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glial cells missing: A Genetic Switch That Controls Glial versus Neuronal Fate

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Summary

The *glial cells missing* (*gcm*) gene in Drosophila encodes a novel nuclear protein that is transiently expressed early in the development of nearly all glia. In loss-of-function *gcm* mutant alleles, nearly all glia fail to differentiate, and, where we can follow them in the PNS, are transformed into neurons. In gain-of-function *gcm* conditions using transgenic constructs that drive ectopic gcm expression, many presumptive neurons are transformed into glia. Thus, *gcm* appears to function as a binary genetic switch for glia versus neurons. In the presence of gcm protein, presumptive neurons become glia, while in its absence, presumptive glia become neurons.

Introduction

Neurons play the leading roles in processing and transmitting information in the nervous system, while glia provide the supporting cast, wrapping and insulating neurons, providing them with nourishment, regulating them with cytokines and growth factors, maintaining their ionic homeostasis, and helping to establish and maintain the blood–brain and blood–nerve barriers. Although neurons and glia are such different cell types, they often arise from common lineages in both the central nervous system (CNS) and the peripheral nervous system (PNS). What makes glia different from neurons? Does a genetic switch (or switches) exist that can turn presumptive neurons into glia or presumptive glia into neurons? Do different CNS and PNS lineages use different genes to direct some of their offspring into the glial fate, or is a common switch called forth in a variety of different lineages?

In Drosophila, much is known about the genes that control the development of the specific patterns and identities of neurons (e.g., Jan and Jan, 1993; Campos-Ortega, 1993; Goodman and Doe, 1993). Nevertheless, little is known about the genes that control the decision of neuronal versus glial cell fate. In the PNS, sensory organ precursors (SOPs) divide several times to generate specific neurons, glia, and support cells (e.g., Bodmer et al., 1989; Jan and Jan, 1993). Two PNS lineages that figure prominently in this study are the SOP lineages giving rise to the lateral chordotonal (CH) organs, in which each SOP undergoes several divisions to generate a neuron and three support cells (a ligament cell, a sheath or scolopale cell, and a cap cell), and the SOP lineage giving rise to the dorsal bipolar dendrite (BD) neuron, in which the SOP appears to divide only once to generate the BD neuron and its glial support cell. Other peripheral glia wrap and insulate the axons in the peripheral nerves.

In the Drosophila CNS, most neurons arise from ganglion mother cells born by the asymmetric division of neuroblasts (NBs), although a few neurons arise from midline mesectodermal progenitors (Goodman and Doe, 1993). Glia have similar origins. The longitudinal glia arise from a lateral NB-like cell, the longitudinal glioblast (Jacobs et al., 1989). Other glia arise from NBs that generate mixed lineages (Udolph et al., 1993). Midline glia arise from midline mesectodermal progenitors (Klämbt et al., 1991; Bossing and Technau, 1994). In the grasshopper, midline glia arise from the median NB that generates both neurons and glia (Condron and Zinn, 1994). The switch in the median NB generation of these two cell types is controlled by the activity of both *engrailed* and protein kinase A (Condron et al., 1994; Condron and Zinn, 1995).

Three different nuclear proteins have been identified that are expressed in different subsets of glia in the developing embryo. The most ubiquitous glial marker is the homeodomain protein encoded by the *repo* gene. Repo (also called RK2) is expressed in nearly all PNS and CNS glia except midline glia (Xiong et al., 1994; Campbell et al., 1994; Halter et al., 1995). Longitudinal glia also express the homeodomain protein encoded by the *prospero* (*pros*) gene (Doe et al., 1991) and the P1 form of the Ets domain transcription factor encoded by the *pointed* (*pnt*) gene. Midline glia neither express repo nor pros, but they do express the P2 form of the pnt protein (Klämbt, 1993; Klaes et al., 1994). Mutations in these three genes do not prevent glia from developing, but do lead to defects in aspects of their differentiation.

In this paper, we describe the cloning, molecular characterization, and genetic analysis of the *glial cells missing* (*gcm*) gene in Drosophila. *gcm* encodes a novel nuclear protein that is transiently expressed in nearly all PNS and CNS glia (except midline glia). In loss-of-function *gcm* mutants, nearly all CNS and PNS glia (except midline glia) fail to differentiate. In the CH organ lineage in the PNS, the normally repo-positive ligament cells are transformed into additional CH neurons. In the BD neuron lineage, the normally repo-positive glial cell is transformed into an additional neuron. In gain-of-function *gcm* conditions using transgenic constructs that drive ectopic *gcm* expression in presumptive neurons throughout the CNS and PNS, many presumptive neurons are transformed into glia. We observe numerous extra repo-positive glia in the CNS. In the BD lineage in the PNS, the presumptive neuron is transformed into another repo-positive glial cell. Because glia are missing from the outset in *gcm* mutant embryos,

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this mutant provides the opportunity to assess the role of glia in establishing axon pathways and in neuronal survival and differentiation.

Thus, *gcm* functions as a binary genetic switch for glia versus neurons. The same gene has been cloned and characterized by Y. Hotta and his colleagues, and in a companion paper (Hosoya et al., 1995 [this issue of *Cell*]), they present results that support these same conclusions. In addition, in a screen for mutations affecting the PNS, P element 84/12 was identified as being inserted in *gcm* (Kania et al., 1995).

Results

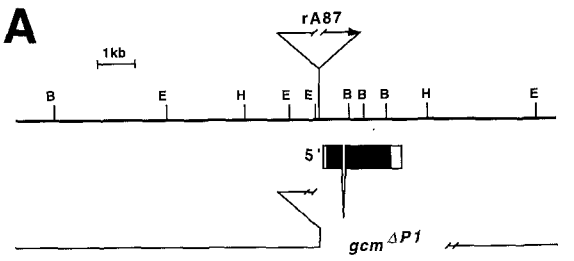
Identification and Cloning of the *gcm* Gene

Our studies on *gcm* began with the ethyl methanesulfonate–induced allele gcm^{1308} isolated in a systematic screen for mutations that disrupt the organization of CNS axon pathways in Drosophila (Seeger et al., 1993). We observed that late stage *gcm* mutant embryos have a CNS with abnormal and occasionally missing longitudinal axon pathways; subsequent analysis revealed that they also have a reduced number of glial cells. gcm^{1308} was mapped to 30B9-12 on the second chromosome by its failure to complement *Df(2L)200* (Lane and Kalderon, 1993). A second allele ($gcm^{N7\text{-}4}$) was discovered in a collection of diepoxybutane-induced mutations that map to the 30A–30C region (Uemura et al., 1989). This allele has more severe pathway defects and almost completely lacks glia; this phenotype is not enhanced when placed over *Df(2L)200*.

While surveying the genomic region containing *gcm*, we discovered that we had in our collection of enhancer trap lines a P[ry^+ *lacZ*] line, rA87 (from Klämbt and Goodman, 1991), that expresses β-galactosidase (β-gal) in glia (see Figure 2H) and maps to 30B9-12. To test whether this insert is in or near *gcm*, we cloned the associated gene and generated imprecise excisions of the rA87 P element insertion.

