Glial cell development in the *Drosophila* embryo

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Summary

Glial cells play a central role in the development and function of complex nervous systems. *Drosophila* is an excellent model organism for the study of mechanisms underlying neural development, and recent attention has been focused on the differentiation and function of glial cells. We now have a nearly complete description of glial cell organization in the embryo, which enables a systematic genetic analysis of glial cell development. Most glia arise from neural stem cells that originate in the neurogenic ectoderm. The bifurcation of glial and neuronal fates is under the control of the glial promoting factor *glial cells missing*. Differentiation is propagated through the regulation of several transcription factors. Genes have been discovered affecting the terminal differentiation of glia, including the promotion glial–neuronal interactions and the formation of the blood–nerve barrier. Other roles of glia are being explored, including their requirement for axon guidance, neuronal survival, and signaling. *BioEssays* 23:877–887, 2001.

Introduction

The development of a functional nervous system requires the correct specification, precise organization and interaction of a large number of neural cell types. These cell types fall into two major categories, neurons and glia. While most research on the nervous system has focused on the development and behavior of neurons, there has been a growing interest and appreciation of their lesser-understood partners, glial cells. Glial cells are intimately associated with neurons, and in primates constitute some 50% of brain mass. Such numbers suggest that the diversification of glial cells has contributed greatly to the evolution of complex nervous systems.

This observation is being born out by the increasing number of roles being assigned to glia as essential components of nervous system function.[1] These roles include structural support, wrapping and insulating neurons, and regulating them with cytokines and growth factors. Glia maintain the ionic homeostasis of neurons, and they establish and maintain the blood–brain and blood–nerve barriers, thereby providing a suitable environment for the propagation of action potentials. Glia have ion channels, neurotransmitter transporters and receptors, which, in addition to regulating the proper ionic environment, are implicated in modulating synaptic activity and regulating synapse numbers.[2–5] Developing glia undergo extensive migrations and cell shape changes and also act as cues and substrata for neuronal migrations and axon pathfinding.[6]

In addition, much has been learned recently about the origins of glial cells. One general rule that has emerged from lineage analysis of neurogenesis in vertebrates and invertebrates is that neurons and glia often share common progenitors.[7–13] Much effort has been made to identify such progenitors and the mechanisms controlling their fates.

The intent of this review is to highlight recent research on the fruit fly *Drosophila melanogaster* that has contributed to our understanding of glial cell origins and differentiation. *Drosophila* has proved excellent for the study of the mechanisms underlying neural development. In addition to its sophisticated classical and molecular genetic tools, much has been learned about the lineages, patterns and identities of glia and neurons, and about the projections and pathways taken by axons in the developing CNS and PNS. Neurons and glia are arranged in a stereotypical pattern repeated in each segment. They are easily identified by position, and by a large array of markers. Through systematic screens for mutations that disrupt nervous system development, research in *Drosophila* has been especially fruitful in identifying genes controlling early developmental events in the nervous system.

This review will focus primarily on the origins, specification and differentiation of glial cells in the trunk region (abdomen and thorax) of the *Drosophila* embryo emphasizing what is known about the molecular mechanisms underlying these processes.

Classification of glial cells

Many different glial cell types have been described CNS and PNS of the embryo and first instar larva based on their position and morphology, and the expression of molecular markers (Fig. 1).[10–18] In the CNS, there are approximately 30 glial cells and 350 neurons per abdominal hemisegment (duplicated on either side of the midline). In the abdominal PNS, 8–10 peripheral glial cells ensheath the motor and sensory axons of the major PNS nerve tracks. In addition, sensory neurons of the PNS have specialized support cells.[19]

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Enveloping the outer layer of CNS and peripheral glia is the perineurium, a thin sheath of connective tissue consisting of perineurial cells that secrete an extracellular neural lamella.[20,21] The perineurium has been excluded from the glial cell population by several authors by the criteria that it does not directly contact neurons and is mesodermal in origin.[20,22]

Ito et al.[16] proposed a common nomenclature and classification of glial cells, falling into three major categories by position and morphology (Fig. 1B). First, surface glia form a continuous covering around the central nervous system and peripheral nerves. They comprise of the peripheral glia and exit glia that ensheath the peripheral nerves, the subperineurial glia that enclose the CNS and the channel glia that line the vertical channels running through the ventral nerve cord at segment boundaries. Second, neuropile glia form a sheath around the neuropile, commissures and nerve roots. They include the interface glia that ensheath the longitudinal nerve tracks (also known as the longitudinal glia, LG), the midline glia that separate and ensheath the commissural nerve tracks, and the nerve root glia that ensheath the roots of the intersegmental and segmental nerves. Finally, cortex glia include cell body glia associated with the soma of neurons in the cortex of the ventral nerve cord.

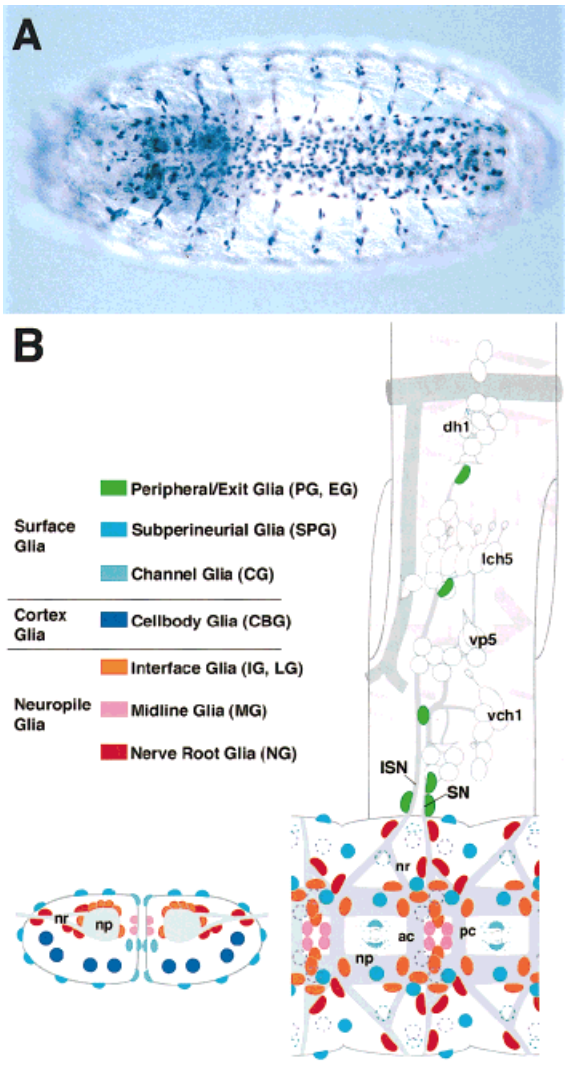

Figure 1. Pattern and classification of glial cells in the central and peripheral nervous system. **A:** Ventral view of a late stage *Drosophila* embryo labeled with an antibody against the Repo protein, a glial-specific homeodomain transcription factor that reveals the pattern of glia nuclei (black) in the CNS and PNS, excluding the midline glia. Anterior is to the left. **B:** Map of glial cells in a mature embryo. Right panel shows a flattened view of an abdominal hemisegment, dorsal midline at the top. Muscles, trachea, sensilla, peripheral nerves and neuropile are shown in gray. Ventral nerve cord is viewed from the dorsal surface at the bottom. Glial cells near the dorsal surface cells are represented by solid colors, which show the approximate position of glial nuclei; dotted circles represent glial cells below the dorsal surface. Lower left panel shows a cross section of the ventral nerve cord. Different classes of glial cells are shown in different colors; key is at upper left. See text for details on glia. Nr, nerve roots; np, neuropile; ac, anterior commissure; pc, posterior commissure; SN, segmental nerve; ISN, intersegmental nerve; vch1, ventral chordotonal organ; vp5, ventral papilla; lch5, lateral chordotonal organ; dh1, dorsal hair sensillum. Panel B is reprinted from Campos-Ortega and Hartenstein[17] p. 262, Fig. 11.12 (used with permission from Springer-Verlag).

