Advanced Review

The many glia of a tiny nematode: studying glial diversity using *Caenorhabditis elegans*

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Glia constitute a major, understudied population of cells in the nervous system. Currently, it is appreciated that these cells exhibit vast morphological, functional, and molecular diversity, but our understanding of glial biology is limited. Some key unanswered questions include how glial diversity is generated during development and what functions distinct glial subtypes serve in the mature nervous system. The nematode *Caenorhabditis elegans* contains a defined set of glia, which have clear morphological and molecular differences, and thus provides a simplified model for understanding glial diversity. In addition, recent experiments suggest that the molecular mechanisms underlying the generation of glial diversity in *C. elegans* are conserved with those in mammals. In this review, we summarize the surprising diversity of glial subtypes present in this simple organism, and highlight current thinking about what roles they perform in the nervous system. We emphasize how genetic approaches may be used to identify the mechanistic origins of glial diversity, which is key to understanding how glia function in health and disease.

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INTRODUCTION

Glia Exhibit Vast Morphological, Molecular, and Functional Diversity

Once thought to be passive 'scaffolding' of the brain, glia have emerged over the last decade as morphologically, functionally, and molecularly diverse cells that control many aspects of neuronal development and function. Morphological differences among glia were first appreciated over a hundred years ago by Ramón y Cajal and Golgi; currently, the catalog of mammalian glial morphologies ranges from the classical star shape of astrocytes to the long, unbranched bipolar shape of radial glia. Substantial morphological diversity is present even within a subtype (i.e., astrocytes) and is tied to anatomical identity. For example, different brain regions can be distinguished based solely on the morphology of their astrocytes.[1,2] Further, expression profiling of glia in mice has led to an appreciation of their molecular diversity,[3–5] which likely corresponds to functional diversity. Mammalian glia carry out a myriad of functions which include: promotion and guidance of axon outgrowth,[6–8] secretion of factors that stabilize and strengthen synapses,[9,10] sculpting of neuronal circuitry via synapse elimination,[11,12] modulation of neurotransmission at the 'tripartite synapse',[13–15] and establishment of the blood–brain barrier.[16] Emerging studies in mammalian systems are providing evidence that region-specific molecular differences in glial cells have important effects on neuronal development and function. For example, Molofsky and colleagues recently demonstrated that α-motor neurons, but not their neighbors, require the expression of a guidance molecule in a distinct population of ventral spinal cord astrocytes for proper circuit formation.[17]

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How Is the Diversity of Glial Subtypes Generated?

Most mammalian glia arise from the same progenitor cells that produce neurons, with the 'glia versus neuron' decision controlled by a Notch-dependent switch that activates glial-specific genes including Sox9 and NFIA.[18,19] Much less is known about how different glial types are then specified. The best understood example is the developing neural tube, in which oligodendrocytes and three different types of astrocytes are specified by integration of extrinsic spatial and temporal cues including Shh, BMP, and Wnt signaling.[20] These extrinsic signals activate combinatorial expression of Olig2, Pax6, Nkx2.2, Nkx6.1, and other transcription factors along the dorsal–ventral axis of the embryo.[20–24] Glial subtypes in other parts of the mammalian brain are likely specified in a similar manner, but how this transcriptional regulatory network is modified to generate distinct morphological and molecular cell identities is not known.

Caenorhabditis elegans Offers a Powerful Model System to Study Glial Diversity

Recent studies in *Drosophila* and *C. elegans* show that invertebrate glia are highly diverse and perform functions similar to vertebrate glia, in some cases using shared molecular mechanisms. As in mammals, invertebrate glia are generated by the same precursors that give rise to neurons, guide neuronal and axon migration,[25] and undergo highly coordinated morphogenesis with the neurons they ensheathe.[26] Invertebrate glia also regulate neuronal activity,[27] promote synaptogenesis,[28] dictate the maintenance of synapse locations during growth,[29] and shape neuronal connections by engulfing synaptic material.[30] Thus, invertebrate glia provide rich models for understanding how glia develop and function.

DIVERSITY OF *C. ELEGANS* GLIA

In contrast to the multitude of uncharacterized glial cell types in the vertebrate nervous system, the *C. elegans* nervous system contains exactly 50 glia[31] and develops in a stereotyped, tractable manner. The majority of glial cells are found in the four types of sense organs in the head, which are called the amphid (AM), cephalic (CEP), outer labial (OL), and inner labial (IL) organs (see Box 1). In each of these sense organs, one or more sensory neurons are partnered with exactly two glia: the sheath and socket (Figure 1). These sheath and socket glia extend unbranched processes collateral with the dendrites of their neuronal partners, terminating at the tip of the nose. The anterior-most aspect of the sheath glial process forms an elaborate 'goblet' structure that completely ensheathes the dendritic endings of its associated sensory neurons[31] (Figure 1, inset). Neurons penetrate the base of the goblet and form apical junctions onto the sheath glial cell. In turn, the sheath glial cell forms apical junctions onto the socket glial cell, which forms a cuticle-lined pore at the tip of the nose through which some of the neuronal sensory cilia protrude[31–33] (Figure 1, inset). Together, the two glial cell types thus create and maintain a nerve channel in which the dendritic endings of sensory neurons receive input from the environment.