Of genomic DNA flanking the rA87 insertion site, 6 kb was recovered by plasmid rescue. This fragment was used to isolate overlapping genomic clones. Fragments from either side of the rA87 P element were used to isolate embryonic cDNA clones, several of which mapped to the same EcoRI–BamHI fragment as the rA87 P element insert (Figure 1A). cDNA clone *gcm-1* and genomic DNA from the corresponding region hybridize on Northern blots to a single transcript of approximately 2.2 kb, the same size as our longest cDNA clone. Using cDNA *gcm-1* for in situ hybridization, we detect embryonic transcripts in glia similar to the β-gal expression in line rA87 (Figure 2).

The rA87 P element insert is homozygous viable as well as viable over *gcm* alleles. We generated 200 excision lines of rA87 and recovered 10 lethal excisions that fail to complement both gcm^{1308} and $gcm^{N7\text{-}4}$. All 10 alleles ($gcm^{\Delta P1}$ to $gcm^{\Delta P10}$) express little or none of the glial-specific transcript. $gcm^{\Delta P1}$ contains a small 3.5 kb deletion that specifically removes the entire glial-specific transcript (see Figure 1A) and has a phenotype identical to $gcm^{N7\text{-}4}$. Thus, the glial-specific transcript identified by rA87 is the product of *gcm*, and $gcm^{\Delta P1}$ and $gcm^{N7\text{-}4}$ both represent the complete loss-of-function of *gcm*.



B

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MVLNGMPITM  PVPMPVPMPV  PSPPATKSRV  AIDWDINDSK   40
MPSVGEFDDF  NDWSNGHCRL  IYSVQSDEAR  KHASGWAMRN   80
TNNHNVNILK  KSCLGVLLCS  AKCKLPNGAS  VHLRPAICDK  120
ARRKQQGKQC  PNRNCNGRLE  IQACRGHCGY  PVTHFWRRDG  160
NGIYFQAKGT  HDHPRPEAKG  STEARRLLAG  GRRVRSLAVM  200
LARESALSDK  LSSLRPTKRQ  AKTQSIQESK  RRRMGASDVL  240
ETKQELVVPP  TTYLPTSTPT  HSTNFNQSQG  SYVPAGQGSV  280
ISQWNREIHY  ETEDPCYANG  MYSYDMLHSP  LSAHSSTGSY  320
YQENKPQQLQ  HSQYQQQLSP  QQHVPVSYDP  SQPISSSLQC  360
GMPSYEICDD  TSSLTSSSGY  CSEDYGYYNG  YLPNSLDVSN  400
GSQSQNLSQD  ASIYTTSSEI  FSVFESTLNG  GGTSGVDLIY  440
DEATAYQQHQ  QQGTFPHLTN  YQQEPQDQMQ  SADYYYSNTG  480
VDNSWNIQMD  ATYHPVNSTD  PIYC                    504
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Figure 1. Map of *gcm* Locus and Sequence of Gcm Protein

(A) Map of the *gcm* locus represented by restriction enzyme sites BamHI (B), EcoRI (E), and HindIII (H). The location of the P[ry^+ *lacZ*] element (rA87) insertion is indicated by the vertical line under an inverted triangle, with an arrow representing the direction of *lacZ* transcription (this insertion is 109 bp 5′ to the end of our longest cDNA clone). The position of the *gcm* transcription unit is indicated by the boxes below the map; the translated region is indicated by the closed portion. The bottom horizontal line represents genomic DNA from the P excision mutant $gcm^{\Delta P1}$; the break in the line indicates deleted DNA. $gcm^{\Delta P1}$ deletes 3.5 kb of genomic DNA, including the entire *gcm* transcript, but retains approximately 1 kb of the P element.

(B) Deduced amino acid sequence of gcm protein.

gcm Encodes a Novel Nuclear Protein

The complete nucleotide sequence of the 1.9 kb cDNA *gcm-1* was determined, and the 2.2 kb cDNA *gcm-2* was sequenced from both ends to cover the stop codons flanking the open reading frame (ORF). *gcm* contains an ORF that encodes a protein of 504 amino acids (see Figure 1B). Gcm has no homology to other known proteins and no internal repeats. We generated antibodies (Abs) against a gcm fusion protein and used them to show that gcm protein is localized to the nucleus (Figure 2G).

Expression of *gcm* Transcript and Gcm Protein

We followed *gcm* expression using in situ localization of transcripts with digoxygenin-labeled antisense RNA probes, immunocytochemistry using antisera against gcm protein, and the more persistent β-gal expression as driven by the rA87 enhancer trap P element as a marker of the cells that transiently express *gcm*. The patterns of *gcm* transcript and gcm protein expression are identical.

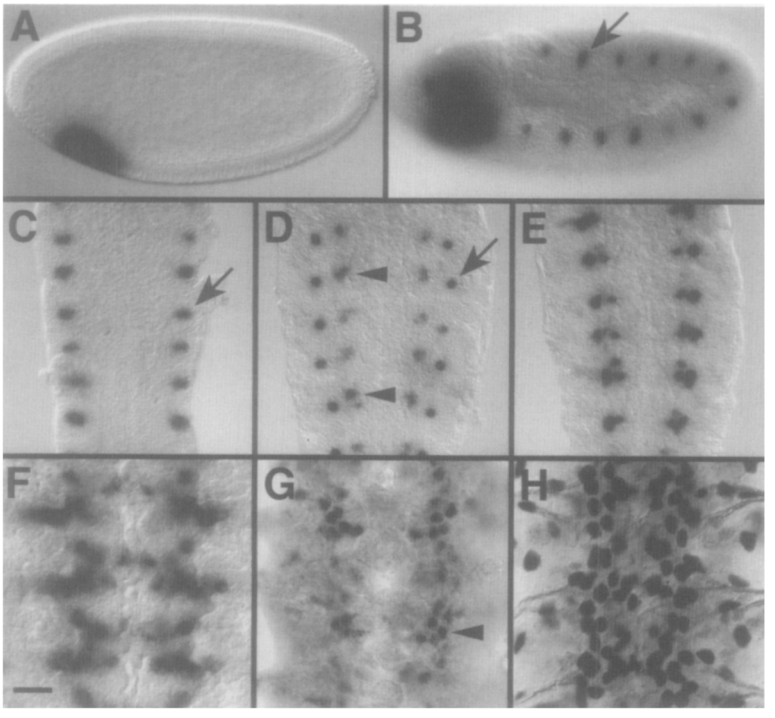

Figure 2. *gcm* Expression

(A–F) In situ localization of *gcm* transcripts showing lateral views of whole-mount embryos (dorsal up, anterior to left) (A and B) and frontal views of dissected embryos (anterior up) (C–F).

(A) Blastoderm stage. *gcm* is expressed in an anterior ventrally restricted patch approximately 16 cells wide by 12 cells in dimension.

(B) Stage 10. *gcm* is transiently expressed in segmentally repeated patches of three to five ectodermal cells (arrow).

(C) Same stage as in (B) but dissected.

(D) Early stage 11. *gcm* expression in the segmentally repeated patches fades as *gcm* becomes restricted to a large glioblast as it delaminates from each patch (arrow). A second patch of *gcm*-expressing cells arises medial to the first glioblast (top arrowhead) and gives rise to a second glioblast (bottom arrowhead). We tentatively call the first cell the peripheral glioblast and the second cell the longitudinal glioblast (see text).