Glia arise from neural stem cells

Nearly all glia in the embryo are derived from neural stem cells that originate in the ventral neurogenic ectoderm and the peripheral ectoderm lateral to either side of the midline.[19,23,24] The exceptions are the midline glia, which are derived from the mesectoderm, a structure analogous to the vertebrate floorplate. These mesectoderm-derived glia have specialized functions in organizing the midline of the CNS. Their specification and developmental history are divergent from other glia (see Ref. 25 for a recent review). Therefore, I will limit this review to the development the lateral glia of the PNS and CNS.

In the PNS, neural stem cells called sensory organ precursors (SOPs) delaminate from the ectoderm and undergo a series of cell divisions that generate specific types of neurons, glia, and other support cells.[19] In the CNS, neural stem cells called neuroblasts and neuroglioblasts delaminate from the ectoderm and cycle through a series of 1–12 asymmetric divisions, producing a ganglion mother cell (GMC) with each event (Fig. 2). Each GMC divides to yield either neurons and/or glia.[10–13,26] An exception are progenitors that delaminate from the neuroectoderm and to generate only glial cells, called glioblasts; these include the lateral glioblast that generates the longitudinal glia.[12–14] For

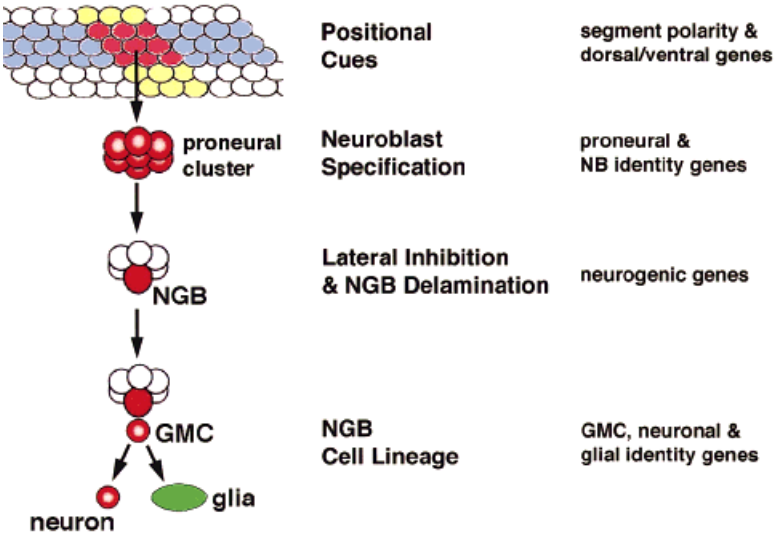

Figure 2. Formation of a typical neuroglioblast lineage. Positional cues (blue and yellow) in the neurogenic ectoderm provide unique positional values to clusters of cells (red). Segment polarity genes provide cues dividing each hemisegment into four rows along the anteroposterior axis. Dorsoventral genes divide the neuroectoderm into three columns along the dorsoventral axis. Clusters of ectodermal cells express proneural genes (red) that confer the potential to become a neuroblast on all cells in the proneural cluster. Specification of neuroblasts requires the combination of proneural and neuroblast (NB) identity genes. One of the cells in the proneural cluster enlarges and delaminates from the neurogenic ectoderm. Those with both neuronal and glial potentials are termed neuroglioblasts (NGB). As the enlarging NGB is formed, it prevents the other cells in the cluster from developing into NGBs by lateral inhibition; these cells lose expression of both proneural and neuroblast identity genes. Neurogenic genes of the Notch signaling pathway mediate lateral inhibition. The NGB divides as a stem cell to produce a series of ganglion mother cells (GMC), which divide into pairs of neurons and/or glia. Unique combinations of identity genes specify the GMCs, neurons and glia. Glial specification requires the expression of the glial identity gene, *glial cells missing*.

simplicity in the next section, I will term all CNS neural stem cells "neuroblasts" (NBs).

Specification of neural stem cells

The genetic mechanisms that control the formation of neuroblasts (Fig. 2) and SOPs have been well studied in *Drosophila*.[19,23,24] The balance of proneural and neurogenic gene activity regulates the initial selection of neuroblasts and SOPs from the ectoderm. Proneural genes are bHLH transcription factors that are expressed in clusters of around 4–7 ectodermal cells where they promote neural stem cell formation. The neurogenic genes of the Notch signaling pathway act to restrict stem cell formation to a single cell within a proneural cluster by lateral inhibition.

The first wave of neuroblasts that delaminate in each abdominal hemisegment are arranged in a "neuroblast array," an orthogonal grid of four rows along the anteroposterior axis, and three columns along the dorsoventral axis.[26] Ultimately, around 30 neuroblasts per abdominal hemisegment are generated within these rows and columns. Each neuroblast is uniquely specified by a combination of nuclear transcription factors and secreted growth factors expressed in striped patterns within each row and column prior to the delamination of the neuroblast from the neuroectoderm.[26]

Apart from this initial ectodermal specification, how each neural stem cell generates stereotypic patterns of multiple cell types in their lineage is not well understood. What is known is that neuroblasts express different combinations of transcription factors depending on the timing of their delamination from the neuroectoderm, and as they generate early-, mid- and late-born GMCs.[27–30] In addition, as GMCs divide from neuroblasts, they inherit the localized determinants Prospero and Numb. Prospero is a homeobox transcription factor that is localized to the basal cortex of the neuroblast, and is asymmetrically distributed to the GMC, where it enters the nucleus and promotes GMC cell fate.[31–34] Numb is a localized determinant that is distributed by similar mechanisms as Prospero, and acts to inhibit Notch signaling, which, after initial neurogenesis, is required to differentiate sibling cell fates derived from GMCs and SOPs.[33,35,36] Thus, each GMC and its daughter cells inherit different combinations of factors that presumably determine the ultimate pattern of neuronal and glial cells. These studies indicate that both intrinsic and extrinsic factors, and the timing of gene

expression determine the differentiation patterns of neuroblast lineages.

Lineage analysis has shown that of the 30-odd neuroblasts per hemisegment, nearly all of those derived from the dorsalmost column at the lateral edge of the neuroectoderm give rise to the majority of the glial cells, including most of the peripheral glia that migrate out and ensheath the peripheral nerves. Two of these are glioblasts giving rise to glial progeny exclusively, while the other 5–7 are neuroglioblasts that give rise to mixed lineages of glia and neurons. These data imply that molecular cues specifying the dorsalmost column of neuroblasts may be important for directing gliogenesis from many neuroblasts.

How all these factors come together to produce different patterns of glial versus neuronal progeny is poorly understood. What is common amongst almost all glial cell progenitors, however, is that the decision of whether to make a glial cell instead of a neuron requires activation of the glial-determining factor, *glial cells missing*.

gcm initiates glial cell differentiation in the neuroectoderm

The *glial cells missing gene (gcm)* is the primary regulator of glial cell determination in *Drosophila*.[37–39] It encodes a novel nuclear factor that is transiently expressed in all glia except for the midline/mesectoderm-derived glia and many of the specialized support cells of PNS sensory neurons. In *gcm* mutant embryos, presumptive glial cells are transformed into neurons. Conversely, when *gcm* is ectopically expressed, presumptive neurons become glia (Fig. 3). Thus, *gcm* functions as a binary genetic switch for glia versus neurons.