Morphological Diversity of *C. elegans* Glia

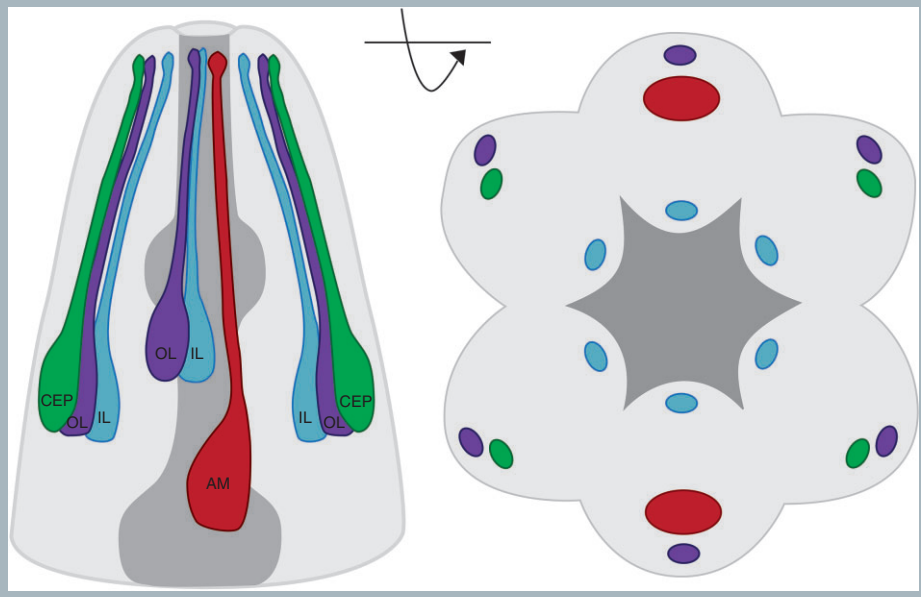
While *C. elegans* glia have simpler shapes than their vertebrate counterparts, glia in different anatomical locations nevertheless exhibit morphological diversity and pair with specific neuronal partners, analogous to the region-specific differences observed among glia in the vertebrate brain (Figure 2). The amphid sheath glia are physically larger than other sheath glia, and interact with more sensory neurons (Figure 2). Electron microscopy shows that these cells are full of vesicles that deposit matrix into the nerve channel[31] (Figure 2, inset). Other sheath glial cells are smaller in size and form lamellae with deep invaginations in the nerve channel[31] (Figure 2, inset). The CEP sheath glia are distinguished by their bipolar morphology: in addition to the anterior process that ensheathes the sensory endings of CEP neurons, they also extend a posterior, sheet-like process that envelops the nerve ring, the main neuropil of the nervous system (Figure 2). Some glia also form close contacts with neurons they do not ensheathe: the lateral IL socket glia are wrapped by specialized extensions of the BAG and FLP neurons[31,32,35](Figure 2, inset); dorsal IL socket glia are juxtaposed to the dendrite of URX; and IL and OLQ sheath glia are closely apposed to dendrites of the nonciliated neurons URA and URB. The function of these contacts is not known. This diversity of glial morphologies and neuronal pairings strongly suggests that, as in vertebrates, glia of *C. elegans* are not interchangeable, but rather provide functions uniquely tailored to their environment.

GLIAL FUNCTION IN *C. ELEGANS*

In addition to morphological diversity, glial cells in different sense organs are molecularly heterogeneous, hinting at functional diversity among *C.*

BOX 1

ANATOMY AND CHARACTERISTICS OF *C. ELEGANS* SENSE ORGANS



Schematic depicts positioning of the four major sense organs in the *C. elegans* head from longitudinal and transverse views: AM (red), CEP (green), IL (blue), and OL (purple). Each sense organ consists of sensory neuron(s) and two glial cell types, the sheath and socket. The amphid sense organ is located laterally in the head with bilateral symmetry. It is the largest sense organ and consists of twelve neurons, which are ensheathed by two glial cells. Eight of the 12 amphid neurons extend nonmotile sensory cilia that are exposed to the environment to sense chemical and mechanosensory stimuli,[34] whereas the ciliary endings of four amphid sensory neurons terminate in pockets within the sheath glial cells. The OL and IL sense organs are both located ventrally, dorsally, and laterally with sixfold symmetry. Each OL organ consists of one mechanosensory neuron ensheathed by two glial cells, and each IL organ is comprised of one chemosensory and one mechanosensory neuron, also ensheathed by two glial cells. The CEP sense organ is located ventrally and dorsally with fourfold symmetry. Each CEP organ consists of one dopaminergic mechanosensory neuron and two glial cells.

elegans glia. In some cases, molecular differences are so complete that cell type-specific reporters have been identified that uniquely mark a single class of glial cell. The amphid sheath glial cell–the only glial cell so far to have been transcriptionally profiled–uniquely expresses many small peptides that are predicted to be secreted; at least one of which is deposited on the neuronal endings.[27] There are even molecular differences within a single glial subtype: e.g., the ventral CEP sheath glial cells express *unc-6/netrin*, while their dorsal counterparts do not.[36,37] Although our catalog of gene expression in *C. elegans* glial cells is currently limited, these examples of molecular markers unique to specific types of glial cells provide a foot in the door to determining whether specialized glial types provide different functions, both in the developing and mature nervous system.