(E) Late stage 11. The longitudinal glioblast has divided, and additional *gcm*-expressing cells begin to appear.

(F) Stage 12. *gcm*-expressing cells continue to appear and divide, and the presumtive longitudinal glia migrate medially.

(G) Stage 14. Localization of gcm protein as detected by anti-gcm Ab. Gcm is expressed in the nuclei of the longitudinal glia (arrowhead) and other glia.

(H) β-Gal expression in rA87 stage 16 embryo as detected by anti-β-gal Ab. β-Gal is expressed in the nuclei of nearly all CNS glia (except midline glia).

Scale bar for (A)–(E) is 50 μm, for (F)–(H) is 10 μm.

gcm is initially expressed in an anterior ventral region of the cellular blastoderm, in a patch approximately 16 cells wide and 12 cells in length (Figure 2A). During gastrulation these cells invaginate at the end of the ventral furrow, just anterior to the cephalic furrow, in an area giving rise to the presumptive cephalic mesoderm. After stage 10, cephalic expression rapidly fades. We have not detected any mutant phenotype associated with this expression.

During stages 10–11, in each hemisegment, two patches of three to five ectodermal cells sequentially and transiently express *gcm* and from each patch delaminates a single large blast cell that maintains *gcm* expression (Figures 2B, 2C, and 2D). The expression of β-gal in line rA87 acts as a good lineage marker for these two blast cells and shows that all of their progeny become glia (Figure 2H). The first more-lateral glioblast appears to give rise to most, if not all, of the exit and peripheral glia, and thus we tentatively call it the peripheral glioblast. The second more-medial glioblast appears to give rise to the longitudinal glia, and thus we tentatively call it the longitudinal glioblast (Jacobs et al., 1989). This pattern of *gcm* expression clearly shows that there are two glioblasts in each hemisegment, but sorting out the precise identities of their progeny will require future lineage analysis. By late stage 11, both glioblasts have divided. The progeny that generate the longitudinal glia have begun migrating medially, while the progeny that generate the peripheral glia have begun migrating distally. Additional *gcm*-expressing cells have begun to appear (Figure 2E), increasing rapidly in number. Besides the two large glioblasts and their progeny (the exit and peripheral glia and the longitudinal glia), we do not know the identity of the other *gcm*-expressing cells, other than, as indicated by β-gal expression in rA87, they all become glia.

gcm is expressed in the progeny of the longitudinal glioblast as they migrate medially to become the longitudinal glia (Figures 2F and 2G). *gcm* transcripts and gcm protein (Figure 2G) are last detected in the longitudinal glia as they take their final positions covering the longitudinal tracts around stage 14. By stage 15, gcm protein has disappeared, even though β-gal continues to be expressed in line rA87 in all CNS glia (except midline glia) as a persistent marker of previous *gcm* expression (Figure 2H). In the brain lobes, *gcm* expression remains high in a complex pattern through stage 17. In the PNS (as detected by transient expression of *gcm* transcripts and persistent expression of β-gal in rA87), *gcm* is transiently expressed in all repo-positive glia along peripheral axon pathways and associated with sensory organs (e.g., Figure 6A).

gcm Function in the CNS

In $gcm^{\Delta P1}$ mutant stage 16 and 17 embryos, there are only a few repo-positive cells remaining in the CNS (compare Figure 3B with 3A and Figure 3E with 3D). The wild-type CNS contains 60 repo-positive glia per segment, while *gcm* mutants have two to three per segment ($n = 28$) in a random pattern. The only CNS glia consistently remaining are the repo-negative midline glia (as detected with en-

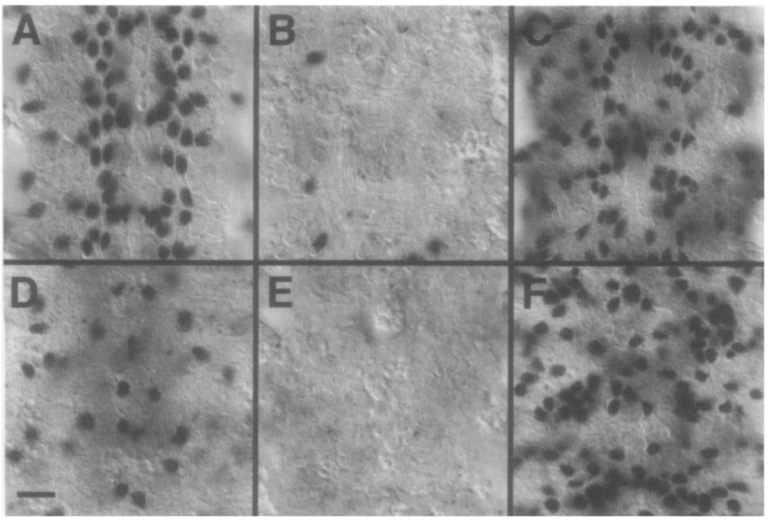

Figure 3. CNS Glia in Wild-Type, *gcm* Loss-of-Function, and *gcm* Gain-of-Function Embryos

Dissected stage 16 embryos (two segments; anterior is up) stained with anti-repo antisera showing nearly all CNS glia. (A)–(C) are focused on the inside surface of the CNS at the level of the longitudinal pathways, while (D)–(F) are focused on the outside surface of the CNS. (A and D) Wild-type embryo. (B and E) $gcm^{\Delta P1}$ loss-of-function mutant embryo. There are very few repo-expressing cells. (C and F) *C155–GAL4; UAS–gcm* gain-of-function embryo. There is an increase in the number of repo-expressing cells, particularly at the outside surface of the CNS (F). Scale bar, 10 μm.

hancer trap line AA142; Klämbt et al., 1991). Electron microscopic analysis of *gcm* mutant embryos confirms that there are no glia covering the longitudinal tracts (Figures 4B and 4D) and no glia ensheathing the nerve cord (Figure 4C), but there still are midline glia.

$gcm^{N7\text{-}4}$ has an identical phenotype to $gcm^{\Delta P1}$ and is thus likely to be a complete loss-of-function mutation. Nevertheless, this allele produces *gcm* transcripts. As detected by in situ hybridization, in $gcm^{N7\text{-}4}$, *gcm* transcripts are observed in the same initial pattern of presumptive CNS glioblasts and glia. Although a cell appears in the normal location of the peripheral glioblast and divides as normal, its progeny fail to migrate distally. In the absence of wild-type *gcm* function, *gcm* transcripts in $gcm^{N7\text{-}4}$ appear to fade more rapidly than normal.