Gcm is a DNA-binding protein that recognizes an octamer consensus DNA sequence that is found repeated in the putative regulatory regions of a number of glial-specific genes.[40–43] Two mammalian Gcm homologs have been identified, *Gcm1/GCMa* and *Gcm2/GCMb*.[40,44,45] They share a unique highly conserved N-terminal 153 amino-acid DNA-binding GCM domain.[40,41] There are potent activation domains at the C terminus of Gcm proteins. Taken together, Gcm proteins have all the characteristics of sequence-specific DNA-binding transcriptional activators.

Expression of rat or mouse *Gcm1* in *Drosophila* promotes glial cell differentiation, indicating that they have conserved biochemical properties,[45,46] yet there is little evidence to date that they promote gliogenesis in vertebrates. Mouse *Gcm1* is required for the development of placental labyrinthine trophoblasts,[47] and mouse *Gcm2* is a master regulator of parathyroid gland development.[48] Low levels of *Gcm1* RNA can be detected in the CNS,[45] but its role has not been fully explored, as *Gcm1* mutant mice die early during development due to placental failure, precluding a thorough examination of *Gcm1* function in nervous system development. Experiments with mosaic mice that bypass the placental defect are needed to fully determine whether *Gcm1* has a role in vertebrate gliogenesis.

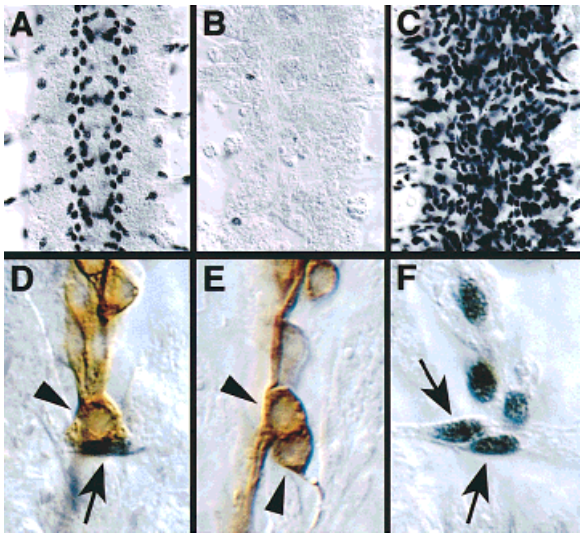

Figure 3. *glial cells missing (gcm)* is a master regulator of glial cell fate in *Drosophila*, and acts as a binary switch between glia and neurons. **A–C:** Glial cells in the embryonic CNS as revealed by anti-Repo staining in four adjacent abdominal segmental neuromeres. A: Wild-type embryo. B: *gcm* loss-of-function results in the absence of glial cell development and loss of Repo expression. C: Panneural expression of *gcm* causes nearly all CNS cells to develop into glial cells expressing Repo. **D–F:** The dorsal bipolar dendrite (dbd) lineage in the peripheral nervous system. In the dbd neuron lineage, the normally Repo-positive glial cell is transformed into a dbd neuron in *gcm* mutants. When *gcm* is ectopically expressed in presumptive neurons, the presumptive dbd neuron is transformed into a Repo-positive glial cell. D: dbd neuron (arrowhead) and glial support cell (arrow) in a wild-type *Drosophila* embryo. E: *gcm* loss-of-function mutant embryo showing glia-to-neuron transformation (arrowheads). (D) *gcm* gain-of-function embryo, in which a transgenic construct drives ectopic *gcm* expression in presumptive neurons transforms neurons into Repo-positive glia (arrows). Data for A–C reprinted from Kim et al.[45] and D–F from Jones et al.[38] (used with permission from Elsevier Science).

These studies indicate that Gcm proteins act as tissue-specific transcriptional regulators of several tissue types. Indeed in *Drosophila, gcm* is also required in hematopoiesis, acting as a factor promoting the development of a specific blood cell type.[49] Thus, *gcm*'s ability to promote glial cell development is context dependent. The additional factors that are required along with *gcm* to promote glial cell development are not known.

The transient expression of *gcm* RNA and protein, and the fact that *gcm* is capable of positively regulating its own expression,[42] suggests that *gcm* expression is tightly regulated. This tight regulation may be especially important

in progenitors that give rise to both neurons and glia. Because *gcm* is transiently expressed, it is likely that it only initiates glial cell differentiation. Downstream genes must accomplish the differentiation and maintenance of glial cell fate. Major questions raised by these studies are the following. How is *gcm* activated in stereotypic patterns in different neural lineages during neurogenesis? How is *gcm* regulated in neural stem cells that generate both glia and neurons? What are the downstream genes that carry out glial cell differentiation?

Timing and mode of *gcm* activation determines the type of glial progenitor

Recent studies have shown that the gliogenic properties of CNS neural progenitors (described earlier as "neuroblasts") differ in the timing and mode of activation of *gcm* expression. Two different types of glial progenitors are easy to distinguish: glioblasts (GBs), which give rise to only glial cells, and neuroglioblasts (NGBs), which divide asymmetrically and produce mixed glial/neuronal lineages (Fig. 4).

Furthermore, neuroglioblasts can be subdivided into at least two types with different modes of division based on the few lineages that have been studied carefully; Udolph et al.[50] termed these "type 1" and "type 2" NGBs (Fig. 4). Type 1 NGBs (as exemplified by NB 6-4 T) bifurcate at the first division to produce a glioblast and a neuroblast, each of which is a precursor with a restricted developmental potential that gives rise to either glial cells or to neurons. Type 2 NGBs (as exemplified by NB 1-1) behave like typical neuroblasts: they generate a series of GMCs that divide once to yield either two sibling neurons or a neuron–glia sibling pair. While this classification into two basic types of NGBs may be oversimplified, as some NGBs, like NB 7-4, exhibit characteristics of both types of division patterns, it serves to illustrate different mechanisms underlying glial patterning.

The timing and mode of expression of *gcm* distinguishes all three types of glial progenitors. In the case of glioblasts (like the lateral glioblast), *gcm* expression precedes their delamination from the neuroectoderm.[38] Patches of 3–5 ectodermal cells transiently expresses *gcm* RNA and protein and, from each patch, a single progenitor maintains *gcm* expression and delaminates from the ectoderm. This *gcm* expression mirrors the pattern of proneural gene activation and subsequent restriction to a single precursor cell mediated by Notch signaling. In *Notch* mutant embryos, an excess of lateral glioblasts are formed at the expense of epidermis. This hypertrophy of glioblasts occurs before they divide to produce their glial progeny,[14] implying that *Notch*-mediated lateral inhibition restricts *gcm* expression to a single progenitor cell in the proneural field. In *gcm* mutant embryos, glioblasts are transformed into neuroblasts.[37] Thus, glioblasts require early and strong expression of *gcm* to prevent them from adopting a neuroblast fate and promoting a glioblast fate.