Glial Function in the Developing Nervous System

Currently, little is known about the roles of glia during *C. elegans* development. CEP sheath glial cell function during development has been the best characterized, because of its unique association with the nerve ring that resembles vertebrate neuron–glia

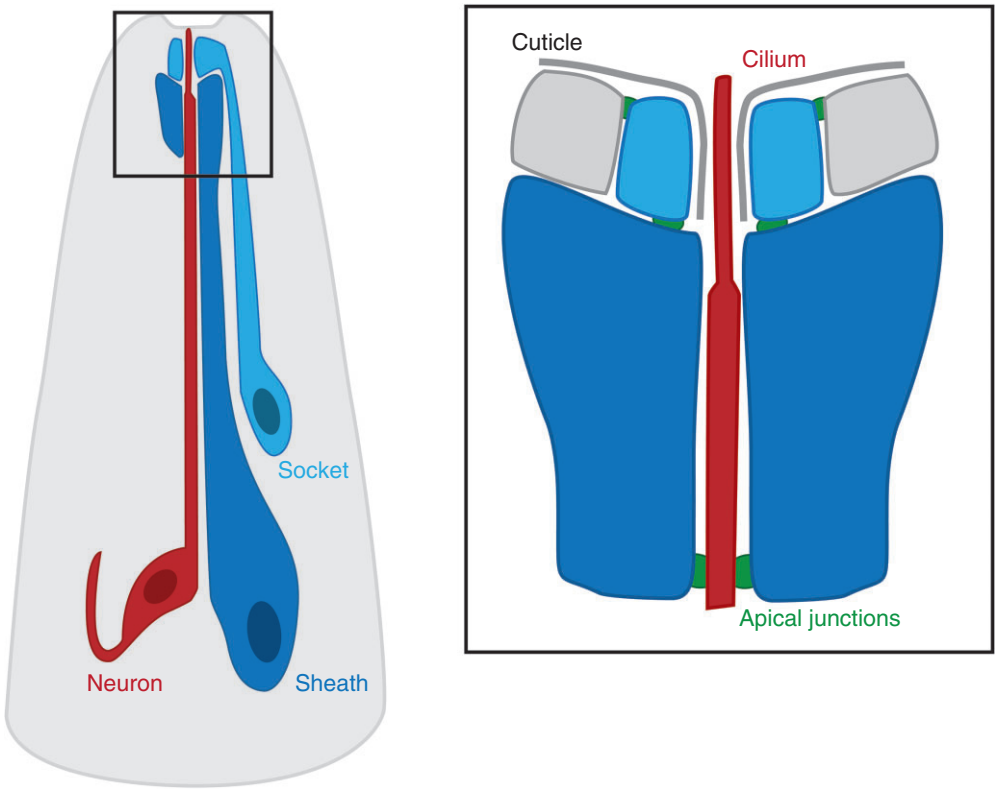


FIGURE 1 | ***Caenorhabditis elegans*** sense organs consist of three major cell types. Schematic of a generalized sense organ, showing a sensory neuron (red), sheath glial cell (dark blue), and socket glial cell (light blue). An unbranched dendrite extends through a channel in the sheath and socket glia, such that the nonmotile sensory cilium at its tip is exposed to the external environment. Apical junctions (green) exist between the neuron and sheath glial cell; the sheath and socket glia; and the socket glial cell and hypodermis, or skin (gray). The socket cell channel is lined by cuticle that is continuous with the cuticle of the hypodermis.

interactions (Figure 2). When CEP glial cells are absent during development, the axons of sensory neurons become misguided and do not branch properly, the nerve ring becomes highly disorganized, and neuronal cell bodies are displaced.[37] At finer resolution, CEP sheath endfeet at the nerve ring dictate the location of synapse formation between two interneurons, AIY and RIA, through an UNC-6/netrin and UNC-40/DCC-dependent process.[28] During larval development, abnormal CEP sheath contacts with the AIY neuron can also induce the formation of ectopic synaptic sites.[29]

Glial cells in other sense organs do not extend processes to the nerve ring and contact synapses, but their formation and maintenance of the sheath-socket channel is important for the establishment and function of sensory neuron dendrites. A molecular pathway, related to Hedgehog (Hh) signaling in vertebrates, has been implicated in the formation of a functional nerve channel. Although the canonical Hh pathway is not conserved in *C. elegans*, the genome is predicted to encode dozens of Hedgehog- and Patched-related proteins, many of which are expressed in a remarkably cell-type-specific manner by different glial sheath and socket cells.[37–39] One of these factors, the Patched-related protein DAF-6, localizes to the lumen of the nerve channel formed by the amphid sheath and socket glial cells. In *daf-6* mutants, the amphid sheath and socket channels become malformed, the sheath glial cell fills with matrix-rich vacuoles, and the cilia of sensory neurons bend abnormally due to the physical constraints of the closed channel.[39] The *daf-6* phenotype can be suppressed by loss of *lit-1*, a Nemo-like kinase that normally promotes expansion of the amphid sheath glial channel.[40] The function of Hedgehog- and Patched-related proteins in other glia has not been explored. Given the role of DAF-6 in setting up the amphid sheath-socket channel, one possibility is that combinations of these proteins mediate glia–glia or neuron–glia communication specific to each sense organ. Intriguingly, Hh signaling has emerged as a regulator of vertebrate glial development in the neural tube and forebrain,[20,41,42] suggesting that glial cells in different organisms may use shared molecular programs.