Using the enhancer detection/GAL4 system (Brand and Perrimon, 1993), we generated embryos that express *gcm* ectopically in all presumptive neurons. We constructed a fusion gene that places the cDNA *gcm-1* under the control of an upstream activating sequence (UAS) (*UAS–gcm*) that allows the reporter (*gcm*) to be activated by the GAL4 transcriptional activator. Two *UAS–gcm* reporter lines were generated. Ectopic expression of *gcm* in presumptive neurons was achieved by crossing these reporter lines with a GAL4 effector line (*C155–GAL4*) that directs expression in all embryonic neurons early in their development (Lin and Goodman, 1994). In *C155–GAL4; UAS–gcm* embryos, ectopic expression of *gcm* in presumptive neurons throughout the CNS causes a striking increase in the number of repo-positive cells (compare Figure 3C with 3A and Figure

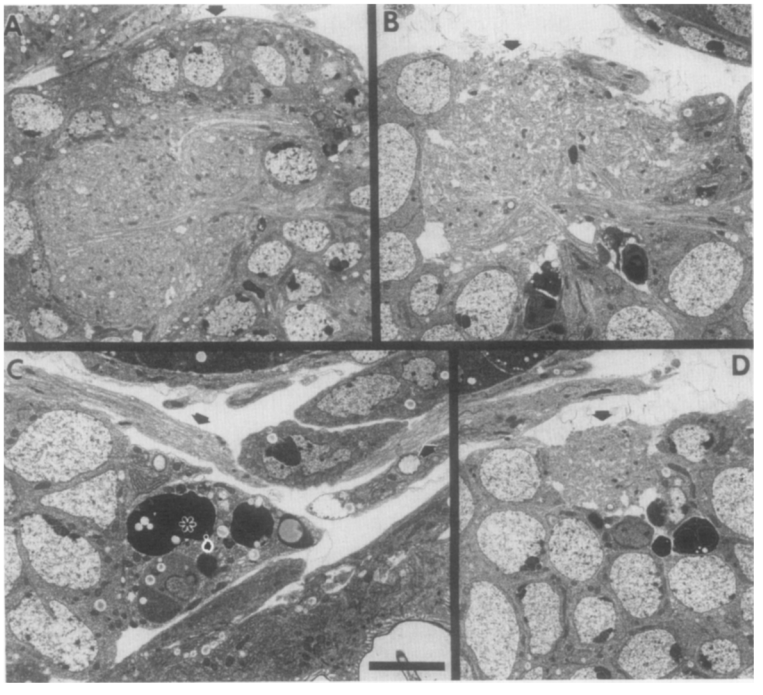

Figure 4. *gcm* Mutant Embryos Lack Longitudinal Glia and Other Glia

Electron micrographs of the CNS of wild-type (A) and $gcm^{\Delta P1}$ mutant (B–D) embryos at late stage 16 showing the longitudinal axon tracts (A, B, and D) and the edge of the CNS and ISN (C). (A) In wild-type embryos, the longitudinal tracts are covered by longitudinal glia (arrow). (B and D) In *gcm* mutant embryos, the longitudinal glia are missing (arrows), the longitudinal tracts are exposed to the hemolymph (arrows), and in some segments the longitudinal tracts are thinner (D) or are missing. (C) In *gcm* mutant embryos, the sheath glia that surround the CNS are missing, the ISN is missing the glia that normally wrap it (arrows), and large macrophages surround the CNS (asterisk). Scale bar indicates 4 μm in (A), (B), and (D) and 3 μm in (C).

3F with 3D), suggesting that many presumptive neurons are transformed into glia. Such embryos have 214 repo-positive glia per CNS segment (n = 3), an increase of 350% over the wild-type level of 60 per segment.

Late stage *gcm* mutant embryos exhibit defects in axon pathways in the CNS in which the longitudinal connectives are sometimes reduced in width and occasionally completely missing between segments (compare Figures 4B and 4D with 4A and Figure 5B with 5A). To determine the cause of this phenotype, we used MAb 1D4 against fasciclin II to examine the development of the first two longitudinal axon pathways (the MP1 and vMP2 pathways) and subsequent additional fasciclin II–positive pathways (Goodman and Doe, 1993; Lin et al., 1994) (Figures 5C–5F).

The pCC axon, which normally pioneers the vMP2 pathway, extends anteriorly at late stage 12/early stage 13 in a normal fashion in 100% of hemisegments (n = 128) in *gcm* embryos (Figure 5C). In 82% of hemisegments (n = 158), the MP1 and vMP2 pathways form normally at late stage 13/early stage 14 (arrowheads in Figures 5D and 5E). However, in 18% of hemisegments, one or both of these pathways is incomplete or missing between segments (compared with <1% in wild type; n = 110) (arrows in Figures 5D and 5E). By stage 16, more severe defects are observed in the longitudinal tracts. The normal three fasciclin II–positive longitudinal pathways are present in 65% of hemisegments (n = 62) (arrowheads in Figure 5F), but in 35%, one, two, or all three of them are missing or severely disrupted (arrows in Figure 5F). Thus, in *gcm* embryos, there is a progressive increase in defects observed in the developing longitudinal axon pathways, from a normal pCC at early stage 13, to a low percentage of segments with abnormal MP1 and vMP2 pathways at late stage 13/early stage 14, to a higher percentage of segments with abnormal longitudinal tracts by stage 16.

In the CNS of late stage *gcm* mutant embryos, we also observe increased neural cell death. Stage 16 embryos have fewer neurons compared with wild type, causing the CNS to be reduced and variable in width. We also observe that the mutant CNS is surrounded by an unusually large number of macrophages that are greatly enlarged and vacuolated compared with wild type (see Figures 4C, 5G, and 5H), consistent with studies by Tepass et al. (1994), who reported that mutants with increased cell death have larger macrophages containing more vacuoles due to the increased uptake of cellular debris.

gcm Function in the PNS

We focused our genetic analysis in the PNS on the development of two sensory organs: the lineage giving rise to the dorsal BD neuron and supporting glial cell, and the lineages giving rise to the pentascolopidial lateral CH organs (LCH5) (Ghysen et al., 1986; Bodmer et. al., 1989).

In the dorsal BD neuron lineage, the BD neuron is 22C10 positive (Fujita et al., 1982), whereas the glial support cell is repo positive. In each wild-type abdominal segment, the BD neuron has distinctive bipolar dendrites that extend laterally along with the processes of the repo-positive glial cell (Figure 6B). Enhancer trap line rA87 (in *gcm*) also

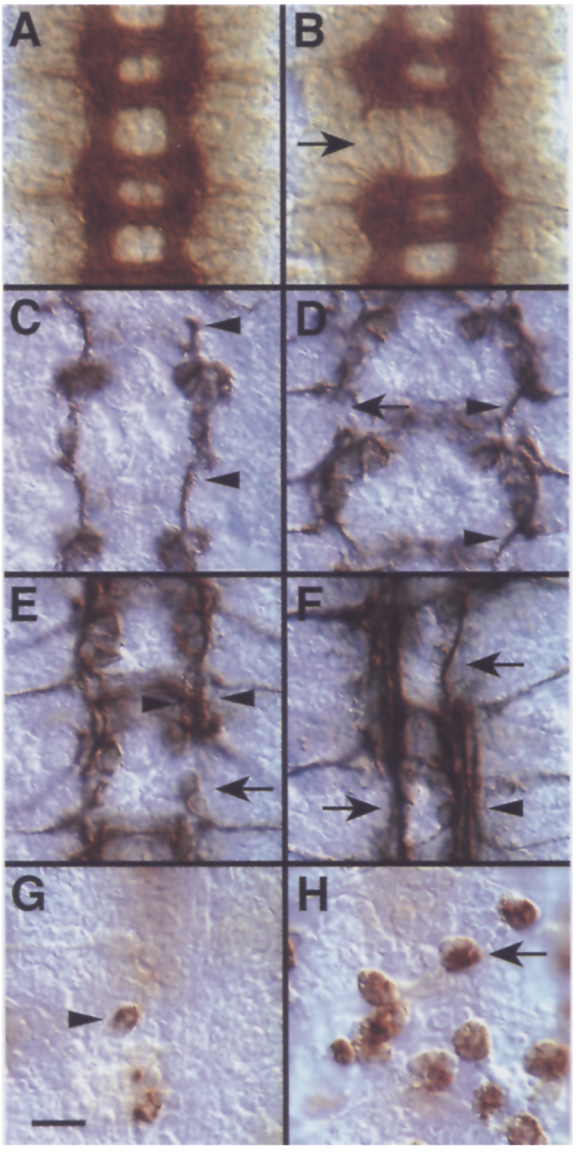

Figure 5. The CNS of *gcm* Mutant Embryos

Dissected embryos (pairs of segments; anterior is up).

