitor synaptic activity and suggests that  $\text{Ca}^{2+}$  release and LTD may be related. Robitaille (Bourque and Robitaille, 1998; Robitaille, 1998) hypothesized that Schwann cells at the neuromuscular junction detect presynaptic activity using G-protein-coupled receptors (GPCR), such as muscarinic acetylcholine receptors, to sense neurotransmitter release. To assess whether the activity of such G-proteins influenced LTD, he injected Schwann cells with GTP- $\gamma$ S, a G-protein activator, to mimic GPCR activation. Following presynaptic stimulation, an excessive decrease in synaptic activity was observed, consistent with a reduction in neurotransmitter release by the presynaptic cell or increased turnover of released transmitter. Thus, a mimic of GPCR activation was sufficient to cause LTD-like synaptic changes. Furthermore, injection of GTP- $\beta$ S, a G-protein antagonist, into the Schwann cell resulted in increased synaptic activity, consistent with a predicted decrease in LTD. Taken together, these results suggest that specific manipulation of synaptic glia can affect synaptic activity.

The mechanism by which perisynaptic Schwann cells might regulate neurotransmitter dynamics in the *Xenopus* neuromuscular junction is not clear. However, this instructive activity could very well be modulated at the level of synaptic neurotransmitter levels. There are now numerous examples of how glia might modulate the concentration of neurotransmitter at the synaptic cleft. Glia as well as neurons express a variety of neurotransmitter transporters (Bergles and Jahr, 1997; Huang and Bergles, 2004; Rothstein *et al.*, 1994). Perhaps the most-studied glial transporters have been those involved in clearance of glutamate and glycine. However, glial transporters for gamma amino butyric acid (GABA) have been described as well (Minelli *et al.*, 1995, 1996). Glutamate is the major excitatory neurotransmitter in the vertebrate CNS, and its levels at the synaptic cleft are tightly controlled. Studies of glutamate signaling in the magnocellular nuclei of the rat hypothalamus have provided evidence that glial clearance of glutamate is important in synaptic transmission. The magnocellular nuclei undergo a stereotypical retraction of astrocyte processes from synaptic areas in lactating females (Hatton, 2002; Theodosis and Poulain, 1993). Oliet *et al.* described a feedback mechanism for non-lactating animals whereby pharmacological inhibition of glutamate transporters, causing an increase in synaptic glutamate, resulted in decreased transmitter release from presynaptic neurons (Oliet *et al.*, 2001). The same experiment performed in lactating rats yielded little change in transmitter release, suggesting that the glutamate transporters on astrocytes are responsible for clearance and maintenance of presynaptic neurotransmitter release (Oliet *et al.*, 2001). Although other interpretations of this result are possible, these studies were an important attempt to study glial function in a natural, *in vivo* setting.

Evidence that astrocyte glutamate transporters are important for clearance of synaptic glutamate has also come from antisense studies in the rat. Both *in vitro* and *in vivo* administration of antisense oligonucleotides against the GLAST or GLT-1 glial glutamate transporters resulted in elevated glutamate levels. In living rats, such a blockade resulted in neurodegenerative features characteristic of glutamate-induced neurotoxicity and progressive paralysis (Rothstein *et al.*, 1996). Similar results were observed in mice harboring targeted lesions in transporter genes (Tanaka *et al.*, 1997; Watase *et al.*, 1998).

Glial clearance of glycine, a major CNS inhibitory neurotransmitter, from synapses is also important for regulating synaptic activity. Two glycine transporters, GlyT1 and GlyT2, have been identified in mammals (Guastella *et al.*, 1992; Liu *et al.*, 1992, 1993; Smith *et al.*, 1992). Expression studies suggest that the GlyT1 transporter is widely expressed on glia of the CNS, whereas GlyT2 expression is restricted to CNS neurons (Adams *et al.*, 1995; Zafra *et al.*, 1995a,b). Strikingly, targeted disruption of the GlyT1 transporter leads to severe motor and respiratory deficits in newborn homozygous mice (Gomez



*et al.*, 2003), indicating that glycine uptake by glia may be essential for neuronal function. Taken together, the results discussed here suggest that glial uptake of neurotransmitters is essential for proper synaptic activity, raising the possibility that regulated uptake could modulate synaptic function.

In addition to clearance of neurotransmitter using transporters, glia can also release neurotransmitter inhibitors. For example, in the fresh water snail *Lymnaea stagnalis*, a soluble glia-derived protein similar to the acetylcholine receptor (AChR) can bind synaptic acetylcholine. Although mutant animals lacking this AChR mimic have not been described, *in vitro* studies strongly suggest that this protein can modulate synaptic responses (Smit *et al.*, 2001).