The expression of *gcm* in neuroglioblast lineages differs depending on whether it is a type 1 or type 2 NGB. In type 1 NGBs (like NB 6-4T), *gcm* RNA and Gcm protein are expressed in the parental neuroglioblast.[51,52] After an asymmetric division, strong *gcm* expression is found in only one of the daughter cells. This *gcm*-positive daughter cell behaves as a glioblast and generates several glial cells, while the other daughter cell behaves like a neuroblast, generating several GMCs that give rise to neurons. These results suggest that *gcm* RNA and protein levels are regulated, and are either

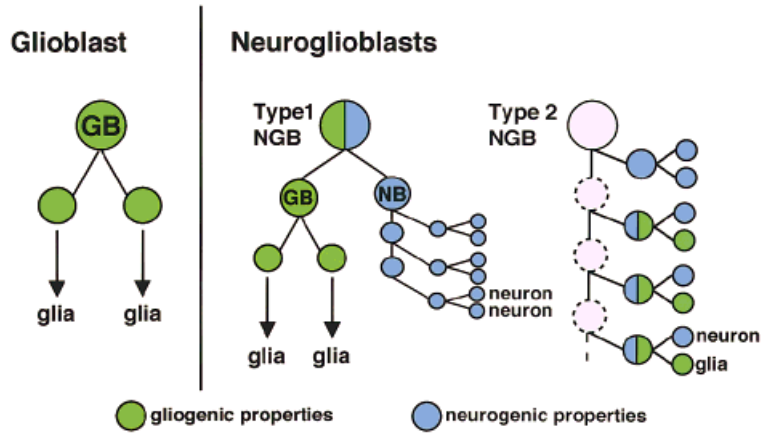

Figure 4. Three modes of glial origin in the CNS. Glia are derived from progenitors called glioblasts (GB) and neuroglioblasts (NGB). GBs generate only glial cells. They express *gcm* prior to delamination from the neuroectoderm. NGBs generate both neurons and glia. At least two types of NGB division patterns have been described. Type 1 NGBs, like NB 6-4T, bifurcate into a GB and neuroblast (NB) at the first division that are precursors with restricted glial or neuronal potential. *gcm* is expressed at low levels and its expression is upregulated in the daughter cell that becomes a GB. This upregulation of *gcm* requires the asymmetric distribution of Prospero from the NGB into the GB daughter cell. Type 2 NGBs, like NB 1-1, generate a series of ganglion mother cells (GMC) that divide once to yield either two sibling neurons or a neuron/glia sibling pair. Glial cell differentiation requires *gcm* expression in the GMC lineage, and is promoted by Notch signaling. Some NGBs exhibit characteristics of both types of division patterns. Since only subsets of NGBs require Prospero and Notch to produce glial cells, it is likely that other modes of *gcm* activation are utilized as well. See text for details. Adapted from Udolph et al.[50] (used with permission from The Company of Biologists Ltd).

preferentially segregated to one daughter cell and/or rapidly degraded in the other. In addition, *gcm* levels are upregulated in the daughter cell that becomes a glioblast.

This upregulation of *gcm* expression in one daughter cell requires the expression of Prospero protein in the cortex of NB 6-4T and its asymmetric segregation into the *gcm* expressing daughter cell.[53,54] In *prospero* mutant embryos, there is a loss of *gcm* expression and a failure to produce glial cells. Mutations in *miranda* and *inscuteable*, genes required for the proper asymmetric distribution of Prospero, also result in a failure to correctly regulate *gcm*. Thus, in type 1 NGBs, the bifurcation between glial and neuronal cell fates uses the same molecular apparatus that generates asymmetry between neuroblasts and GMCs, where the GMC takes on glioblast fate due to the strong *prospero*-dependent expression of *gcm*. Whether Prospero acts directly to maintain or upregulate *gcm* transcription, or acts indirectly by stabilizing *gcm* RNA and protein through intermediate factors is not known.

In type 2 NGBs (like NB 1-1), *gcm* is not expressed in the neuroglioblast, but is expressed later in GMC lineages that generate neuron/glia sibling pairs. Thus, the bifurcation between neuronal and glial cell fates occurs at the division of the GMC. This asymmetric differentiation of neuron/glia sibling GMC daughter cells is under the control of Notch signaling.[50] Notch is activated as the cells differentiate, and one of the daughter cells inherits the asymmetric factor Numb, which acts to inhibit Notch signaling in that cell. In *Notch* mutant embryos, these GMCs produce two neurons, instead of producing a neuron/glia pair. Conversely, in *numb* mutants, two glia are produced. Genetic epistasis demonstrates that *Notch* acts upstream of *gcm*. Thus, Notch signaling promotes glial cell fate through the activation of *gcm*, while Numb acts as its antagonist by inhibiting Notch-mediated *gcm* activation.[50]

As mentioned earlier, some NGBs exhibit characteristics of both type 1 and 2 modes of division within their lineage.[50,53] For instance, NB 7-4 first generates several neuroblasts that give rise to neurons. *gcm* is then activated in the NGB, which then generates several GMCs that are gliogenic. Like NB 6-4T, this *gcm* expression requires Prospero; in *prospero* mutants there is a reduction in glial cells produced from NB 7-4.[53] Subsequently, NB 7-4 goes on to produce GMCs that generate both neurons and glia. Some of these glia are Notch dependent, suggesting that they are the product of neuron/glia sibling pairs.[50] Since only subsets of NGBs require Prospero and Notch to produce glial cells, it is likely that other modes of *gcm* activation are also utilized.

The same system of Notch/Numb control of neuron/glia sibling differentiation is likely to apply to at least one lineage of SOPs in the embryonic PNS. In the dorsal bipolar dendrite (dbd) neuron lineage, a single SOP divides once to generate the dbd neuron and associated glial cell (Fig. 3D). In *numb* mutant embryos, instead of a neuron/glia sibling pair, the SOP generates two glial cells.[55] This phenotype is identical to that of ectopically expressing *gcm* in the presumptive dbd neuron, and the opposite phenotype of *gcm* mutant embryos, where the SOP generates two neurons (Fig. 3E,F). Since Numb acts by antagonizing Notch, it is probable that the activation of *gcm* in this SOP lineage is promoted by Notch signaling.

It is interesting to note that Notch signaling has been described to play an instructive role in gliogenesis in mammals. Activation of Notch promotes the differentiation of many different glial cell types, including Schwann cells, radial glia, Müller glia, and astrocytes.[56–59] One must be cautious in describing Notch as instructive for gliogenesis, however, as in the *Drosophila* its ability to promote gliogenesis is entirely context dependent. In the PNS of the adult wing of *Drosophila*, for example, Notch activation represses glial cell development and *gcm* activation.[60] In this case, the glial cell precursor produced in the wing SOP lineage is not the product of a neuron/glia sibling pair. Thus, Notch signaling during the differentiation of sibling cells results in different outcomes depending on the specific lineage in which it is operating. How these different outcomes are controlled is not understood.

Differentiation of glia downstream of *gcm*

The initiation of gliogenesis is thought to occur through the direct activation of downstream target genes of *gcm*. To date, several genes have been identified that may be immediately downstream of *gcm*, and nearly all of them are transcription factors. The most ubiquitous is the glial-specific homeobox transcription factor *repo*.[61–63] *repo* is the only known gene to be expressed exclusively in all glia (except midline glia and many PNS support cells) (Fig. 1A), and its expression is *gcm*-dependent (Fig. 3B). Transient expression of *gcm* is followed by maintained expression of *repo* (Fig. 3C). Eleven Gcm-binding sites are found in the regulatory region of *repo* suggesting direct regulation by *gcm*.[40] Mutations in *repo* do not affect initial glial determination, but do affect their differentiation, and the expression of late glial cell markers. Defects associated with *repo* mutations include a reduction in the number of glial cells, disrupted fasciculation of axons, and the inhibition of ventral nerve cord condensation. Some of these phenotypes may be attributed to increased neuronal cell death, as neuronal apoptosis has been observed in optic lobes of *repo* mutant adults.[64] Increased neuronal apoptosis is also observed in *gcm* mutant embryos that lack glia.[38] Thus *repo* appears to control certain aspects of terminal glial differentiation, including glial-dependent neuronal survival.