(A and B) MAb BP102 staining of wild-type (A) and $gcm^{\Delta P1}$ mutant (B) stage 16 embryos showing the longitudinal and commissural axon tracts. In *gcm* embryos the longitudinal axon tracts are abnormal and occasionally missing between segments (arrow).

(C–F) MAb 1D4 (anti-fasciclin II) staining of *gcm* mutant embryos.

(C) Late stage 12 embryo. The pCC axon (arrowheads) extends anteriorly as normal in all hemisegments.

(D) Stage 13 embryo. In most segments the vMP2 pathway forms normally (arrowheads), but is occasionally incomplete (arrow).

(E) Stage 14 embryo. The MP1 (right arrowhead) and vMP2 (left arrowhead) pathways form in most segments, but are occasionally missing (arrow).

(F) Stage 16 embryo. Severe defects are observed in the longitudinal tracts. All three fasciclin II–positive longitudinal pathways are present in many segments (arrowhead), but are missing or disrupted in others (arrows).

(G–H) Macrophages revealed by anti-peroxidasin staining in wild-type (G) and *gcm* mutant (H) stage 16 embryos.

(G) In wild type, small macrophages are observed on the surface of the CNS (arrowhead).

(H) In *gcm* embryos, an increased number of large macrophages are observed on the surface of the CNS (arrow).

Scale bar, 10 μm.

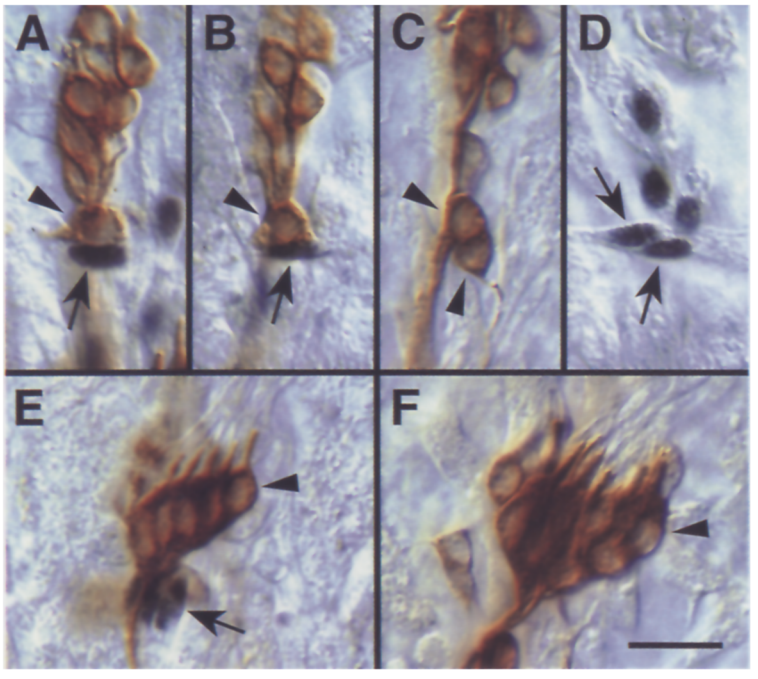

Figure 6. *gcm* Function in the PNS

(A–D) The dorsal cluster of sensory neurons in dissected stage 16 embryos (single segment; dorsal up, anterior to left).

(A) Enhancer trap line rA87 reveals the glial cell (arrow) (the nucleus is black as stained with anti-β-gal Ab) that supports the BD neuron (arrowhead) (the cytoplasm of the BD neuron and other neurons are stained brown with MAb 22C10).

(B) Wild-type embryo; neurons stained with MAb 22C10, glia with anti-repo Ab. Arrowhead marks the BD neuron; arrow marks its glial support cell.

(C) $gcm^{\Delta P1}$ loss-of-function mutant embryo stained with MAb 22C10, showing two 22C10-positive neurons in the position of the BD neuron (arrowheads); anti-repo staining reveals no glia (data not shown).

(D) *C155–GAL4; UAS–gcm* gain-of-function mutant embryo stained with anti-repo Ab, showing two repo-positive glia in the position of the BD neuron (arrows). There are also three extra repo-positive glia in the dorsal cluster.

(E–F) The pentascolopidial lateral CH organ (LCH5) in single abdominal segments in dissected stage 16 embryos (dorsal up, anterior to left).

(E) Wild-type embryo stained with MAb 22C10 and anti-repo Ab. The five CH neurons are visualized with 22C10 (arrowhead). Each CH neuron has a prominent dorsal dendrite. Ventral to the CH neurons are five ligament cells that express repo (arrow).

(F) *gcm* loss-of-function mutant stained with MAb 22C10 reveals at least four extra CH neurons (note absence of ligament cells and many extra CH neurons and dendrites).

Scale bar, 10 μm.

expresses β-gal in the BD glial cell (Figure 6A). In $gcm^{\Delta P1}$ mutant embryos, the repo-positive glial cell is missing and is replaced by a duplicated 22C10-positive BD neuron (100% of hemisegments; n = 40) (Figure 6C). The two BD neurons do not extend their distinctive bipolar dendrites, but do extend axons toward the CNS (Figure 7).

Ectopic expression of *gcm* in the BD neuron lineage transforms the presumptive BD neuron into a glial cell. In *C155–GAL4; UAS–gcm* embryos, in which *gcm* is ectopically expressed in all presumptive neurons, the BD neuron is missing and is replaced by an extra repo-positive glial cell (100% of hemisegments; n = 19) (see Figure 6D). The extra repo-positive glial cell is juxtaposed to and shaped like the normal glial cell. In the *gcm* loss-of-function and gain-of-function analysis of the BD lineage, we either observe two 22C10-positive neurons or two repo-positive glia, respectively, all with the appropriate morphology; we never observe intermediate cell types.

We also observe ectopic repo-positive cells in the dorsal cluster of sensory neurons (see Figure 6D) that normally contains no glia (see Figures 6A and 6B), suggesting that, here too, presumptive neurons are capable of transforming into glia in the presence of *gcm*.