Glia may also influence synaptic activity by secretion of neurotransmitters into the synaptic cleft. There is now ample evidence that glutamate is released from astrocytes in CNS slices and *in vitro* (Araque *et al.*, 1998, 2001; Bezzi *et al.*, 1998; Kang *et al.*, 1998; Liu *et al.*, 2004; Parpura *et al.*, 1994). When this release is studied, it is often coupled to the release of  $\text{Ca}^{2+}$  from intracellular stores within astrocytes (Araque *et al.*, 2001). Recent studies have also begun to elucidate the mechanism by which glutamate is exported out of astrocytes. It seems that a vesicular compartment is involved in release, and that a vesicular glutamate transporter (VGLUT), previously thought to be expressed and functional only in neurons, participates in glutamate release (Bezzi *et al.*, 2004; Montana *et al.*, 2004).

Glia have been observed to synthesize and release other synaptic mediators such as acetylcholine (Heumann *et al.*, 1981; Lan *et al.*, 1996), GABA (Minchin and Iversen, 1974), and ATP (Newman, 2003; Zhang *et al.*, 2003); however, much less is known about the relevance of this release, and whether it also occurs *in vivo*. Additional studies *in vivo*, examining animals deficient in astrocyte-specific neurotransmitter release, should help in assessing the significance of this glial activity.

## V. Glial Effects on Neuronal Conduction

In addition to participating in important regulatory events at the synapse, glia also affect the electrical properties of neurons. Perhaps the best-studied example of such regulation is the role of myelin in insulating axons. Many vertebrate CNS and PNS axons are ensheathed by a specialized glial myelin sheath. Ensheathment is punctuated by gaps, called the nodes of Ranvier. In these gaps, the action potential traveling down the neuron is regenerated. The organization of the nodes, as well as specialized paranodal structures, is mediated by specific neuronal–glial interactions. For example, the neuronal proteins contactin and contactin-associated protein interact with the glial membrane protein neurofascin 155 to form the paranodal regions (Charles

*et al.*, 2002). Axons that have been demyelinated propagate currents inefficiently, which has been attributed to leakage of current as it proceeds down the axon shaft; thus, glia may play important roles as electrical insulators. However, inefficient conduction could also be a consequence of the disorganized localization of the voltage-gated sodium channels and other relevant channels that mediate action potential generation (Arroyo *et al.*, 2002; Ulzheimer *et al.*, 2004). Careful measurements of currents along such demyelinated axons could test the validity of this hypothesis.

The extent of myelin ensheathment directly correlates with axonal diameter and activity. Signaling between the neuronal factor neuregulin-1 (NRG1), and the ErbB receptor family is important for conveying information regarding axon thickness to the surrounding myelin (Michailov *et al.*, 2004). Thus, myelinating glia are able to measure axon dimensions and calculate myelin thickness. Myelin thickness, in turn, is a relevant parameter in assessing axonal conduction efficiencies, further supporting the hypothesis that developmental signals between glia and neurons regulate the conductive properties of neurons.

Glia also affect neuronal excitability by regulating the levels of potassium ions that bathe neurons. For example, in Müller glia in the retina,  $K^+$  released by neurons is taken up from the extracellular environment by a host of glial-specific and non-glial-specific  $K^+$  channels. In the eye there is a correlation between the loss of inwardly rectifying  $K^+$  currents in Müller glia and glaucoma (Francke *et al.*, 1997), suggesting that glial regulation of  $K^+$  levels may be a component of the mechanism leading to neuronal loss and dysfunction in this disease.

In addition to indirect regulation of axonal currents by glia, functional coupling between neurons and glia has also been documented. In mammalian embryonic brain cultures, stimulation of calcium waves in astrocytes can induce current propagation in neurons, suggesting electrical coupling between glia and neurons (Nedergaard, 1994). More direct evidence for such coupling was provided by examination of the rat locus ceruleus (LC) nucleus. Neurons in this brain region have previously been shown to fire synchronously as a result of interneuronal gap junctions. Interestingly, recordings from glia adjacent to synchronously firing LC neurons demonstrated oscillating glial membrane potentials that were temporally correlated with neuronal firing events (Alvarez-Maubecin *et al.*, 2000). Dye injected into LC glia could be found in LC neurons after sufficiently timed incubations. Furthermore, immunoelectron microscopy using antibodies against connexins, the principle components of gap junctions, revealed glial and neuronal connexin immunoreactivity at sites of glia–neuron membrane apposition. Functional studies suggest that glia of the LC reduce neuronal excitability (Alvarez-Maubecin *et al.*, 2000). Thus, it seems that at least in some instances, glia can modulate conductive properties of neurons by direct electrical coupling.