Other transcription factors are expressed in different subsets of *gcm*-dependent glia in the embryonic CNS and may also be direct targets of *gcm*. Among these are *pointed* and *tramtrack*. Like *repo*, mutations in these genes do not prevent the initiation of glial cell development, but have terminal glial differentiation defects. Unlike *repo* these genes are expressed in other tissues as well as glia.

The P1 form of the ETS-domain transcription factor encoded by the *pointed* gene is expressed in most CNS glia, including the longitudinal glia.[65] In *pointedP1* mutant embryos, longitudinal glia fail to differentiate properly. While mutant glia associate with the longitudinal axon tracks, they subsequently fail to flatten and ensheath them. In addition, a subset of neurons that contact the longitudinal axons fail to express a specific neuronal antigen, 22C10. Ectopic expression of *pointedP1* in the CNS leads to additional cells with glial characteristics as well as the induction of 22C10 antigen in neurons contacting those cells. Thus *pointedP1* is able to control certain characteristics of neighboring neurons through a non-cell-autonomous mechanism.

The p69 form of the zinc-finger transcriptional repressor encoded by *tramtrack gene* (*ttkp69*) is expressed in all CNS glia, as well as many other tissues, including muscles and epidermis.[66] *ttkp69* acts as a repressor of neuronal differentiation. In *ttkp69* mutants, neuronal antigens are ectopically expressed in CNS glia, as well as in muscles and in epidermis. Glial cell development is initiated in *ttkp69* mutants, but mutant glia express neuronal antigens, and exhibit aberrant behavior.

Taken together these data support a model whereby *gcm* promotes glial cell differentiation by turning on glial-specific genes like *repo* and *pointed*, while simultaneously repressing neuronal development by activating the transcriptional repressor *ttkp69* (Fig. 5) .[66] The specification of various glial subtype identities is controlled by unknown factors.

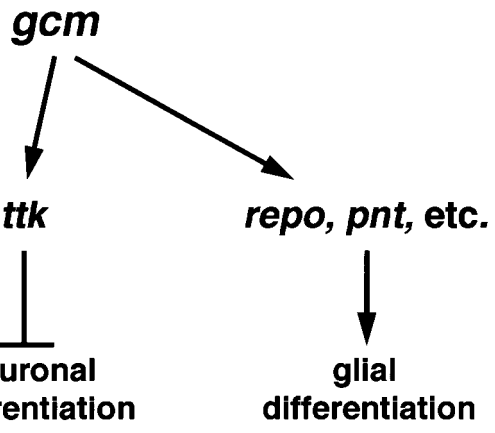

Figure 5. *gcm* initiates glial cell development through the simultaneous activation of glial differentiation and repression of neuronal differentiation. Glial cell differentiation is promoted by the factors *repo*, *pointed* (*pnt*) and others (see text). The repression of neuronal differentiation is mediated by the p69 form of the zinc-finger transcriptional repressor encoded by the *tramtrack* gene (*ttk*).

Terminal differentiation of glia, glial ensheathment and the blood–nerve barrier

In addition to these transcription factors, several other genes have been identified that are required for the terminal differentiation of glia. In the CNS, longitudinal glia both express and require the activity the FGF receptor encoded by the *heartless* gene.[67] In *heartless* mutant embryos, longitudinal glial cells do not flatten and fail to ensheath the longitudinal nerve tracks. It is possible that an FGF-like ligand is presented by axons to promote glial morphogenesis and ensheathment, though such a ligand has not been identified. Complementary studies in grasshopper embryos strongly support this hypothesis.[68] Ectopic Heartless/FGF receptor signaling in grasshopper by application of vertebrate FGF2 causes glial cells to round up in a manner that is similar to *heartless* mutants, suggesting that localized Heartless/FGF receptor signaling is required for proper longitudinal glia morphogenesis.

A similar phenotype is observed in mutants of the *loco* gene, which encodes a family member of the Regulators of G-Protein Signaling (RGS) proteins.[69] *loco* is expressed in longitudinal glia, as well as subperineurial glia, and its expression is directly regulated by both *gcm* and *pointed*.[43] Like *heartless* and *pointed*, *loco* mutant embryos show a failure of longitudinal glia to properly ensheath the longitudinal axon tracks. They also show defects in subperineurial glia (SPGs); SPGs do not form tight contacts with each other, failing to complete the formation of a barrier sheath around the CNS. Whether Heartless/FGF receptor signaling is mediated by a G-protein-coupled mechanism involving Loco is not known, but remains a possibility.

In addition to *loco*, several other genes have been identified that are critical for the proper ensheathment of nerves and the formation of the blood–nerve barrier. *neurexin IV* (*nrx IV*) encodes a transmembrane protein that is an integral component of pleated septate junctions (pSJs) .[70] pSJs are found at the junctions between the SPGs that form a continuous sheath around the ventral nerve cord and in the peripheral and exit glia that ensheath the peripheral nerves (Fig. 6).[21] In *nrx IV* mutants, the pSJs are absent, and the blood–nerve barrier is disrupted, exposing axons to the high K^+ environment of the surrounding hemolymph. This permeability of K^+ results in failure of action potentials and paralysis. Thus, the proper formation of pSJs is required for a selective-diffusion barrier at glial junctions. Interestingly, a vertebrate homolog of Neurexin IV, Caspr/Paranodin, is localized to septate-like junctions at the paranodal region of the nodes of Ranvier of Schwann cells, where it plays an essential role in barrier formation and linking myelenating glial cells to axons.[71,72]

In the PNS, the formation of a complete glial sheath around peripheral nerves also requires the *gliotactin* gene.[73] Gliotactin is expressed in the peripheral glia and a subset of midline glia. In *gliotactin* mutants, ensheathment of the

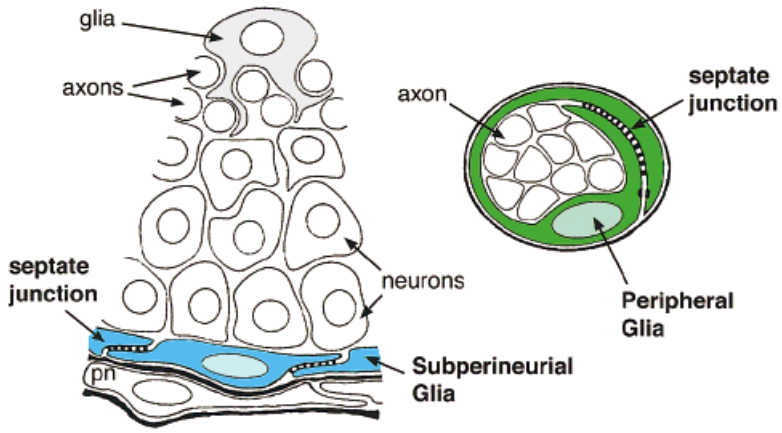

Figure 6. Blood–nerve barrier. Schematic representation of cross sections of the nervous system of late-stage embryos, showing the surface glia ensheathing the ventral nerve cord (left) and peripheral nerves (right) that form the blood–nerve barrier. Pleated septate junctions, found between the subperineurial glia (blue) that form a continuous covering around the CNS, and in peripheral glia (green) that ensheath the peripheral nerves, are required to form a selective-diffusion barrier at glial junctions. Disruption of this barrier by mutations in *neurexin IV* (in the CNS and PNS) and *gliotactin* (in the PNS) exposes the nervous system to the high K^+ environment of the surrounding hemolymph, resulting in action potential failure. Pn, perineurium. Adapted from Tepass and Hartenstein[21] (used with permission from Academic Press).

peripheral nerves is initiated, but not completed. At the points where the ensheathing glial cell should form a tight contact with itself and form pSJs, *gliotactin* mutant glial wrappings show gaps and openings. Because of these gaps, there is a failure of the blood–nerve barrier. Motorneurons fail to propagate action potentials in high K^+ hemolymph and the embryos are paralyzed.

