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Review

Development and organization of glial cells in *Drosophila melanogaster*

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Glial cells constitute the second major cell type of the nervous system. They wrap the neuronal somata and axons with their cytoplasmic processes and play a number of roles in neuronal development, activity and during regeneration. Even though glial cells have been identified in higher invertebrates such as Annelids, Arthropods and Molluscs, they have been studied for a long time in vertebrate model systems. This is due to the fact that invertebrate glial cells are highly heterogeneous, which hampers an easy classification. In addition, the identification of these cells often relies on the use of electron microscopy. Recently, however, enormous progress has been made in insect neurobiology and developmental biology due to the availability of genetic and molecular tools developed in *Drosophila melanogaster*. These technological improvements have facilitated the discovery of glial specific markers and genes. In addition, a number of mutations affecting glial development has also been identified, enabling us to define key steps of the glial differentiation program. The analysis of these mutations has also clarified the precise roles played by glial cells.

Glial cell organization in *Drosophila melanogaster*

The existence of glial cells in insects has been known for a long time (Wigglesworth, 1959; Strausfeld, 1976; Saint Marie *et al.,* 1982) but a lack of specific markers has limited the study of glial development and organization in small insects such as *Drosophila* (Poulson *et al.,* 1950; Fredieu and Mahowald, 1989). One of the most important breakthroughs in understanding glial development and function in *Drosophila melanogaster* has been the use of engineered transposons and germline transformation (Rubin and Spradling, 1982; O'Kane and Gehring, 1987; Wilson *et al.,* 1989). Enhancer trap lines are stocks of flies in which a transposon carrying the *E. coli* ß-galactosidase (ß-gal) gene is inserted in the fly genome. In this way it has been possible to identify markers specific for all or subsets of glial cells (Fig. 1). Using enhancer trap markers it has been possible to define the organization of glial cells in the embryonic nervous system (Klämbt and Goodman, 1991; Nelson and Laughon, 1993; Ito *et al.,* 1995) (Fig. 1). In addition, the enhancer trap approach has provided markers for specific developmental stages and also for different subcellular components such as the nucleus (O'Kane and Gehring, 1987) or the cytoplasm (Giniger *et al.,* 1993; Ito *et al.,* 1995). This has made it possible to explore in detail the morphology of neuronal and glial cells.

The embryonic nervous system of *Drosophila melanogaster* includes the central nervous system which consists of the brain and nerve cord (a metameric structure subdivided into neuromeres), the stomatogastric nervous system and the peripheral nervous system. The nerve cord shows bilateral symmetry with longitudinal connectives running on each side along the antero-posterior axis. Fibers from the longitudinal connectives crossing the midline and reaching the contralateral connectives form the anterior and posterior commissures present in each neuromere (Fig. 1). Motor and sensory fibers form the intersegmental and segmental nerves on each side of the cord. Each neuromere contains an average of 60 glial cells, the number being variable between segments and individuals. Glial cells have been subdivided into three major classes depending on their position within the ventral nerve cord (Meyer *et al.,* 1987; Ito *et al.,* 1995) (Fig. 1). The first class is constituted by surface associated glial cells which include the outermost glial cells, perineural and subperineural cells. These cells form a sheath around the ventral cord functioning as a blood-brain barrier. Surface associated glial cells also include channel glial cells at the segment border. The second class includes the cortex glial cells, or cell body glial cells, found underneath the surface glial cells and associated with neuronal cell bodies. The third class of glial cells, neuropile associated glial cells, are found along the axonal structures and provide a scaffold for the axon bundles. To this class belong midline glial cells, wrapping the anterior and posterior commissures, longitudinal glial cells, lining the longitudinal connectives, and nerve root glial cells, which lie along the peripheral nerve root.

Glial cells are also present in the embryonic as well as the adult peripheral nervous system where they wrap sensory and motor fibers (Figs. 1 and 2). Peripheral glial cells resemble non-myelinating Schwann cells in vertebrates in that they ensheathe multiple axons and are devoid of myelin (see Fig. 2A,B,C,E; Murray *et al.,* 1984; Auld *et al.,* 1995). Finally, glial cells which originate from the optic lobe display a rather complex organization, which reflects the "layer structure" of axonal fibers and neuronal somata within the optical ganglia. Depending on the position in the ganglia, glial cells have been subdivided into different sub-types, most of which have been characterized in detail in large insects (Strausfeld, 1976; Saint Marie and Carlson, 1983; Winberg *et al.,* 1992).