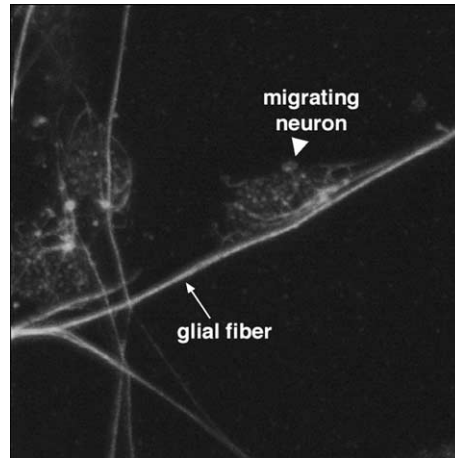
Synchronous firing of neurons can also be achieved by secretion of glutamate from astrocytes. Studies of hippocampal CA1 neurons have shown that glutamate secreted onto these neurons and binding to extrasynaptic NMDA receptors may allow synchronous CA1 firing (Fellin *et al.*, 2004).

The experiments described here suggest that firing properties of neurons can be regulated by glia by both direct electrical and chemical coupling, or by control of myelin development. As with other studies presented here, the consequences of these glial activities in intact animals have not yet been analyzed; however, the identification of specific pathways and molecules involved in these processes should aid in designing the relevant *in vivo* experiments.

## **VI. Glial Regulation of Neuronal Migration and Process Outgrowth**

It has long been hypothesized that glia play important roles in directing neurons and their processes to appropriate locations and targets within the nervous system (Cajal, 1911; Chotard and Salecker, 2004). Pioneering work by Rakic (Rakic, 1971) based principally on static observations of granule cell migration in the developing cerebellum led to the hypothesis that granule neuron migration was guided by glia (Fig. 2). Similar observations suggested that within the cerebral cortex, radial glial cells, which extend processes from the subventricular zone to the pial surface, serve as tracks along which newly generated neurons migrate to reach their destinations (Rakic, 1988). It is now known that glia-guided migration is not the only mechanism of neuronal migration in developing nervous systems (reviewed in Hatten, 2002); nonetheless, it is a major aspect of nervous system development. Studies in which murine cerebellar glia and granule neurons were purified and plated together clearly showed both tight association of neurons with glial fibers and neuronal movement along these fibers (Edmondson and Hatten, 1987). Furthermore, a recent study in which mouse embryos were infected with a retrovirus encoding green fluorescent protein demonstrated not only that radial glia in the cortex give rise to neurons, and thus function as stem cells, but also that neurons generated by these glia proceed to migrate along radial glia fibers *in vivo* (Noctor *et al.*, 2001).

How glia and neurons establish affinity and how migration is executed are important questions for which answers are now emerging. To define the neuronal proteins involved in glial fiber recognition, postnatal cerebellar cells were used to raise antibodies recognizing cell surface moieties. One such immune activity blocked the formation of stable neuron–glia interactions in cultured cells, suggesting that it might recognize a neuronal epitope



**Figure 2** Cerebellar granule cell migrating on a Bergmann glia fiber. Green,  $\beta$ -tubulin in a neuron, marking the glial fiber track; red, dynein intermediate chain in the nucleus and centrosome of the migrating neuron. (Image courtesy of David Solecki and Mary Beth Hatten.) (See Color Insert.)

essential for adhesion to glial fibers (Edmondson *et al.*, 1988; Fishell and Hatten, 1991). Further studies led to the cloning of a neuronal protein containing multiple extracellular protein-binding domains, termed astrotactin (Zheng *et al.*, 1996). The glial ligand for astrotactin has not yet been determined; however, the brain lipid-binding protein (BLBP) gene may be involved in supporting neuronal migration, since its expression in radial glia throughout the CNS is correlated with neuronal differentiation and migration along glial fibers (Feng and Heintz, 1995; Feng *et al.*, 1994). Additional neuronal components involved in adhesion and cytoskeleton organization have also been extensively defined (Hatten, 2002).

Some studies have suggested that neuronal processes can serve to guide migrating glial cells. For example, time-lapse imaging of migrating glia in zebrafish embryos revealed that these cells are guided by axons of the lateral line neurons. Ablation or misrouting of axons in these embryos prevented glial migration or caused abnormal misrouted migration, strongly indicating that glia follow neuronal tracks (Gilmour *et al.*, 2002). Similar effects on glial migration have also been documented in *Drosophila* embryos in mutants defective in sensory axon extension (Giangrande, 1994).