In each abdominal segment of wild-type embryos, the five CH neurons of LCH5 are 22C10 positive (see Figure 6E). Each of these five CH neurons has an axon that extends ventrally towards the CNS and a prominent dorsal dendrite that inserts into one of five sheath (scolopale)

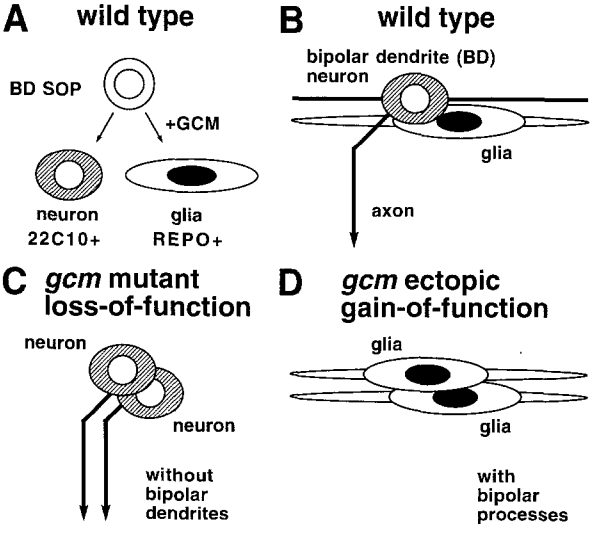

Figure 7. Diagram of the BD Neuron and Glial Cell in Wild-Type, *gcm* Loss-of-Function, and *gcm* Gain-of-Function Embryos

(A) The BD SOP divides once to generate the BD neuron and glial support cell (Bodmer et al., 1989).

(B) In wild type, the BD neuron and its glial support cell both have bipolar processes.

(C) In *gcm* loss-of-function mutants, the glial support cell is transformed into a neuron; both neurons have axons, but neither has a lateral dendrite.

(D) In *gcm* gain-of-function mutants, the BD neuron is transformed into another glia cell; both glia extend bipolar processes.

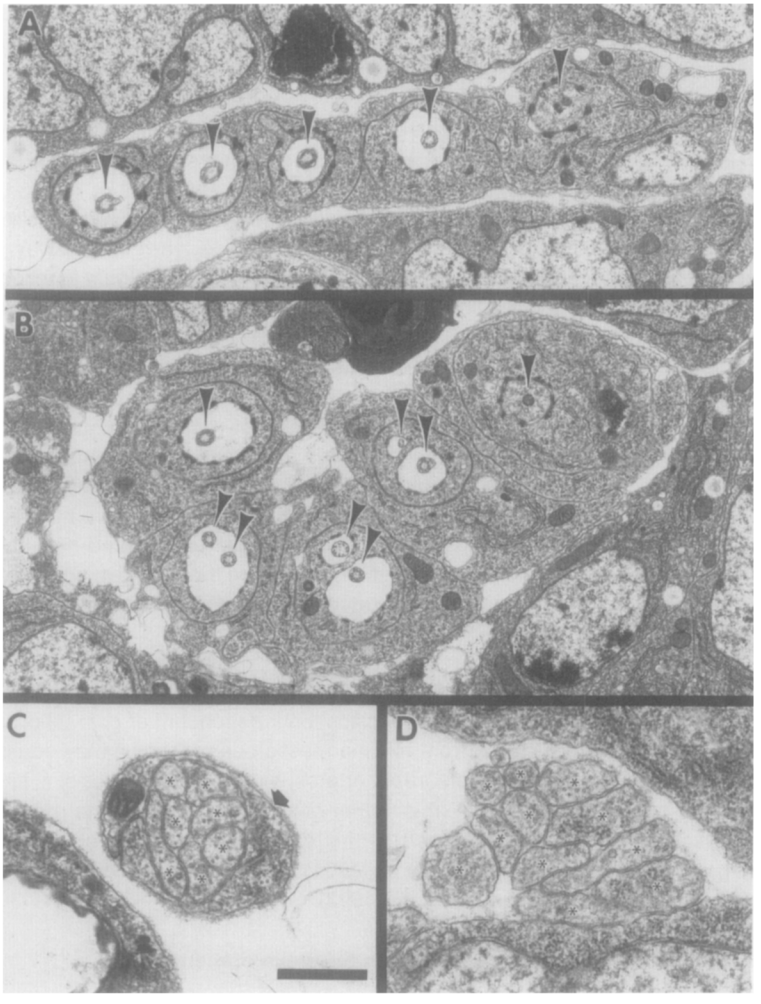

Figure 8. In *gcm* Mutant Embryos, Ligament Support Cells Transform into Extra CH Neurons

Electron micrographs of the PNS of wild-type (A and C) and $gcm^{\Delta P1}$ mutant (B and D) embryos at late stage 16 showing cross sections of the dendrites of the lateral CH neurons (arrows) inserted in the sheath cells (A and B) and of the LCH5 nerve branch between the ISN and the CH neurons (C and D).

(A) Wild-type LCH5 contain five CH neurons (arrows), each with a single dendrite inserted into a single sheath cell.

(B) *gcm* mutant embryos contain as many as 10 neurons; the ligament cells are missing. This section shows eight CH neuron dendrites (arrows) inserted into the normal five sheath cells, such that three of the sheath cells each has two dendrites from two different CH neurons inserted into them.

(C) In wild-type embryos, we observe seven axons (asterisks) in the LCH5 nerve, five from the CH neurons and two from other neighboring neurons; the nerve is surrounded by glia (arrow).

(D) In a *gcm* mutant embryo, the nerve contains 11 axons (asterisks); the extra four axons are presumably from four extra CH neurons. The nerve lacks glial wrappings.

Scale bar is 1.33 μm in (A) and (B) and 0.5 μm in (C) and (D).

cells, which contact five cap cells that contact the epidermis. Just ventral to the five CH neurons are the five ligament glial cells that have smaller nuclei and are 22C10 negative and repo positive.

In $gcm^{\Delta P1}$ mutant embryos, the repo-expressing ligament cells are absent (100% of hemisegments; n = 56), and instead we observe a range of extra CH neurons that are 22C10 positive and have large nuclei and dorsal dendrites (see Figure 6F): 10 neurons, 29%; nine neurons, 34%; eight neurons, 26%; seven neurons, 10%; six neurons, 1%; and five neurons, 0% (n = 42).

We examined these CH organs in stage 16 wild-type and $gcm^{\Delta P1}$ mutant embryos by serial section electron microscopy. In wild-type LCH5 organs, we consistently observe five CH neurons, each with a single dorsal dendrite inserted into a single sheath cell (Figure 8A). We randomly picked and reconstructed two *gcm* mutant LCH5 organs. In both we observe 10 neuron-like cells with large nuclei and no ligament cells. In both we observe eight dorsal dendrites inserted into the normal five sheath cells, such that three of the sheath cells each have two dendrites from two CH neurons inserted into them (Figure 8B). The two neuron-like cells that lack dorsal dendrites are on the ventral side of the cluster, furthest from the sheath cells. We also counted the axons in cross sections of the LCH5 nerve branch after it leaves the CH neurons but before arriving at the intersegmental nerve (ISN). In wild-type embryos, we count seven axons, five from the CH neurons and two from other neighboring neurons (Figure 8C). In one *gcm* mutant segment, we count 11 axons (Figure 8D); we presume that the extra four axons are from four of the extra CH neurons. Moreover, the LCH5 nerve branch in *gcm* embryos lacks glial wrappings (Figure 8D).