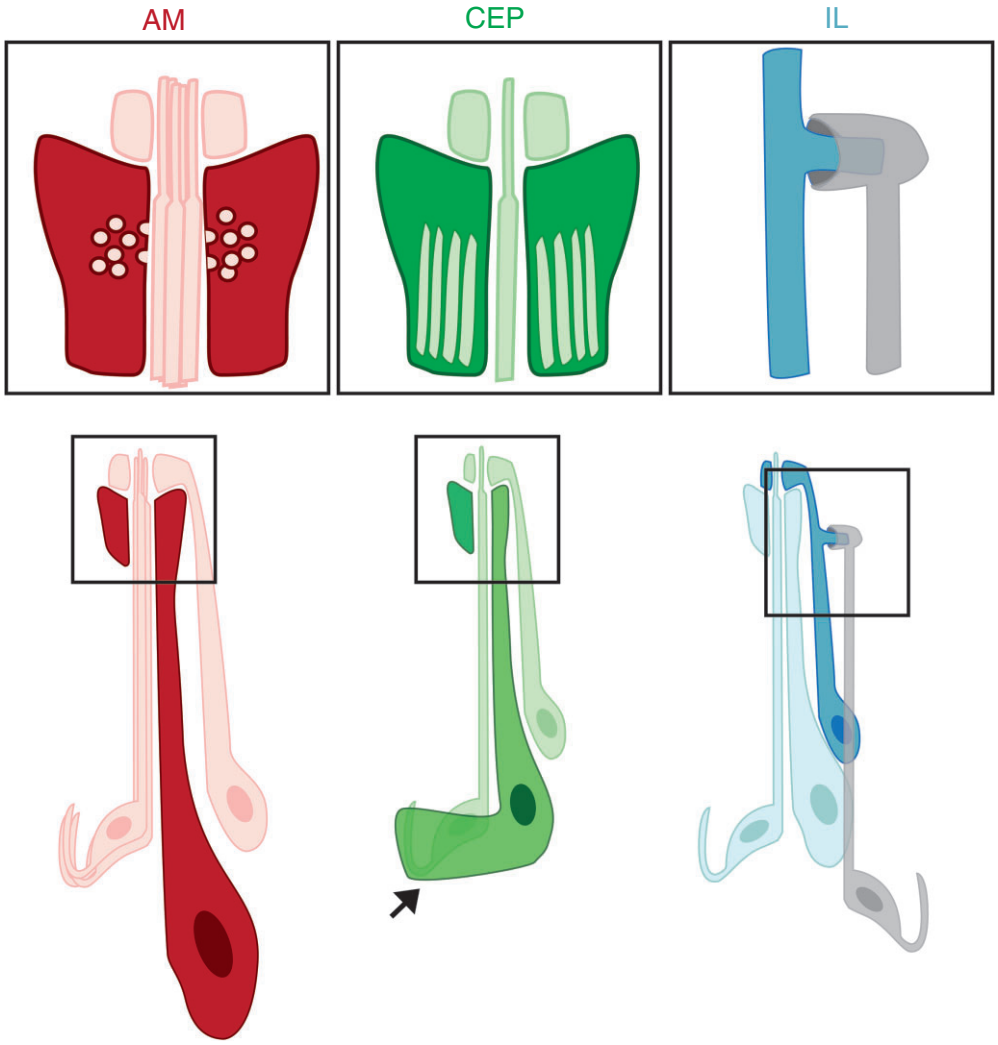


FIGURE 2 | Subtypes of sheath and socket glia. Examples of glial diversity: the amphid sheath glial cell (AM, red), the cephalic sheath glial cell (CEP, green) and the lateral inner labial socket glial cell (IL, blue). The amphid sheath glial cell is larger than its counterparts in other sense organs and contains many matrix-filled vesicles (inset). The CEP sheath glial cell has a bipolar morphology with a process that ensheathes the CEP neuron dendrite at the nose tip and a sheet-like process that extends posteriorly to contact the nerve ring (arrow). CEP sheath glia have deep invaginations that form lamellae in the nerve channel (inset), a feature it shares with outer and inner labial (OL/IL) sheath glial cells (not pictured). The lateral IL socket glial cell is uniquely wrapped by an extension of the BAG neuron (inset).

Another interesting but unexplored question is how glial cells pair with appropriate partner neurons during development. Mutations that disrupt CEP sheath function lead to shortened sensory dendrites, indicating that neuron-glia pairing may be required for dendrite extension.[37] Additionally, the non-canonical manner in which head sense organs develop may provide a hint as to when pairing occurs. The morphologies of both neurons and glial cells form using an 'anchor and stretch' mechanism. Both cell types are born near the presumptive nose tip, anchor there, and then their cell bodies migrate posteriorly, stretching out dendrites and glial processes behind them as they migrate.[26] This anchoring requires an extracellular molecule, DYF-7, and *dyf-7* mutant animals have shortened sensory dendrites and sheath glial processes.[26] Interestingly, in *dyf-7* mutants, the amphid sheath glial cell still ensheathes the shortened dendritic endings of the amphid neurons.[26] These observations suggest that neuron-glia pairing occurs early in embryonic development, before dendrite and process extension. Furthermore, ablations of precursors of glial cells and neurons during development in OL/IL/CEP sense organs caused neurons that had lost their normal glial partner to associate with different glia.[43] The ability of 'abandoned' neurons to find new partners suggests that neurons, glia, or components of the extracellular

matrix (ECM) contain long-range cues that can confer specificity for cell–cell associations. However, the factors that regulate proper neuron-glia pairing are unknown.

Glial Function in the Mature Nervous System

Recent studies in vertebrate and invertebrate model systems have determined that proper glial function is important for many aspects of nervous system activity. Importantly, in contrast to other organisms, ablation of glial cells in *C. elegans* does not result in neuron death, providing the opportunity to study the function of these cells without grossly disrupting the nervous system.

Animals in which the CEP sheath glial cells are not properly specified display defects in several CEP neuron-regulated behaviors, including egg-laying, feeding, and gustatory-associated learning.[44,45] Similarly, animals mutant for DELM-1 and DELM-2 (degenerin-like mechanosensory linked) channels, which are expressed in glia that associate with mechanosensory neurons, exhibit defects in nose touch and foraging behaviors.[46] These results demonstrate that CEP sheath glial cells contribute to normal CEP neuron function.