Glia also seem to be important for directing proper axon outgrowth and pathfinding. For example, the netrin guidance molecules are expressed in glia of *C. elegans* (Wadsworth *et al.*, 1996), *Drosophila* (Jacobs, 2000), and vertebrates (Serafini *et al.*, 1996), suggesting important roles in directing axon pathfinding. Expression of the chemorepellent Slit in midline glia of

the *Drosophila* CNS is critical for preventing midline recrossing by axons (Kidd *et al.*, 1999). Guidance proteins produced by glia-like cells of the vertebrate floor plate also act to control midline crossing (Serafini *et al.*, 1996). In *Drosophila*, a subset of neurons in animals lacking the glial cells missing (*gcm*) gene fail to extend their normal processes (see also Hidalgo *et al.*, 1995), suggesting a possible role for their associated glia (whose processes are closely aligned with the neuronal processes) in process extension (Hosoya *et al.*, 1995; Jones *et al.*, 1995).

In *Drosophila* and vertebrates, once axons have reached the vicinity of their targets, extensive pruning takes place, whereby excessive processes are degraded. Interestingly, in the fruit fly, glia play an active role in this process. Glia infiltrate regions in which pruning will take place and engulf fragmented axonal processes. Temporary inactivation of these glia using a glial-targeted temperature-sensitive mutation in the *Drosophila* homolog of the vesicle pinching protein dynamin transiently prevented pruning, demonstrating an active role for glia in this process (Awasaki and Ito, 2004; Watts *et al.*, 2004).

Although a thorough molecular description of the roles of glia in neuronal and axonal migration is still lacking, recent studies have demonstrated the importance of glia in these processes. The identification of some proteins regulating neuronal movement should serve as an inroad to a more complete description of these glia–neuron interactions.

## VII. Reciprocal Control of Cell Survival between Neurons and Glia

As described in the beginning of this chapter, glia are often required, both *in vitro* and *in vivo*, for the survival of the neurons with which they interact. Indeed, primary cultures of neurons are invariably mixed with glia, which presumably provide both trophic and nutritive support. Removal of glia without the addition of specific survival factors results in neuronal death (Meyer-Franke *et al.*, 1995). *In vivo*, loss of the glial sheaths associated with neurons can severely affect neuronal survival. For example, overexpression of a dominant negative form of the ErbB receptor in non-myelinating Schwann cells (cells that ensheath neurons but do not form a myelin sheath) of adult transgenic mice resulted in the death of these Schwann cells, followed by a loss of unmyelinated axons and the subsequent death of sensory neurons (Chen *et al.*, 2003).

*In vitro*, the ratio of glial cells to neurons required for neuronal survival is generally not stoichiometric. Thus, cultures with 5% glia are sufficient for robust neuronal survival (e.g., Fishell and Hatten, 1991). This observation supports previous assertions that glia-regulated neuronal survival is mediated, at least in part, by soluble factors.

Glial support of neuronal survival has been mainly explored in two areas: nutritive and trophic factor support. In vertebrates, glucose plays a key role as an energy source for cellular metabolism. Both glia and neurons possess the relevant glycolytic enzymes required to break down this sugar and use it for the production of ATP. Thus, it has long been assumed that in vertebrates, neuronal energy is supplied by sugar carried in the blood. Recent studies, however, have challenged this notion, suggesting instead that the primary energy source for active neurons is lactic acid, generated by glia. Interestingly, individual astrocytes in the CNS often contact both blood vessels and neurons and are thus perfectly situated to be energy mediators. The lactate shuttle hypothesis suggests that uptake of glutamate by astrocytes, following a bout of neuronal activity, stimulates astrocytic glycolysis and lactic acid production. Lactic acid, in turn, leaves the astrocyte and is taken up by the adjacent neuron, to be used as an energy source (Pellerin and Magistretti, 1994; Pellerin *et al.*, 1998). Although this hypothesis is attractive, since it allows neurons to tightly couple activity to energy utilization, it remains controversial. Objectors do not refute the idea that lactate could be used as a source of energy; however, they question whether it is the main energy source. Thus, it is possible that glucose is normally taken up directly by neurons, without astrocyte mediation (Chih *et al.*, 2001).

Trophic support of neurons was originally described by a series of now-classic papers by Rita Levi-Montalcini and Viktor Hamburger (Hamburger and Levi-Montalcini, 1949; Levi-Montalcini and Levi, 1943). Their pioneering work led to the discovery of nerve growth factor (NGF) and eventually to a host of other related neurotrophins. In culture, the requirement of glia for neuronal survival can be bypassed by incorporation of neurotrophins, including BDNF, in the culture medium. Furthermore, glia have been shown to express neurotrophin genes in culture (Condorelli *et al.*, 1995; Furukawa *et al.*, 1986; Gonzalez *et al.*, 1990; Yamakuni *et al.*, 1987), suggesting a role in survival of neurons. In *Drosophila*, neurons in animals carrying mutations in the *gcm* gene eventually die; however, whether death is a direct result of glial loss or a secondary consequence is unclear (Hosoya *et al.*, 1995; Jones *et al.*, 1995). Direct roles for glial-derived survival factors in neuronal survival *in vivo* have not been convincingly demonstrated in any organism.