Gliotactin is homologous to vertebrate Neuroligins, a family of neural transmembrane proteins shown to form heterophilic intercellular junctions with β-neurexins.[74–76] Neuroligins were initially thought to be expressed in neurons. However, recent studies demonstrate that some Neuroligins are also expressed in glial cells and that one, Neuroligin 3, is expressed in a wide range of glial cells, including Schwann cells, retinal astrocytes, spinal cord astrocytes, and olfactory ensheathing glia.[76] *Drosophila* peripheral glia share many characteristics with vertebrate nonmyelinating Schwann cells and olfactory ensheathing glia, and the presence of conserved molecules implicates homologous mechanisms underlying their development.

Given the presence of Neurexin IV at the glial junction, it is possible that there is an interaction between Gliotactin and Neurexin IV. To date, however there is no evidence for heterophilic binding of the two molecules,[70] nor do either show homophilic interactions.[70,73] Thus the ligands for these molecules have yet to be identified. It is tempting, however, to speculate that complexes of Neuroligin–Neurexin molecules are involved in completing the formation of the blood–nerve barrier and also in interactions of nerve and glia, since an additional Neuroligin and other Neurexins and have been identified in *Drosophila*. As Gliotactin is expressed in peripheral glia, but not in SPGs, other mechanisms must be in place to promote the formation of the blood–nerve barrier in SPGs.

In addition to forming a blood–nerve barrier sheath encompassing the peripheral nerves, mature peripheral glia in *Drosophila* elaborate processes that ensheath individual axons and axon fascicles within the peripheral nerves. The complete development of these glial elaborations has recently been shown to require the activity of the Fray protein expressed in peripheral glia.[77] In *fray* mutants, glial processes elaborate and extend into the nerve, begin to surround axons and axon fascicles, but fail to form complete glial wraps encircling axons. This phenotype indicates that Fray is involved in a terminal event of axon ensheathment. Fray belongs to a novel family of serine/threonine kinases related to vertebrate PAK kinases. Most interesting, a Fray homolog, the rat PASK protein, can rescue the *Drosophila fray* mutant phenotype when expressed in glia, indicating functional conservation. Whether PASK is involved in mammalian glial development is not known, but these results demonstrate that a conserved signaling pathway is shared in *Drosophila* and vertebrates, and its role in glial ensheathment needs to be explored.

Glial–neuronal interactions: migration of glia, axon guidance and glial/neuronal survival

In the developing nervous system of *Drosophila*, there are many reciprocal interactions between neurons and glia governing their differentiation. Glia go through extensive migrations in close contact with neurons; reciprocally, the growth cones of axons use glia as intermediate substrata as they extend towards their targets. For example, as the growth cones of motor axons pioneer the peripheral nerves, they first contact glia and then grow past them. Subsequently, the peripheral glia migrate along the motor axons from the lateral edge of the CNS and ultimately ensheath the peripheral nerves.[18]

In the CNS, longitudinal glia, born from the lateral glioblast, move medially to contact axons that pioneer the longitudinal axon fascicles. They then migrate together with growth cone extensions and serve as attractive cues to fasciculating axons. In the absence of glia, caused either by *gcm* mutation or by targeted ablation, pathfinding in the longitudinal tracks is disrupted.[37,78] While pioneering axon tracks will form in the absence of glia, there is a high frequency of errors that, when compounded, lead to fasciculation defects. These findings suggest that longitudinal axons are guided by multiple cues, some on glia, which acting together, ensure the high fidelity of axon pathways observed in normal development. The molecular cues and mechanisms that govern the migration of glia and their interaction with neurons during longitudinal axon pathfinding have yet to be identified.

Mutual interactions also govern the survival of both glia and neurons. In *gcm* mutant embryos that lack glia, there is an increase in neuronal apoptosis.[38,79] Similarly, *repo* mutants affecting the terminal differentiation of glia also show increased neuronal apoptosis.[61–64] Targeted ablation of glia and neurons have revealed mutually dependent interactions for their survival.[79] Ablation of longitudinal glia induces neuronal cell death in the longitudinal fascicles. Interestingly, targeted ablation of longitudinal glia only affects subsets of neurons; the pioneer neurons that initiate the longitudinal fascicles do not require glia for survival, whereas the later extending follower neurons do. Ablation of the pioneer neurons has a reciprocal effect on the survival of longitudinal glia. In the absence of early forming neurons, glia undergo apoptosis. Thus, in the case of the longitudinal glia, initial contact with neurons is required for their survival. Subsequently, later-born neurons require glia for their survival. Such interactions may regulate proper glial and neuronal numbers, and form a possible link between trophic support and axon guidance. The similarities between these trophic mechanisms in *Drosophila* and cell survival systems in vertebrate nervous systems is striking, yet the molecular mechanisms controlling trophic support in the nervous system of *Drosophila* are not known.

Conclusions

Much has been learned recently about the origins, differentiation and functions of glial cells during the embryonic development of *Drosophila*, yet this analysis is still in its infancy. We now know that glia arise from neural stem cells using the same apparatus that generates neurons, and that neuronal and glial cell fates bifurcate through the activity of the transcription factor *gcm*. As in vertebrates, Notch signaling plays a critical role in the terminal differentiation of many glia. The propagation of differentiation is carried out through several transcription factors, by promoting glial cell differentiation and preventing neuronal differentiation. Factors that govern the development of specific glial subtypes remain to be identified.

We have discovered a number of genes affecting the terminal differentiation of glia, primarily involved in their proper morphogenesis, including axonal ensheathment and the formation of the blood–nerve barrier. Many of these genes have conserved counterparts in vertebrates, indicating that at least some aspects of glial morphogenesis are conserved.

There is growing evidence that glia are used as intermediate cues for axon guidance, that glia go through extensive migrations using neurons as substrates, and that there is an intimate reciprocal trophic relationship between neurons and glia governing their survival. The mechanisms controlling these glial–neuronal interactions remain largely undiscovered discovered.

Glia have been proposed to modulate the function of neurons, including the efficacy of synaptic activity. The strengths of *Drosophila* have always been the powerful classical and molecular genetic methods that can be brought to bear on biological processes. The emerging evidence of conserved mechanisms underlying glial morphogenesis in *Drosophila* and vertebrates makes *Drosophila* a model system with great potential to contribute to our understanding of glial cell biology. Future genetic analysis of glial cell differentiation and functions in *Drosophila* will enable the unraveling of complex processes underlying the interaction of neurons and glia.