Despite the absence of glia in *gcm* mutants, peripheral sensory nerves as visualized with MAb 22C10 and peripheral motor nerves as visualized with MAb 1D4 (anti–fasciclin II) develop in a relatively normal fashion in $gcm^{\Delta P1}$ mutant embryos.

Discussion

The results presented here support a model in which the *gcm* gene functions as a genetic switch to control glial versus neuronal cell fate in both the Drosophila CNS and PNS. In the presence of gcm protein, presumptive neurons become glia, while in its absence, presumptive glia be-

come neurons. In the CNS, nearly all glia (except midline glia) are missing in the absence of gcm; many extra glia appear after ectopic expression of gcm. However, our most striking evidence supporting this model comes from genetic analysis of *gcm* function in the PNS.

In the dorsal BD neuron lineage, a single SOP is thought to divide once to generate the BD neuron and its glial support cell (Bodmer et al., 1989). In loss-of-function *gcm* mutants, the presumptive glial cell is transformed into a neuron. In gain-of-function *gcm* conditions produced by ectopic expression of *gcm*, the presumptive BD neuron is transformed into a glial cell. In the CH organ lineage, in loss-of-function *gcm* mutants, the presumptive ligament cell (which normally expresses the glial marker repo) is transformed into a CH neuron.

The specific types of glia and neurons generated in any given lineage appear to differ by only the transient expression of *gcm*. In the absence of gcm, the presumptive glial cell does not transform into a random neuron, but rather transforms into the appropriate type of neuron for that particular lineage. Thus, in the absence of gcm, the presumptive glial cell presumably expresses the appropriate combination of neural cell fate specifiers so that it can differentiate into an appropriate neuron. In the CH lineage, in the absence of gcm, the presumptive ligament cell differentiates into a normal CH neuron.

Conversely, in the presence of gcm, the presumptive neural cell does not transform into a random glial cell, but rather transforms into the appropriate type of glial cell for that particular lineage. In the BD lineage, in the presence of gcm, the presumptive BD neuron is transformed and differentiates like the normal glial cell associated with the BD neuron. Thus, although glia and neurons have very different properties, the specific kinds of glia and neurons generated within any particular lineage share many common aspects of their genetic specification. Their differences appear to be controlled by the presence or absence of gcm.

In Drosophila, although glia arise from a variety of different kinds of lineages in the CNS and PNS, nearly all of them appear to be specified by a common genetic switch controlled by *gcm*. *gcm* functions to control the specification of the longitudinal glia that arise from the longitudinal glioblast, other CNS glia that arise from a variety of different NBs generating both neurons and glia, the sheath glia surrounding the CNS, the exit glia and peripheral glia that arise from the peripheral glioblast, and the glial support cells for sensory organs that arise from SOPs. These glia normally transiently express gcm and then maintain expression of repo, a glial homeodomain protein; in *gcm* mutants, all of these glia are absent and repo is not expressed.

The longitudinal glioblast in the CNS appears to be restricted to generate only glia (the same may be true of the peripheral glioblast), while most other stem cells in the CNS and PNS are multipotent and generate both neurons and glia (Jacobs et al., 1989; Udolph et al., 1993; Bodmer et al., 1989). *gcm* expression correlates with these different patterns of restriction, being expressed in the two glioblasts and their progeny from the outset, but not being expressed in the multipotent NBs in the CNS or SOPs in the PNS, but rather only appearing in their progeny destined to be glia (as observed by the transient expression of *gcm* mRNA and persistent expression of *lacZ* from the P element inserted in *gcm*).

We do not know precisely when *gcm* is required in the life history of any single cell to send it down the glial pathway, if and when neural cells become refractory to *gcm* function, and whether cells other than presumptive neurons can also be turned into glia. However, a number of observations lead us to speculate that *gcm* is required early in the life of a cell and that as neurons differentiate they become refractory to *gcm* function. First, *gcm* is normally transiently expressed at early stages and disappears by the time glia fully differentiate. Second, in our ectopic expression experiments, some but not all presumptive neurons transform into glia. Most new glia are located near the outside surface of the CNS, where the youngest presumptive neurons are located, supporting the notion that timing may be critical for *gcm* function.

Our results confirm that midline glia are different from all other CNS and PNS glia, by showing that midline glia have a unique specification independent of *gcm*. Midline glia arise from midline mesectodermal cells; they do not express gcm, repo, or pros, but they do express sim, slit, and argos (which other glia do not). The midline glia are specified in part by the *spitz* group genes (Crews et al., 1988; Klämbt et al., 1991) and the P2 transcript from the *pnt* gene (Klämbt, 1993).

The Role of Glia in the Development of Axon Pathways

In the absence of glia, most axon pathways can develop, although there is increased variability from segment to segment, and in some segments the pathways may be missing or abnormal.

When the longitudinal glia were discovered (Jacobs et al., 1989), it was thought that they prefigure the first longitudinal pathways (Jacobs and Goodman, 1989); however, subsequent analysis revealed that the repo-positive longitudinal glia follow rather than precede the first growth cone, pCC (Lin et al., 1994; R. D. F., C. Kopczynski, V. Auld, and C. S. G., unpublished data), consistent with the results presented here that in *gcm* mutant embryos, the pCC growth cone extends anteriorly in a normal fashion in 100% of hemisegments. In the absence of longitudinal glia, in 82% of hemisegments, the initial two longitudinal axon pathways (the MP1 and vMP2 pathways) develop normally; in the remaining 18%, these two pathways either do not form or are highly abnormal. In 65% of hemisegments, relatively normal longitudinal connectives form; in the remaining 35%, they either do not form or are very thin and highly abnormal. Thus, the longitudinal glia are not absolutely required for the formation of the longitudinal axon pathways, but they do help to facilitate the formation of these pathways.

Glia Are Required for Specific Aspects of Neuronal Differentiation

Previous in vitro studies in vertebrates have shown that glial cells (and specific factors they secrete) can be required for both neuronal survival and differentiation (e.g., Hatten et al., 1988). Moreover, it has been shown that specific aspects of neuronal differentiation can be influenced by the presence or absence of glia in cell culture. For example, rat sympathetic neurons grown in vitro extend single axons in the absence of glia, but only extend dendrites in the presence of glia (Tropea et al., 1988).

The *gcm* mutant has allowed us to address these issues in the developing organism. By examining neural development in the absence of glia, it has been possible to show that certain aspects of neuronal differentiation require the presence of glia. In the BD lineage in the PNS, normally both the BD neuron and its glial support cell extend bipolar lateral processes. In *gcm* loss-of-function mutants, the presumptive glial cell is transformed into another neuron, and although both neurons extend axons, neither neuron develops the characteristic of the BD neuron (Figure 7). In contrast, in *gcm* gain-of-function conditions, the presumptive BD neuron is transformed into another glial cell, and both glial cells extend bipolar lateral processes. These results suggest that it is the glial cell and not the neuron that leads the way in establishing the bipolar lateral processes and that the glial processes either induce or stabilize the dendrites of the neuron.