Roles for neurons in promoting the survival of glia have been surprisingly well established both *in vivo* and *in vitro*. Many glial cell types express neurotrophin receptors (reviewed in Althaus and Richter-Landsberg, 2000), and signaling pathways within these cells in response to trophic factor stimulation have been elaborated (Althaus and Richter-Landsberg, 2000; Heumann, 1994). Perhaps the clearest example of trophic support provided by neurons to glia comes from studies of midline glia in *Drosophila* (Bergmann *et al.*, 2002; Hidalgo *et al.*, 2001). In the *Drosophila* embryo, the midline glia are important for separating and ensheathing commissural

axons. Initially, about ten glia are generated in each segment. Eventually, most of these die by apoptosis, leaving approximately three glia per segment. Apoptosis of midline glia is initially prevented by the action of the neuronally generated transforming growth factor (TGF)- $\alpha$ -like ligand SPITZ. SPITZ activates the conserved epidermal growth factor (EGF) signaling pathway within midline glia, resulting in inhibition of the proapoptotic protein HID by phosphorylation. Midline glia apparently compete for a limited amount of SPITZ ligand, so that those receiving little signal eventually die in a head irritation defective (HID)-dependent fashion.

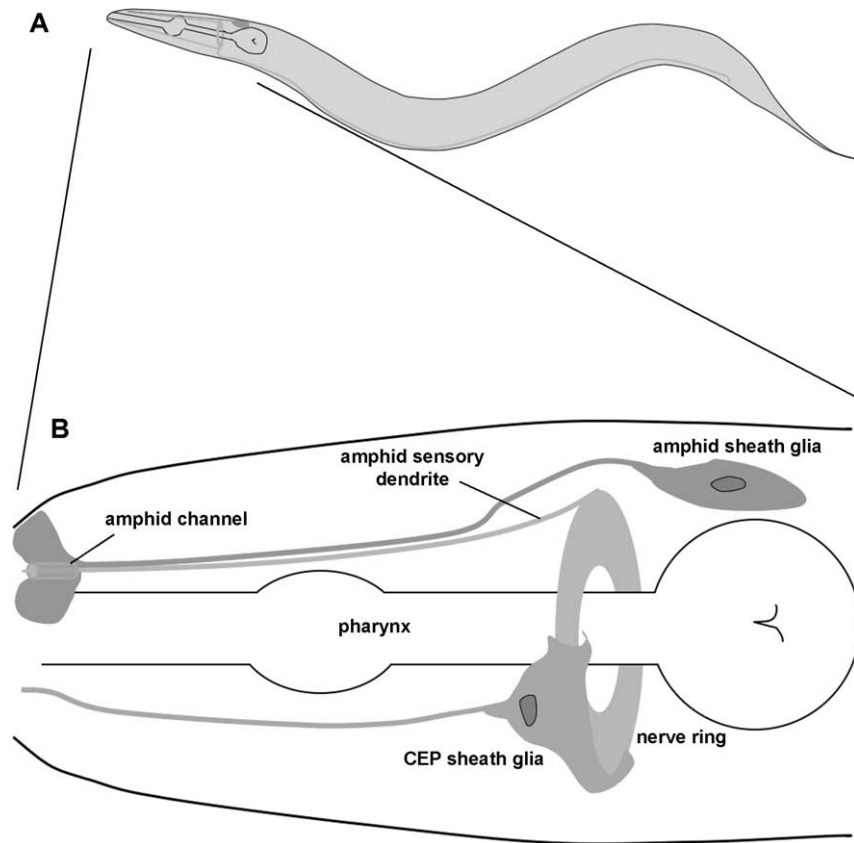
## VIII. Genetic and Functional Studies of Glia in the Nematode *Caenorhabditis elegans*

Few functional studies of glia have been conducted in invertebrate animals. Recent studies, however, suggest that the nematode *C. elegans* may serve as an excellent organism from which both functional and molecular insights regarding glial roles in the nervous system may be gained.