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References

1. Laming PR, Kimelberg H, Robinson S, Salm A, Hawrylak N, Muller C, Roots B, Ng K. Neuronal-glial interactions and behaviour. Neurosci Biobehav Rev 2000;24:295–340.
2. Pfrieger FW, Barres BA. Synaptic efficacy enhanced by glial cells in vitro. Science 1997;277;1684–1687.
3. Bergles DE, Jahr CE. Glial contribution to glutamate uptake at Schaffer collateral-commissural synapses in the hippocampus. J Neurosci 1998;18:7709–7716.
4. Ullian EM, Sapperstein SK, Christopherson KS, Barres BA. Control of synapse number by glia. Science 2001;291:657–661.
5. Haydon PG. Glia: listening and talking to the synapse. Nat Rev Neurosci. 2001;2:185–193.
6. Lemke G. Glial control of neuronal development. Annu Rev Neurosci 2001;24:87–105.
7. Turner DL, Cepko CL. A common progenitor for neurons and glia persists in rat retina late in development. Nature 1987;328:131–136.
8. Stemple DL, Anderson DJ. Isolation of a stem cell for neurons and glia from the mammalian neural crest. Cell 1992;71:973–985.
9. Williams BP, Price J. Evidence for multiple precursor cell types in the embryonic rat cerebral cortex. Neuron 1995;14:1181-1188.
10. Udolph G, Prokop A, Bossing T, Technau GM. A common precursor for glia and neurons in the embryonic CNS of *Drosophila* gives rise to segment-specific lineage variants. Development 1993;118:765–775.
11. Bossing T, Udolph G, Doe CQ, Technau GM. The embryonic central nervous system lineages of *Drosophila melanogaster*. I. Neuroblast lineages derived from the ventral half of the neuroectoderm. Dev Biol 1996;179:41–64.
12. Schmidt H, Rickert C, Bossing T, Vef O, Urban J, Technau GM. The embryonic central nervous system lineages of *Drosophila melanogaster*. II. Neuroblast lineages derived from the dorsal part of the neuroectoderm. Dev Biol 1997;189:186–204.

13. Schmid A, Chiba A, Doe CQ. Clonal analysis of *Drosophila* embryonic neuroblasts: neural cell types, axon projections and muscle targets. Development 1999;26:4653–4689.
14. Jacobs JR, Hiromi Y, Patel NH, Goodman CS. Lineage, migration, and morphogenesis of longitudinal glia in the Drosophila CNS as revealed by a molecular lineage marker. Neuron 1989;2:1625–1631.
15. Klämbt C, Goodman CS. The diversity and pattern of glia during axon pathway formation in the Drosophila embryo. Glia 1991;4:205–213.
16. Ito K, Urban J, Technau GM. Distribution, classification and development of Drosophila glial cells during late embryogenesis. Roux Arch Dev Biol 1995; 204:284–307.
17. Campos-Ortega JA, Hartenstein V. The Embryonic Development of Drosophila melanogaster. Berlin Heidelberg: Springer-Verlag. 1997.
18. Sepp KJ, Schulte J, Auld VJ. Developmental dynamics of peripheral glia in *Drosophila melanogaster*. Glia 2000;30:122–133.
19. Jan YN, Jan LY. The peripheral nervous system. In Bate M, Martinez-Arias A, ed; The Development of Drosophila melanogaster. New York: Cold Spring Harbor Press. 1993. p 1207–1244.
20. Edwards JS, Swales LS, Bate M. The differentiation between neuroglia and connective tissue sheath in insect ganglia revisited: the neural lamella and perineurial sheath cells are absent in a mesodermless mutant of *Drosophila*. J Comp Neurol 1993;333:301–308.
21. Tepass U, Hartenstein V. The development of cellular junctions in the *Drosophila* embryo. Dev Biol 1994;161:563–596.
22. Hoyle G. Glial cells of an insect ganglion. J Comp Neurol 1986;246:85–103.
23. Campos-Ortega JA. Early neurogenesis in *Drosophila melanogaster*. In Bate M, Martinez-Arias A, ed; The Development of Drosophila melanogaster. New York: Cold Spring Harbor Press. 1993. p 1091–1129.
24. Goodman CS, Doe CQ. Embryonic development of the *Drosophila* central nervous system. In Bate M, Martinez-Arias A, ed; The Development of Drosophila melanogaster. New York: Cold Spring Harbor Press. 1993. p 1131–1206.
25. Jacobs JR. The midline glia of *Drosophila*: a molecular genetic model for the developmental functions of glia. Prog Neurobiol 2000 Dec;62:475–508.
26. Skeath JB. At the nexus between pattern formation and cell-type specification: the generation of individual neuroblast fates in the *Drosophila* embryonic central nervous system. Bioessays 1999;21: 922–931.
27. Broadus J, Skeath JB, Spana EP, Bossing T, Technau G, Doe CQ. New neuroblast markers and the origin of the aCC/pCC neurons in the *Drosophila* central nervous system. Mech Dev 1995;53:393–402.
28. Cui X, Doe CQ. *ming* is expressed in neuroblast sublineages and regulates gene expression in the *Drosophila* central nervous system. Development 1992;116:943–952.
29. Kambadur R, Koizumi K, Stivers C, Nagle J, Poole SJ, Odenwald WF. Regulation of POU genes by *castor* and *hunchback* establishes layered compartments in the *Drosophila* CNS. Genes Dev 1998;12:246–260.
30. Brody T, Odenwald WF. Programmed transformations in neuroblast gene expression during *Drosophila* CNS lineage development. Dev Biol 2000; 226:34–44.
31. Doe CQ, Chu-LaGraff Q, Wright DM, Scott MP. The *prospero* gene specifies cell fates in the *Drosophila* central nervous system. Cell 1991; 65:451–464.
32. Vaessin H, Grell E, Wolff E, Bier E, Jan LY, Jan YN. *prospero* is expressed in neuronal precursors and encodes a nuclear protein that is involved in the control of axonal outgrowth in *Drosophila*. Cell 1991;67: 941–953.
33. Knoblich JA, Jan LY, Jan YN. Asymmetric segregation of Numb and Prospero during cell division. Nature 1995;377:624–627.
34. Hirata J, Nakagoshi H, Nabeshima Y, Matsuzaki F. Asymmetric segregation of the homeodomain protein Prospero during *Drosophila* development. Nature 1995;377:627–630.
35. Uemura T, Shepherd S, Ackerman L, Jan LY, Jan YN. *numb*, a gene required in determination of cell fate during sensory organ formation in *Drosophila* embryos. Cell 1989;58:349–360.
36. Rhyu MS, Jan LY, Jan YN. Asymmetric distribution of Numb protein during division of the sensory organ precursor cell confers distinct fates to daughter cells. Cell 1994;76:477–491.
37. Hosoya T, Takizawa K, Nitta K, Hotta Y. *glial cells missing*: a binary switch between neuronal and glial determination in Drosophila. Cell 1995;82:1025–1036.
38. Jones BW, Fetter RJ, Tear G, Goodman CS. *glial cells missing*: a genetic switch that controls glial versus neuronal fate. Cell 1995;82:1013–1023.
39. Vincent S, Vonesch J-L, Giangrande A. *glide* directs glial fate commitment and cell fate switch between neurones and glia. Development 1996;122:131–139.
40. Akiyama Y, Hosoya. T, Poole AM, Hotta Y. The gcm-motif: a novel DNA-binding motif conserved in *Drosophila* and mammals. Proc Natl Acad Sci USA 1996;93:14912–14916.
41. Schreiber J, Sock E, Wegner M. The regulator of early gliogenesis *glial cells missing* is a transcription factor with a novel type of DNA-binding domain. Proc Natn Acad Sci USA 1997;94:4739–4744.
42. Miller AA, Bernardoni R, Giangrande A. Positive autoregulation of glial promoting factor *glide/gcm*. EMBO J 1998;17:6316–6326.
43. Granderath S, Bunse I, Klämbt C. *gcm* and *pointed* synergistically control glial transcription of the *Drosophila* gene *loco*. Mech Dev 2000; 91:197–208.
44. Altshuller Y, Copeland NG, Gilbert DJ, Jenkins NA, Frohman MA. Gcm1, a mammalian homolog of Drosophila glial cells missing. FEBS Lett 1996; 393:201–204.
45. Kim J, Jones BW, Zock C, Chen Z, Wang, H, Goodman CS, Anderson DJ. Isolation and characterization of mammalian homologs of the *Drosophila* gene, *glial cells missing*. Proc Natl Acad Sci USA 1998;95: 12364–12369.
46. Reifegerste R, Schreiber J, Gulland S, Ludemann A, Wegner M. mGCMa is a murine transcription factor that overrides cell fate decisions in *Drosophila*. Mech Dev 1999;82:141–150.
47. Schreiber J, Riethmacher-Sonnenberg E, Riethmacher D, Tuerk EE, Enderich J, Bosl MR, Wegner M. Placental failure in mice lacking the mammalian homolog of *glial cells missing*, GCMa. Mol Cell Biol 2000;20: 2466–2474.
48. Gunther T, Chen ZF, Kim J, Priemel M, Rueger JM, Amling M, Moseley JM, Martin TJ, Anderson DJ, Karsenty G. Genetic ablation of parathyroid glands reveals another source of parathyroid hormone. Nature 2000;406: 199–203.
49. Lebestky T, Chang T, Hartenstein V, Banerjee U. Specification of *Drosophila* hematopoietic lineage by conserved transcription factors. Science 2000;288:146–149.
50. Udolph G, Rath P, Chia W. A requirement for *Notch* in the genesis of a subset of glial cells in the *Drosophila* embryonic central nervous system which arise through asymmetric divisions. Development 2001;128:1457–1466.
51. Akiyama-Oda Y, Hosoya T, Hotta Y. Asymmetric cell division of thoracic neuroblast 6-4 to bifurcate glial and neuronal lineage in *Drosophila*. Development 1999;126:1967–1974.
52. Bernardoni R, Kammerer M, Vonesch JL, Giangrande A. Gliogenesis depends on *glide/gcm* through asymmetric division of neuroglioblasts. Dev Biol 1999;216:265–275.
53. Akiyama-Oda Y, Hotta Y, Tsukita S, Oda H. Mechanism of glia-neuron cell-fate switch in the *Drosophila* thoracic neuroblast 6-4 lineage. Development 2000;127:3513–3522.
54. Ragone G, Bernardoni R, Giangrande A. A novel mode of asymmetric division identifies the fly neuroglioblast 6-4T. Dev Biol 2001;235:74–85.
55. Brewster R, Bodmer R. Origin and specification of type II sensory neurons in *Drosophila*. Development 1995;121:2923-2936.
56. Furukawa T, Mukherjee S, Bao ZZ, Morrow EM, Cepko CL. *rax, Hes1*, and *notch1* promote the formation of Muller glia by postnatal retinal progenitor cells. Neuron 2000;26:383–394.
57. Gaiano N, Nye JS, Fishell G. Radial glial identity is promoted by Notch1 signaling in the murine forebrain. Neuron 2000;26:395–404.
58. Morrison SJ, Perez SE, Qiao Z, Verdi JM, Hicks C, Weinmaster G, Anderson DJ. Transient Notch activation initiates an irreversible switch from neurogenesis to gliogenesis by neural crest stem cells. Cell 2000; 101:499–510.
59. Tanigaki K, Nogaki F, Takahashi J, Tashiro K, Kurooka H, Honjo T. Notch1 and Notch3 instructively restrict bFGF-responsive multipotent neural progenitor cells to an astroglial fate. Neuron 2001;29:45–55.