Likewise, animals in which the amphid sheath glia have been ablated exhibit defects in chemotaxis to various odorants, mediated by the AWA and AWC neurons, and in thermotaxis, mediated by the AFD neuron.[27] The ciliary endings of these neurons are normally embedded in pockets of the sheath glial cell and are deformed in glia-ablated animals, which is likely the major reason these neurons do not function normally. However, glia-ablated animals also exhibit defects in sensory functions mediated by the neurons ASE, ADL, and ASH, which retain wild-type morphology.[27] These observations provide evidence that amphid sheath glial cells can directly affect neuron activity. In accordance with this idea, the transcriptional profile of amphid sheath glial cells is enriched for expression of transmembrane proteins and secreted peptides, which could mediate glia-to-neuron communication.[27] One of these candidates is FIG-1, a secreted protein that contains thrombospondin and EGF-like domains.[27] This molecule is of particular interest because astrocytes secrete thrombospondin to regulate synapse assembly and function in mammals.[9,10] Unlike their wild-type counterparts, amphid sensory neurons of *fig-1* mutants do not take up lipophilic dyes, a defect also observed in glia-ablated animals.[27] Similar to thrombospondin, FIG-1 may be secreted by amphid sheath glial cells to modulate the activity of a 'sensory synapse' at which amphid neurons receive input from the environment.

In addition to modulating neuronal activity, some *C. elegans* glia seem to directly respond to environmental stimuli. For example, amphid sheath glial cells uniquely express the Degenerin/ENaC channel, ACD-1, and animals mutant for this acid-sensitive ion channel are defective in acid sensing.[47,48] Full restoration of acid sensation was achieved only when *acd-1* expression was specifically rescued in amphid sheath glial cells, suggesting that sheath glial cells contribute to this sensory modality. Furthermore, like the thermosensory AFD neuron that it ensheathes, the amphid sheath glial cell also responds to changes in temperature. Increasing ambient temperature results in an upregulation of the *ver-1* gene in the amphid sheath through TTX-1, an OTD/OTX transcription factor.[49] Thermosensation and *ttx-1* expression were previously thought to be specific to AFD neurons; however, amphid sheath glial cells also express *ttx-1* and respond to changes in temperature even in the absence of the AFD neuron.[49] Thus, some glia may sense specific external stimuli, including pH and temperature, and use that information to modulate the activity of their neuronal partners, by mechanisms that could include secretion of small peptides and regulation of the local ionic environment.

SPECIFICATION OF GLIAL DIVERSITY IN *C. ELEGANS*

Although the upstream molecular mechanisms that regulate neuron versus glia cell fate decisions do not appear to be shared between invertebrates and vertebrates, the few known pathways that diversify glial fates in *C. elegans* are conserved in other organisms. *lin-26*, a transcription factor that lacks orthologs in higher-order organisms, is expressed broadly in developing glial cells during *C. elegans* embryogenesis.[50] Functional studies suggest that this transcription factor determines whether cells will become glia or neurons, because in its absence, glial cells develop abnormally, take on a neuronal fate, or die.[50] Further downstream, the process of glial cell subtype specification has only been characterized for developing CEP sheath glial cells. These cells uniquely express *hlh-17*, an ortholog of the oligodendrocyte marker *Olig2*.[37] Analogous to Olig2 activation in the vertebrate neural tube, expression of *hlh-17* in CEP glia is controlled by Pax and Nkx-like factors.[37] In developing ventral CEP sheath glial cells, VAB-3, a transcription factor similar to vertebrate Pax6/7, regulates the expression