Glia in *C. elegans* comprise a group of cells that conform to the criteria outlined in Section II of this chapter. *C. elegans* glia are closely associated with neurons and their processes (Perens and Shaham, submitted; Ward *et al.*, 1975), are not neurons themselves (as assessed by the absence of synaptic or gap junction connectivity to neighboring cells; Ward *et al.*, 1975; White *et al.*, 1986), and are lineally related to neurons. Although cells in the developing *C. elegans* embryo do not form germ layers, the lineage that gives rise to the 959 somatic cells in the adult hermaphrodite is essentially invariant. An examination of the lineal relatives of *C. elegans* glia reveals that sister cells of these are neurons, other glia, or epithelial cells (Sulston *et al.*, 1983), all cells of ectodermal origin in vertebrates. Furthermore, all glia in *C. elegans* extend processes that abut and also ensheath neurons with which they associate. Thus, these cells are highly reminiscent of vertebrate glia.

### A. Anatomy

In the adult *C. elegans* hermaphrodite there are 56 glial cells that associate with specific subsets of the 302 neurons of the animal. The 56 glia can be divided into three classes: sheath glia, socket cells, and GLR cells. Sheath glia extend processes that associate with dendritic projections of sensory neurons. These cells ensheath sensory dendrites at the dendritic tip, where a specialized sensory apparatus is localized (Figs. 3 and 4) (Perkins *et al.*, 1986; Ward *et al.*, 1975). The roles of sheath glia in sensory function are discussed in detail below. Of the 24 sheath glia, four cells, the CEP neuron sheath cells, are bipolar, extending both dendrite-associated processes and processes that

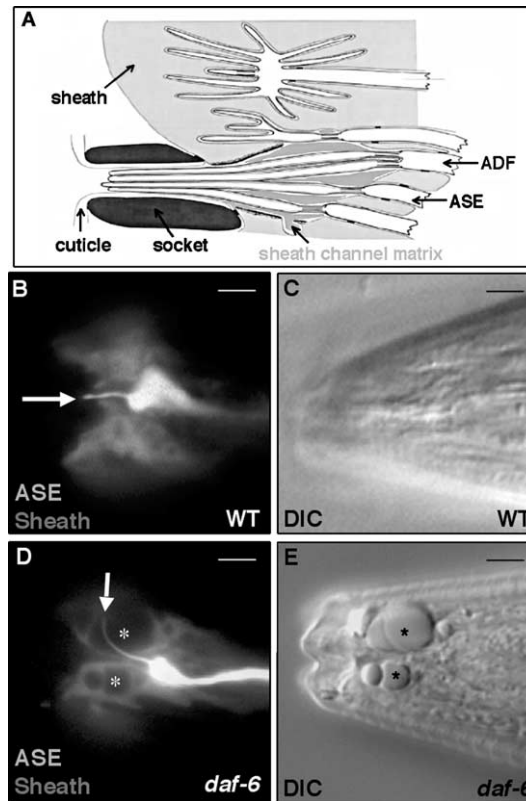


**Figure 3** Glia in *C. elegans*. (A) Schematic of a *C. elegans* adult hermaphrodite. Anterior, left; dorsal, up. Major neural tracts (green) and an amphid sheath glia (red) are depicted. The outline of the pharynx of the animal is also shown. (B) Enlarged view of the anterior region. The nerve ring and an amphid channel neuron dendrite (green), the amphid sheath glia and channel (red), and the CEP sheath glia (pink) are depicted. (See Color Insert.)

envelop the nerve ring (Fig. 4), a discrete neuropil composed of many neuronal processes, that is generally viewed as the animal's brain. Most synaptic interactions between neurons occur in the nerve ring (Ware *et al.*, 1975). In addition to enveloping the nerve ring, the CEP sheath glia also send fine processes into the neuropil, where they can be found closely apposed to a small number of synapses (White *et al.*, 1986). The ventral CEP sheath cells express the *C. elegans* netrin UNC-6, suggesting that they may have roles in axon guidance within the nerve ring (Wadsworth *et al.*, 1996).

Twenty-six glia, termed socket cells, run along the terminal portion of sheath cell processes and surround the dendritic tips of a subset of sensory neurons,





**Figure 4** The glial channel of the *C. elegans* amphid sensory organ is closed in *daf-6* mutants. (A) Schematic showing the dendritic tip region of the amphid (adapted from Ward *et al.*, 1975). A representative neuron embedded within the sheath glia is shown. Two channel neurons, ADF and ASE, are labeled, as are the sheath and socket glia. The sheath secretes a matrix into the channel (green). The socket glia secrete cuticle, which is contiguous with the cuticle on the animal's exterior. This image is an enlarged view of the anterior of Figure 3B. (B) Fluorescence image of a wild-type amphid. Sheath (red) and ASE channel neuron (green) are shown. Arrow points to ASE cilium in the amphid channel. (C) differential interference contrast (DIC) image of animal in (B). (D) Fluorescence image of a *daf-6* mutant amphid. Note absence of exposed channel. (E) DIC image of animal in (D). Asterisks indicate vacuoles accumulating within the sheath glia. (See Color Insert.)

anterior to the sheath cell. In some cases socket cells make a pore through which sensory dendrites can access the animal's environment (Fig. 4).