60. Van De Bor V, Giangrande A. Notch signaling represses the glial fate in fly PNS. Development 2001;128:1381–1390.
61. Xiong W, Okano H, Patel N, Blendy J, Montell C. *repo* encodes a glial-specific homeo domain protein required in the *Drosophila* nervous system. Genes Dev 1994;8:981–994.
62. Campbell G, Göring H, Lin T, Spana E, Andersson S, Doe CQ, Tomlinson A. RK2, a glial-specific homeodomain protein required for embryonic nerve cord condensation and viability in *Drosophila*. Development 1994; 120:2957–2966.
63. Halter DA, Urban J, Rickert C, Ner S, Ito K, Travers A, Technau G. The homeobox gene *repo* is required for the differentiation and maintenance of glia function in the embryonic nervous system of *Drosophila melanogaster*. Development 1995;121:317–332.
64. Xiong WC, Montell C. Defective glia induce neuronal apoptosis in the *repo* visual system of *Drosophila*. Neuron 1995;14:581–590.
65. Klaes A, Menne T, Stollewerk A, Scholz H, Klämbt C. The Ets transcription factors encoded by the *Drosophila* gene *pointed* direct glial cell differentiation in the embryonic CNS. Cell 1994;78:149–160.
66. Giesen K, Hummel T, Stollewerk A, Harrison S, Travers A, Klämbt C. Glial development in the *Drosophila* CNS requires concomitant activation of glial and repression of neuronal differentiation genes. Development 1997;124:2307–2316.
67. Shishido E, Ono N, Kojima T, Saigo K. Requirements of DFR1/Heartless, a mesoderm-specific *Drosophila* FGF-receptor, for the formation of heart, visceral and somatic muscles, and ensheathing of longitudinal axon tracts in CNS. Development 1997;124:2119–2128.
68. Condron B. Spatially discrete FGF-mediated signalling directs glial morphogenesis. Development 1999;126:4635–4641.
69. Granderath S, Stollewerk A, Greig S, Goodman CS, O'Kane CJ, Klämbt C. *loco* encodes an RGS protein required for *Drosophila* glial differentiation. Development 1999;126:1781–1791.
70. Baumgartner S, Littleton JT, Broadie K, Bhat MA, Harbecke R, Lengyel JA, Chiquet-Ehrismann R, Prokop A, Bellen HJ. A Drosophila Neurexin is required for septate junction and blood nerve barrier formation and function. Cell 1996;87:1059–1068.
71. Bellen HJ, Lu Y, Beckstead R, Bhat MA. Neurexin IV, caspr and paranodin–novel members of the neurexin family: encounters of axons and glia. Trends Neurosci 1998;21:444–449.
72. Bhat MA, Rios JC, Lu Y, Garcia-Fresco GP, Ching W, St. Martin M, Li J, Einheber S, Chesler M, Rosenbluth J, Salzer JL, Bellen HJ. Axon-glia interactions and the domain organization of myelinated axons requires Neurexin IV/Caspr/Paranodin. Neuron 2001;30:369–383.
73. Auld VJ, Fetter RD, Broadie K, Goodman CS. Gliotactin, a novel transmembrane protein on peripheral glia, is required to form the blood-nerve barrier in Drosophila. Cell 1995;81:757–767.
74. Ichtchenko K, Hata Y, Nguyen T, Ullrich B, Missler M, Moomaw C, Sudhof TC. Neuroligin 1: a splice site-specific ligand for beta-neurexins. Cell 1995;81:435–443.
75. Ichtchenko K, Nguyen T, Sudhof TC. Structures, alternative splicing, and Neurexin binding of multiple Neuroligins. J Biol Chem 1996;271:2676–2682.
76. Gilbert M, Smith J, Roscams A, Auld VJ. Neuroligin 3 is a vertebrate gliotactin expressed in the olfactory ensheathing glia, a growth-promoting class of macroglia. Glia 2001;34:151–164.
77. Leiserson WM, Harkins EW, Keshishian H. Fray, a *Drosophila* serine/threonine kinase homologous to mammalian PASK, is required for axonal ensheathment. Neuron 2000;28:793–806.
78. Hidalgo A, Booth GE. Glia dictate pioneer axon trajectories in the *Drosophila* embryonic CNS. Development 2000;127:393–402.
79. Booth GE, Kinrade EFV, Hidalgo A. Glia maintain follower neuron survival during *Drosophila* CNS development. Development 2000;127:237–244.