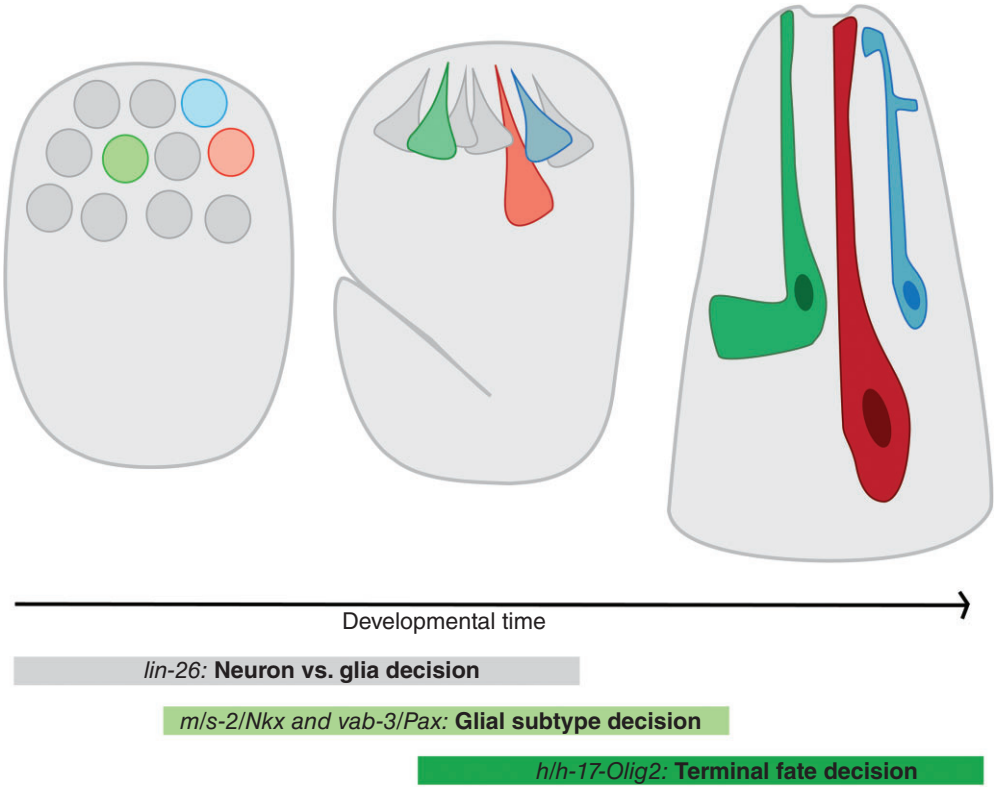


FIGURE 3 | Glial development. Working model of glial subtype specification: cells in neural lineages express the nonconserved factor, *lin-26*, to select a glial rather than neuronal fate. The CEP sheath glial subtype (green) is specified by the conserved transcription factors, *mls-2/Nkx* and *vab-3/Pax*, whose expression starts during embryogenesis. In turn, these factors activate expression of transcription factors, such as *hlh-17*, an *Olig2* ortholog, that may control aspects of mature CEP sheath glial morphology or function. Presumably, similar transcription factor hierarchies specify other glial subtypes (red, blue), but these factors remain to be identified.

of the Nkx-related protein MLS-2, and together these factors promote the expression of *hlh-17*. In developing dorsal CEP sheath glial cells, VAB-3 directly activates *hlh-17*[37] (Figure 3). Another conserved transcription factor, *alr-1*, related to the homeobox factor *aristaless*, is expressed in amphid socket glial cells and is important for maintaining normal amphid sense organ structure. In the absence of *alr-1*, the junctions between amphid sheath and socket glial cells disintegrate, amphid socket morphology becomes abnormal, and mutant animals exhibit sensory defects.[51] How other glial types are specified is not yet known, but the process likely involves the activation of a series of transcription factors that 'lock in' the fate of each particular glial subtype.

Using *C. elegans* to Understand How Glial Diversity Is Generated

Caenorhabditis elegans has proven to be a powerful genetic system for understanding how neuronal fates are specified, and these same approaches may be readily applied to dissecting glial diversity. Understanding how fate specification occurs hinges on the identification of relevant *cis*-regulatory DNA sequences upstream of cell-type-specific genes and the *trans*-acting factors that regulate those sequences. Two experimental approaches in *C. elegans* are key to discovering these elements. First, 'promoter-bashing' approaches can quickly whittle away sequences to identify the important *cis*-regulatory motifs. The *C. elegans* genome is compact, and *cis*-regulatory elements that control cell-type-specific gene expression are typically localized within a few kilobases upstream of the coding sequence. Regulatory sequences of a gene of interest are cloned upstream of a fluorescent reporter (i.e., promoter:GFP) to create fusion constructs that are microinjected into the germline and stably inherited as extrachromosomal arrays in progeny. Transgenesis is rapid, taking less than two weeks to go from a DNA construct to a stable transgenic strain.[52] As the animals are transparent throughout life, activity of a candidate promoter can be easily assayed in whole live animals without the need to mount, dissect, or stain them. Identification of *cis*-regulatory motifs can now further be guided by a

wealth of new genomic resources, including the modENCODE project, that have mapped out transcription factor binding sites across the genome.[53] Second, to identify *trans*-acting factors, forward genetic screens are readily used to identify factors whose disruption results in loss of cell-type-specific promoter:GFP expression. Such a screen may be performed by systematic genome-wide RNAi or by random chemical mutagenesis. In a typical chemical mutagenesis strategy, animals are screened under a fluorescent dissecting microscope, typically at a rate of 5–10 animals per minute, for loss of cell-type-specific GFP expression. Each animal carries an average of five homozygous disruptive mutations, for an overall screening rate of 25–50 mutations per minute, or about 2000 mutations per hour. Thus, in only 10 h of screening time, an average of one disruptive mutation in each of the ~20,000 genes may be examined. With the advent of whole-genome sequencing, interesting mutations may be mapped and cloned in a single step in a matter of weeks, at a current cost of about $500 per mutant.[52]

These unbiased approaches have been used extensively to dissect the basis of neuronal diversity in *C. elegans*. They have led to the prevailing 'terminal selector' model for neuronal fate specification, in which many transient inputs coalesce to activate unique cell-type-specific master regulators or 'terminal selectors' that then activate batteries of genes specific to a given neuron type.[54] Closely related to this model is the observation of hierarchical 'tiers' of neuronal fates, in which increasingly specific fates are layered on top of a broad class type (i.e., neuron > sensory neuron > amphid neuron > ASE neuron).[55] An important major question is whether this paradigm holds for glial fate specification in *C. elegans* and in mammals.

The diversity of glial cell types found in the nematode nervous system, the ability to specifically mark these glial subtypes with promoter:GFP fusions, and emerging studies that show conservation of important glial factors to vertebrate orthologs make *C. elegans* a powerful system for elucidating how the morphological and molecular diversity of glia is generated.