Six glia, termed GLR cells, extend sheet-like projections that contact muscle arms in the head. In some cases GLR extensions have been seen at neuromuscular junctions in the head of the animal (White *et al.*, 1986).

An attractive model for studying the functions of sheath and socket glia is the amphid, the largest sensory organ in *C. elegans*. The amphid

is composed of 12 neurons displaying sensory cilia at their dendritic tips, a single sheath glial cell, and a single socket glial cell (Ward *et al.*, 1975). The sheath glia envelopes all 12 neurons at the dendritic tip. Eight of these neurons (termed ADF, ASG, ASE, ASK, ASJ, ASI, ADL, and ASH) extend through a channel, made by the sheath glia, into the contiguous socket glia channel and are exposed to the outside environment through the socket glia pore. Four amphid neurons (AWA, AWB, AWC, and AFD) are fully embedded within the sheath glia at their dendritic tip. Numerous studies of amphid neurons have revealed roles for these cells in chemotaxis, odor sensation, thermotaxis, mechanosensation, avoidance of high osmolarity, and dauer pheromone sensation (Bargmann and Mori, 1997; Driscoll and Kaplan, 1997; Riddle and Albert, 1997). Thus, numerous molecular markers are available for these cells, and alterations in their functions can be easily scored by sensory behavior abnormalities or structural defects in the neurons.

## B. Functional Studies

*C. elegans* has a small number of cells, each of which generally performs unique functions. Thus, eliminating single cells in this organism can be compared to the removal of entire tissues, or even organs, in vertebrates. Laser ablation of *C. elegans* cells has been an effective tool for studying cell function. Using this method, individual cells within live animals can be ablated at various times in embryos and larvae, and developmental and/or behavioral consequences can be assessed in operated animals. To examine the role of the sheath glia in amphid sensory functions, the bilateral sheath glia have been ablated. Anecdotal reports suggested that amphid sheath glia ablation could result in behavioral and developmental deficits (cited in Bargmann *et al.*, 1990; Vowels and Thomas, 1994). Indeed, recent comprehensive studies have conclusively shown that ablation of amphid sheath glia in animals in which the sensory organ has already formed impaired sensory functions of the organ (T. Bacaj and S. Shaham, unpublished results). Thus, amphid sheath glia are essential for proper neuronal sensory functions in this organism.

Examination of amphid neurons following sheath glia ablations revealed that the neurons did not die, but displayed stereotypic morphological abnormalities at the dendritic tip (T. Bacaj and S. Shaham, unpublished results). These observations are interesting in two respects. First, *C. elegans* amphid neurons can live normally in an intact organism in the absence of their associated glia. Thus, issues of neuronal viability, which have made the study of glia in vertebrates difficult (see Section I), are obviated. Second, the results suggest that glia are intimately involved in the maintenance and

generation of dendritic ending structure. In vertebrates, dendritic spines are often associated with glia, and it is thus possible that the shape of these receptive ends is also controlled by glia. Indeed, a recent study examining spine morphology in the hippocampus demonstrated that mice lacking the ephrin receptor ephA4 have longer spine lengths than wild-type animals. Interestingly, hippocampal neurons express ephrin A3, and their associated astrocytes express ephA4. Furthermore, soluble ephrin A3 can result in spine length reduction (Murai *et al.*, 2003). Thus, as for *C. elegans* sensory endings, spine morphology may be regulated by glia in the hippocampus.

In addition to roles in morphogenesis of the amphid sensory organ, *C. elegans* glia seem to be important for proper nervous system assembly. Preliminary laser ablation studies of the CEP sheath cells suggest that these cells may be important for assembly and morphogenesis of the *C. elegans* nerve ring (S. Yoshimura and S. Shaham, unpublished observations). Similar studies in *Drosophila* suggest glial roles in axon guidance and fasciculation (Hidalgo *et al.*, 1995).