CNS Glia Are Required for the Survival of CNS Neurons

In the absence of CNS glia, many CNS neurons die during later stages of embryonic development, and the CNS becomes surrounded by an unusually large number of enlarged macrophages. Neuronal apoptosis has also been reported in the optic lobes of *repo* mutant adults (Xiong and Montell, 1995). In the absence of functional glia, it is possible that CNS neurons die owing to the lack of the blood–brain barrier. In Drosophila, the glia that sheath the CNS help shield CNS neurons against the high K^+ concentration of the hemolymph. Exposure to high K^+ concentration is likely to depolarize neurons, abolish their excitability, and possibly lead to higher levels of Ca^{2+} influx. For example, gliotactin is expressed on peripheral glia, and in gliotactin mutant embryos, the peripheral glia do not form a blood–nerve barrier and, as a result, peripheral motor axons are exposed to the high K^+ hemolymph, action potentials fail to propagate, and the embryos are paralyzed (Auld et al., 1995). Drosophila glia secrete proteins that regulate NB proliferation (Ebens et al., 1993), and it is possible that CNS glia might provide trophic factors that regulate neuronal survival.

The Control of Glial Cell Fate in Vertebrates

Just as in Drosophila, so too in vertebrates, glia and neurons often share common lineages in both the CNS and PNS. For example, in the neural crest lineage in the PNS, many migrating progenitors appear to retain the potential to give rise to both glia and neurons (e.g., Le Douarin et al., 1991; Frank and Sanes, 1991). A multipotent stem cell isolated from the mammalian neural crest can generate both neurons and glia (Stemple and Anderson, 1992). In the vertebrate retina, individual stem cells are multipotent and can generate both neurons and glia (Turner and Cepko, 1987; Holt et al., 1988; Wetts et al., 1988). Mixed neural and glia lineages have also been observed in the optic tectum (e.g., Galileo et al., 1990) and spinal cord (Leber et al., 1990). Specified precursors that generate homogeneous clones of a single neural or glial cell type have been identified in the mammalian cerebral cortex (e.g., Walsh and Cepko, 1992; Grove et al., 1993; Luskin et al., 1993); however, multipotent precursor cells that can generate both neurons and glia have also been identified in the cortex, possibly at earlier stages (Davis and Temple, 1994; Williams and Price, 1995).

Given that vertebrates and fruit flies use many related genes for the patterning and specification of neurons and neural structures, we would not be surprised if vertebrates use a *gcm*-like gene and similar genetic switch to control glial fate in the variety of CNS and PNS lineages described above. It will be interesting to see to what degree this prediction of homologous gene function and expression is confirmed.

Experimental Procedures

Generation and Analysis of *gcm* Mutations

Alleles $gcm^{\Delta P1}$ to $gcm^{\Delta P10}$ were generated by imprecise excision of a P[*ry*$^+$ *lacZ*] enhancer trap line rA87 (Klämbt and Goodman, 1991) that is homozygous viable and maps just upstream of the *gcm* transcript. rA87 was mobilized by crossing to flies carrying a stable genomic source of transposase, *Δ2-3(99B)*, and *gcm* alleles were isolated by selecting *ry*$^-$ lines that failed to complement the lethalities of gcm^{1308}, $gcm^{N7\text{-}4}$, and *Df(2L)200*. Mutant chromosomes were placed over balancer chromosomes carrying *lacZ* markers *CyO*, P[*ry*$^+$ *elav–lacZ*] and *CyO*, P[*w*$^+$ *actin 5C–lacZ*], which aided in the identification of mutant embryos. To analyze midline glia, lines were constructed that carry $gcm^{N7\text{-}4}$ or $gcm^{\Delta P1}$ and the AA142 line (Klämbt et al., 1991).

Isolation and Analysis of Genomic DNA and *gcm* cDNA Clones

Genomic DNA flanking the enhancer trap rA87 was recovered by plasmid rescue. The flanking 6 kb of DNA was used to isolate overlapping genomic clones from the Tamkun iso-1 cosmid library. Fragments of DNA flanking either side of the rA87 insertion were used to screen the 9–12 hr embryonic cDNA library (Zinn et al., 1988); a single cDNA clone was isolated that mapped within 1 kb of the rA87 insertion site. This 1.9 kb cDNA (*gcm-1*) was sequenced. Additional cDNAs were isolated. The largest of these, a 2.2 kb cDNA *gcm-2* (similar in size to the single band seen on the Northern), was sequenced from both ends to cover the stop codons at the beginning and end of the ORF. Genomic clones covering the entire ORF and the 5′ end up to and past the rA87 insertion site were also sequenced. Genomic DNA from rA87 excision lines was examined by Southern blot analysis to determine the extent of the deletions.

In Situ Transcript Localization

In situ localization of transcripts in whole embryos was performed as described previously (Kopczynski and Muskavitch, 1992) with minor modifications by C. Kopczynski. Digoxigenin-labeled RNA probes were generated from the *gcm-1* cDNA cloned into pBluescript SK(+).

Generation of Antisera

Bacterial fusion protein was produced using QIAGEN QIAexpress. A

702 bp PstI fragment from the *gcm-1* cDNA containing amino acids 99–330 was cloned into pQE-32, which adds 22 amino acids to the N-terminus. This addition contains six histidine residues, which allows for a one-step purification by immobilized Ni^{2+} chelate affinity chromatography. Rats were injected with 100 μg of protein emulsified in RIBI adjuvent (Immunochem Research), and were boosted at 2 week intervals.

Generation and Analysis of Gain-of-Function Conditions

The *UAS–gcm* reporter construct was generated by cloning the entire *gcm-1* cDNA into a pUAST vector that is a P element transformation vector that contains a polylinker flanked by five GAL4-binding sites upstream of the *hsp70* minimal promoter and by an SV40 polyadenylation site. Two *UAS–gcm* reporter lines were generated by P element–mediated transformation. Ectopic expression of *gcm* was achieved by crossing these lines with the *C155–GAL4* effector line that directs expression from early stages in all embryonic neurons (Lin and Goodman, 1994).

Immunohistochemical Detection of Proteins in Embryos

Horseradish peroxidase (HRP) immunohistochemistry and embryo dissections were carried out as previously described (Lin and Goodman, 1994). MAb 22C10 (Fujita et al., 1982), MAb 1D4 (anti–fasciclin II) (G. Helt and C. S. G., unpublished data), and MAb BP102 (A. Bieber, N. Patel, and C. S. G., unpublished data) were detected with HRP-conjugated goat anti-mouse secondary Ab (Jackson Immunoresearch). Anti-RK2/repo antisera (Campbell et al., 1994) and anti-gcm antisera were followed by biotinylated goat anti-rat secondary Ab and detected using Vectastain Elite ABC; the diaminobenzidine reaction was enhanced to give a black color by addition of 0.064% NiCl and 0.02% $CoCl_2$. Anti-β-gal was used and detected as previously described (Lin and Goodman, 1994). Anti-peroxidasin (Abrams et al., 1993; Tepass et al., 1994) was followed by biotinylated goat anti-mouse secondary Ab and Vectastain ABC.

Electron Microscopy

gcm mutant embryos were collected from a $gcm^{\Delta P1}$/*CyO*, P[ry^+ *elav–lacZ*] stock, screened for the 25% homozygous mutant embryos, and fixed and prepared for electron microscopy as previously described (Auld et al., 1995).

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The accession number for *gcm* is U34039.