REFERENCES

1. Emsley JG, Macklis JD. Astroglial heterogeneity closely reflects the neuronal-defined anatomy of the adult murine CNS. *Neuron Glia Biol* 2006, 2:175–186.
2. Tsai H-H, Li H, Fuentealba LC, Molofsky AV, Taveira-Marques R, Zhuang H, Tenney A, Murnen AT, Fancy SPJ, Merkle F, et al. Regional astrocyte allocation regulates CNS synaptogenesis and repair. *Science* 2012, 337:358–362.
3. Cahoy JD, Emery B, Kaushal A, Foo LC, Zamanian JL, Christopherson KS, Xing Y, Lubischer JL, Krieg PA, Krupenko SA, et al. A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. *J Neurosci* 2008, 28:264–278.
4. Doyle JP, Dougherty JD, Heiman M, Schmidt EF, Stevens TR, Ma G, Bupp S, Shrestha P, Shah RD, Doughty ML, et al. Application of a translational profiling approach for the comparative analysis of CNS cell types. *Cell* 2008, 135:749–762.
5. Yeh T-H, Lee DY, Gianino SM, Gutmann DH. Microarray analyses reveal regional astrocyte heterogeneity with implications for neurofibromatosis type 1 (NF1)-regulated glial proliferation. *Glia* 2009, 57:1239–1249.
6. Hatten ME. Central nervous system neuronal migration. *Annu Rev Neurosci* 1999, 22:511–539.
7. Lemke G. Glial control of neuronal development. *Annu Rev Neurosci* 2001, 24:87–105.
8. Rakic P. Guidance of neurons migrating to the fetal monkey neocortex. *Brain Res* 1971, 33:471–476.
9. Christopherson KS, Ullian EM, Stokes CCA, Mullowney CE, Hell JW, Agah A, Lawler J, Mosher DF, Bornstein P, Barres BA. Thrombospondins are astrocyte-secreted proteins that promote CNS synaptogenesis. *Cell* 2005, 120:421–433.
10. Eroglu C, Allen NJ, Susman MW, O'Rourke NA, Park CY, Ozkan E, Chakraborty C, Mulinyawe SB, Annis DS, Huberman AD, et al. Gabapentin receptor $\alpha 2\delta$-1 is a neuronal thrombospondin receptor responsible for excitatory CNS synaptogenesis. *Cell* 2009, 139:380–392.
11. Clarke LE, Barres BA. Emerging roles of astrocytes in neural circuit development. *Nat Rev Neurosci* 2013, 14:311–321.
12. Schafer DP, Lehrman EK, Kautzman AG, Koyama R, Mardinly AR, Yamasaki R, Ransohoff RM, Greenberg ME, Barres BA, Stevens B. Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron* 2012, 74:691–705.
13. Beattie EC, Stellwagen D, Morishita W, Bresnahan JC, Ha BK, Von Zastrow M, Beattie MS, Malenka RC. Control of synaptic strength by glial TNFα. *Science* 2002, 295:2282–2285.
14. Bezzi P, Gundersen V, Galbete JL, Seifert G, Steinhäuser C, Pilati E, Volterra A. Astrocytes contain a

vesicular compartment that is competent for regulated exocytosis of glutamate. *Nat Neurosci* 2004, 7: 613–620.

15. Perea G, Navarrete M, Araque A. Tripartite synapses: astrocytes process and control synaptic information. *Trends Neurosci* 2009, 32:421–431.
16. Abbott NJ, Rönnbäck L, Hansson E. Astrocyte-endothelial interactions at the blood–brain barrier. *Nat Rev Neurosci* 2006, 7:41–53.
17. Molofsky AV, Kelley KW, Tsai H-H, Redmond SA, Chang SM, Madireddy L, Chan JR, Baranzini SE, Ullian EM, Rowitch DH. Astrocyte-encoded positional cues maintain sensorimotor circuit integrity. *Nature* 2014, 509:189–194.
18. Deneen B, Ho R, Lukaszewicz A, Hochstim CJ, Gronostajski RM, Anderson DJ. The transcription factor NFIA controls the onset of gliogenesis in the developing spinal cord. *Neuron* 2006, 52:953–968.
19. Stolt CC, Lommes P, Sock E, Chaboissier M-C, Schedl A, Wegner M. The Sox9 transcription factor determines glial fate choice in the developing spinal cord. *Genes Dev* 2003, 17:1677–1689.
20. Rowitch DH, Kriegstein AR. Developmental genetics of vertebrate glial-cell specification. *Nature* 2010, 468:214–222.
21. Genethliou N, Panayiotou E, Panayi H, Orford M, Mean R, Lapathitis G, Malas S. Spatially distinct functions of PAX6 and NKX2.2 during gliogenesis in the ventral spinal cord. *Biochem Biophys Res Commun* 2009, 382:69–73.
22. Hochstim C, Deneen B, Lukaszewicz A, Zhou Q, Anderson DJ. Identification of positionally distinct astrocyte subtypes whose identities are specified by a homeodomain code. *Cell* 2008, 133:510–522.
23. Muroyama Y, Fujiwara Y, Orkin SH, Rowitch DH. Specification of astrocytes by bHLH protein SCL in a restricted region of the neural tube. *Nature* 2005, 438:360–363.
24. Zhou Q, Anderson DJ. The bHLH transcription factors OLIG2 and OLIG1 couple neuronal and glial subtype specification. *Cell* 2002, 109:61–73.
25. Oland LA, Tolbert LP. Key interactions between neurons and glial cells during neural development in insects. *Annu Rev Entomol* 2003, 48:89–110.
26. Heiman MG, Shaham S. DEX-1 and DYF-7 establish sensory dendrite length by anchoring dendritic tips during cell migration. *Cell* 2009, 137:344–355.
27. Bacaj T, Tevlin M, Lu Y, Shaham S. Glia are essential for sensory organ function in *C. elegans*. *Science* 2008, 322:744–747.
28. Colón-Ramos DA, Margeta MA, Shen K. Glia promote local synaptogenesis through UNC-6 (netrin) signaling in *C. elegans*. *Science* 2007, 318:103–106.
29. Shao Z, Watanabe S, Christensen R, Jorgensen EM, Colón-Ramos DA. Synapse location during growth depends on glia location. *Cell* 2013, 154:337–350.
30. Fuentes-Medel Y, Logan MA, Ashley J, Ataman B, Budnik V, Freeman MR. Glia and muscle sculpt neuromuscular arbors by engulfing destabilized synaptic boutons and shed presynaptic debris. *PLoS Biol* 2009, 7:e1000184.
31. Ward S, Thomson N, White JG, Brenner S. Electron microscopical reconstruction of the anterior sensory anatomy of the nematode *Caenorhabditis elegans*. *J Comp Neurol* 1975, 160:313–337.
32. Doroquez DB, Berciu C, Anderson JR, Sengupta P, Nicastro D. A high-resolution morphological and ultrastructural map of anterior sensory cilia and glia in *Caenorhabditis elegans*. *eLife* 2014, 3:e01948.
33. White JG, Southgate E, Thomson JN, Brenner S. The structure of the nervous system of the nematode *Caenorhabditis elegans*. *Philos Trans R Soc Lond B Biol Sci* 1986, 314:1–340.
34. Bargmann, CI. Chemosensation in *C. elegans*, *WormBook*, ed. *The C. elegans Research Community, WormBook*. doi/10.1895/wormbook.1.123.1. http://www.wormbook.org. (Accessed October 25, 2006).
35. Perkins LA, Hedgecock EM, Thomson JN, Culotti JG. Mutant sensory cilia in the nematode *Caenorhabditis elegans*. *Dev Biol* 1986, 117:456–487.
36. Wadsworth WG, Bhatt H, Hedgecock EM. Neuroglia and pioneer neurons express UNC-6 to provide global and local netrin cues for guiding migrations in *C. elegans*. *Neuron* 1996, 16:35–46.
37. Yoshimura S, Murray JI, Lu Y, Waterston RH, Shaham S. mls-2 and vab-3 Control glia development, hlh-17/Olig expression and glia-dependent neurite extension in *C. elegans*. *Dev Camb Engl* 2008, 135: 2263–2275.
38. Hao L, Johnsen R, Lauter G, Baillie D, Bürglin TR. Comprehensive analysis of gene expression patterns of hedgehog-related genes. *BMC Genomics* 2006, 7:280.
39. Perens EA, Shaham S. *C. elegans* daf-6 encodes a patched-related protein required for lumen formation. Dev. *Cell* 2005, 8:893–906.
40. Oikonomou G, Perens EA, Lu Y, Watanabe S, Jorgensen EM, Shaham S. Opposing activities of LIT-1/NLK and DAF-6/patched-related direct sensory compartment morphogenesis in *C. elegans*. *PLoS Biol* 2011, 9:e1001121.
41. Garcia ADR, Petrova R, Eng L, Joyner AL. Sonic hedgehog regulates discrete populations of astrocytes in the adult mouse forebrain. *J Neurosci* 2010, 30:13597–13608.
42. Orentas DM, Hayes JE, Dyer KL, Miller RH. Sonic hedgehog signaling is required during the appearance of spinal cord oligodendrocyte precursors. *Dev Camb Engl* 1999, 126:2419–2429.
43. Sulston JE, Schierenberg E, White JG, Thomson JN. The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Dev Biol* 1983, 100:64–119.