Studies in *C. elegans* have revealed interesting communication between sheath glia and their associated neurons. The extracellular space surrounding the ciliated endings of amphid neuron dendrites is composed of an electron-dense matrix that is housed in large vesicles within the surrounding sheath glial cell and secreted onto neurons (Ward *et al.*, 1975). Interestingly, mutations that affect cilia formation, such as mutations in genes encoding components of the intraflagellar transport (IFT) system (Sloboda, 2002) or mutations in the *daf-19* gene, which encodes a transcription factor required for cilia formation, result in the accumulation of numerous matrix-laden vesicles within the sheath glia (Perkins *et al.*, 1986). These observations suggest that the sheath glia can monitor and respond to the state of the neurons that they ensheath. Defects in the *che-12* gene suggest that the matrix secreted by sheath glia is important for neuronal function (Perkins *et al.*, 1986; Starich *et al.*, 1995). Animals carrying mutations in *che-12* possess fairly normal glia and neurons, as assessed by ultrastructural studies, yet these animals have profound chemosensory deficits. Furthermore, in wild-type animals, seven amphid neurons have the capacity to take up lipophilic dyes (such as DiI or fluorescein antibody search [FITC]) from the environment. Thus, these dyes can be used as indicators of exposure to the environment or neuronal function (Hedgecock *et al.*, 1985). In *che-12* mutants, these exposed channel neurons show reduced dye uptake (Perkins *et al.*, 1986; Starich *et al.*, 1995). These observations suggest that factors secreted by glia are important for sensory neuron properties and activities. The recent identification of a protein component of the matrix (Perens and Shaham, 2005; Sutherlin *et al.*, 2005) should allow a genetic dissection of this neuron–glia conversation.

Recent work on the amphid sensory organ has also identified important molecular players in generating the glial channel that ensheathes ciliated dendritic endings. Such a channel is formed by glia or specialized epithelia in sensory organs of many animals, including the olfactory, taste, and auditory organs of vertebrates (Burkitt *et al.*, 1993; Jan and Jan, 1993). In *C. elegans*, two genes, *daf-6* and *che-14*, act cooperatively to regulate channel formation (Michaux *et al.*, 2000; Perens and Shaham, 2005). Defects in either *daf-6* or *che-14* result in the inability of the sheath glia to form a channel contiguous with the socket glia channel. As a result, sensory endings of the amphid channel neurons are not exposed to the environment, and animals display profound sensory deficits (Fig. 4). DAF-6 protein is related to the Hedgehog receptor, Patched, and its sequence suggests that it is a member of a sub-family of sterol-sensing domain (SSD)-containing proteins of previously unknown function (Perens and Shaham, 2005). DAF-6 expression is restricted to luminal structures in *C. elegans*, and the protein is localized to the apical surfaces of all tube classes of the animal. In the amphid glia, DAF-6 function is required early during channel formation, and its expression lasts for only a short time during embryogenesis and the earliest larval stage.

CHE-14 protein is expressed in multiple epithelial cell types, including tubular epithelia. CHE-14 is related to the *Drosophila* Dispatched protein required for Hedgehog secretion (Michaux *et al.*, 2000). *che-14* mutants have abnormal amphid structure and display defects in cuticle structure, suggesting a role for *che-14* in the secretion of cuticle components by underlying hypodermal cells (the *C. elegans* equivalents of epidermal cells; Michaux *et al.*, 2000). Interestingly, animals harboring mutations in both *daf-6* and *che-14* exhibit synthetic defects in tube formation in several organs, suggesting that the proteins act together in this process. One attractive hypothesis for glia channel formation assigns a role for *daf-6* in inhibiting endocytosis from apical glial surfaces and for *che-14* in promoting exocytosis during channel formation (Michaux *et al.*, 2000; Perens and Shaham, 2005). These activities result in net membrane gain surrounding the channel, leading to channel opening and expansion.

The studies of amphid channel formation have also revealed that neuronally expressed genes are required for proper channel morphogenesis. Mutants in *daf-19*, a neuronally expressed gene required for cilia formation, possess abnormal amphid channels, displaying irregular shape and size. Furthermore, the glial protein DAF-6 is not properly localized in *daf-19* mutants. Thus, *daf-19*, perhaps through its role in cilia formation, promotes normal glial cell shape.

Although still in its infancy, the study of glia in *C. elegans* has already revealed exciting and essential roles for these cells in the functioning of the nervous system. Continued functional studies using cell-specific ablations and further genetic studies to identify genes required for glia–neuron

interactions should yield a rich understanding of the roles that glia play in the *C. elegans* nervous system. The remarkable conservation of many morphological and molecular features between the nervous systems of *C. elegans* and human beings suggests that glial genes and roles identified in the nematode may teach us much regarding glial genes and roles in humans.

## IX. Summary

In the mammalian brain, there are roughly five times as many glia as there are neurons, yet glial functions and their mechanisms remain mysterious. The studies described in this chapter suggest that glia play essential and complex roles in regulating nervous system structure and function. Recent interest in these cells as active participants in nervous system behavior has led to the development of a number of important model systems to examine glia both *in vitro* and *in vivo*. Continued studies using these assay systems, as well as tractable *in vivo* genetic models, should help to elucidate the roles of these remarkable cells.

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