44. Felton CM, Johnson CM. Modulation of dopamine-dependent behaviors by the *Caenorhabditis elegans* Olig homolog HLH-17. *J Neurosci Res* 2011, 89:1627–1636.

45. McMiller TL, Johnson CM. Molecular characterization of HLH-17, a *C. elegans* bHLH protein required for normal larval development. *Gene* 2005, 356: 1–10.

46. Han L, Wang Y, Sangaletti R, D'Urso G, Lu Y, Shaham S, Bianchi L. Two novel DEG/ENaC channel subunits expressed in glia are needed for nose-touch sensitivity in *Caenorhabditis elegans*. *J Neurosci* 2013, 33:936–949.

47. Wang Y, Apicella A, Lee S-K, Ezcurra M, Slone RD, Goldmit M, Schafer WR, Shaham S, Driscoll M, Bianchi L. A glial DEG/ENaC channel functions with neuronal channel DEG-1 to mediate specific sensory functions in *C. elegans*. *EMBO J* 2008, 27:2388–2399.

48. Wang Y, D'Urso G, Bianchi L. Knockout of glial channel ACD-1 exacerbates sensory deficits in a *C. elegans* mutant by regulating calcium levels of sensory neurons. *J Neurophysiol* 2012, 107:148–158.

49. Procko C, Lu Y, Shaham S. Glia delimit shape changes of sensory neuron receptive endings in *C. elegans*. *Dev Camb Engl* 2011, 138:1371–1381.

50. Labouesse M, Hartwieg E, Horvitz HR. The *Caenorhabditis elegans* LIN-26 protein is required to specify and/or maintain all non-neuronal ectodermal cell fates. *Dev Camb Engl* 1996, 122:2579–2588.

51. Tucker M, Sieber M, Morphew M, Han M. The *Caenorhabditis elegans* aristaless orthologue, alr-1, is required for maintaining the functional and structural integrity of the amphid sensory organs. *Mol Biol Cell* 2005, 16:4695–4704.

52. Boulin T, Hobert O. From genes to function: the *C. elegans* genetic toolbox. *WIREs Dev Biol* 2012, 1:114–137.

53. Gerstein MB, Lu ZJ, Van Nostrand EL, Cheng C, Arshinoff BI, Liu T, Yip KY, Robilotto R, Rechtsteiner A, Ikegami K, et al. Integrative analysis of the *Caenorhabditis elegans* genome by the modENCODE project. *Science* 2010, 330:1775–1787.

54. Hobert O. Regulation of terminal differentiation programs in the nervous system. *Annu Rev Cell Dev Biol* 2011, 27:681–696.

55. Hobert O. Neurogenesis in the nematode *Caenorhabditis elegans, WormBook*, ed. The *C. elegans Research Community, WormBook*. doi/10.1895/wormbook.1.12.2. http://www.wormbook.org. (Accessed October 4, 2010).