A very specialized form of glial cell in the peripheral nervous system is the thecogen cell, one of the accessory cells of the external sensory organ (for a review see Giangrande and Palka, 1990). The origin, the cellular mechanisms of development and the genetic program that lead to thecogen differentiation have been already characterized. This cell is tightly associated with the sensory organ: it is clonally related to the other cells of the sensory organ and requires expression of the same genes that lead to the differentiation of these cells (Fig. 3). The external sensory organ originates from a sensory organ precursor cell, a committed cell that divides twice to give the neuron, the thecogen cell and two other cells, tormogen and trichogen, which secrete the cuticular structures of the sensory organ, the socket and the shaft (Bodmer *et al.,* 1989; Brewster and Bodmer, 1995). In insects, sensory neurons are located at the periphery, beneath the epidermis. These bipolar neurons send a basal process, the axon, towards the central nervous system, and a short apical process, the dendrite, towards the shaft. The axon fasciculates with other fibers to form a nerve wrapped by glial cells whereas the dendrite is wrapped by an electron-dense sheath secreted by the thecogen, which also ensheathes the neuronal cell body (Keil and Steinbrecht, 1986). The lineage association of the thecogen cell with the sensory organ, its position and the fact that it does not express glial specific genes indicate that thecogen cells and glial cells wrapping the axons represent two unrelated types of cells (Giangrande *et al.,*

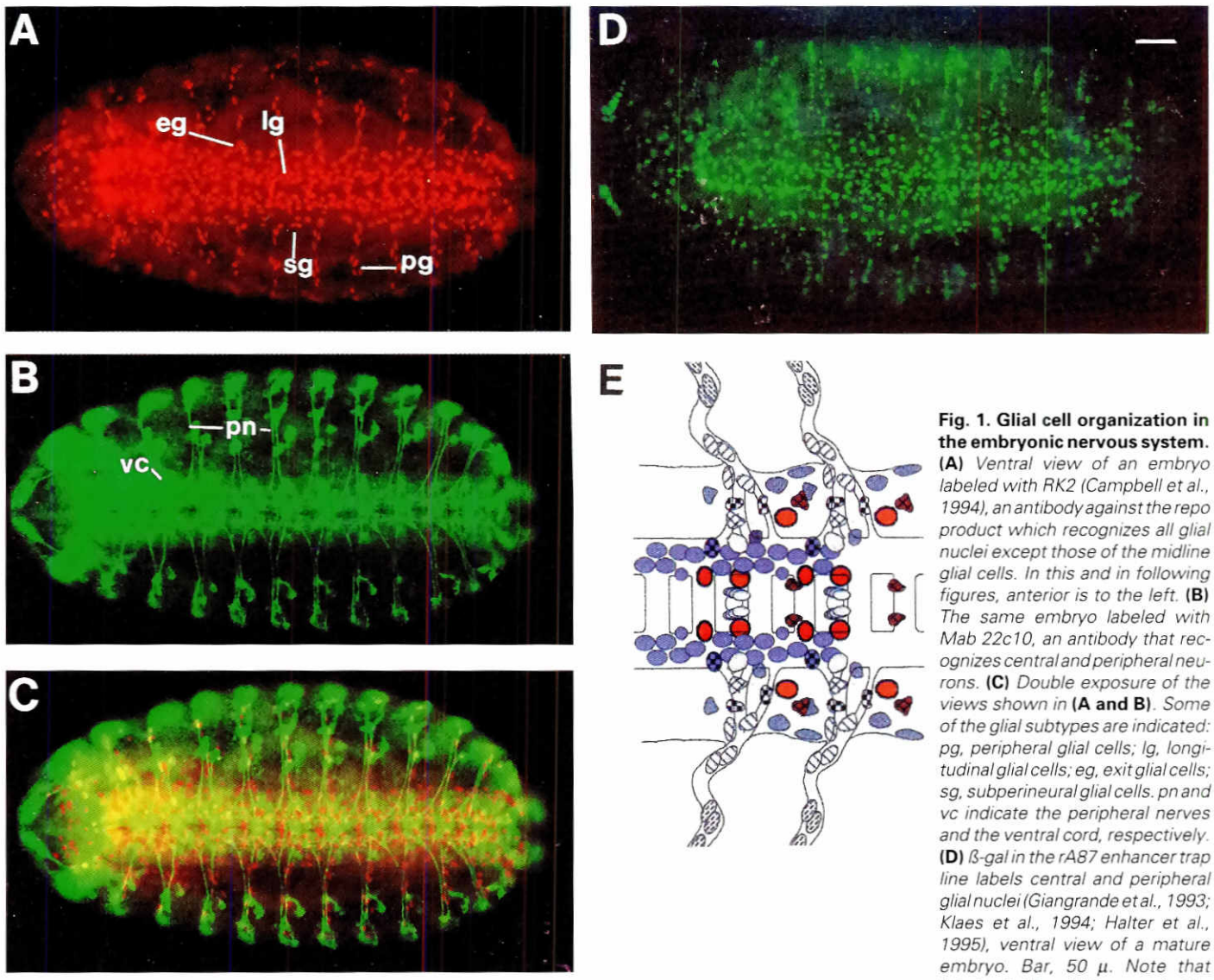
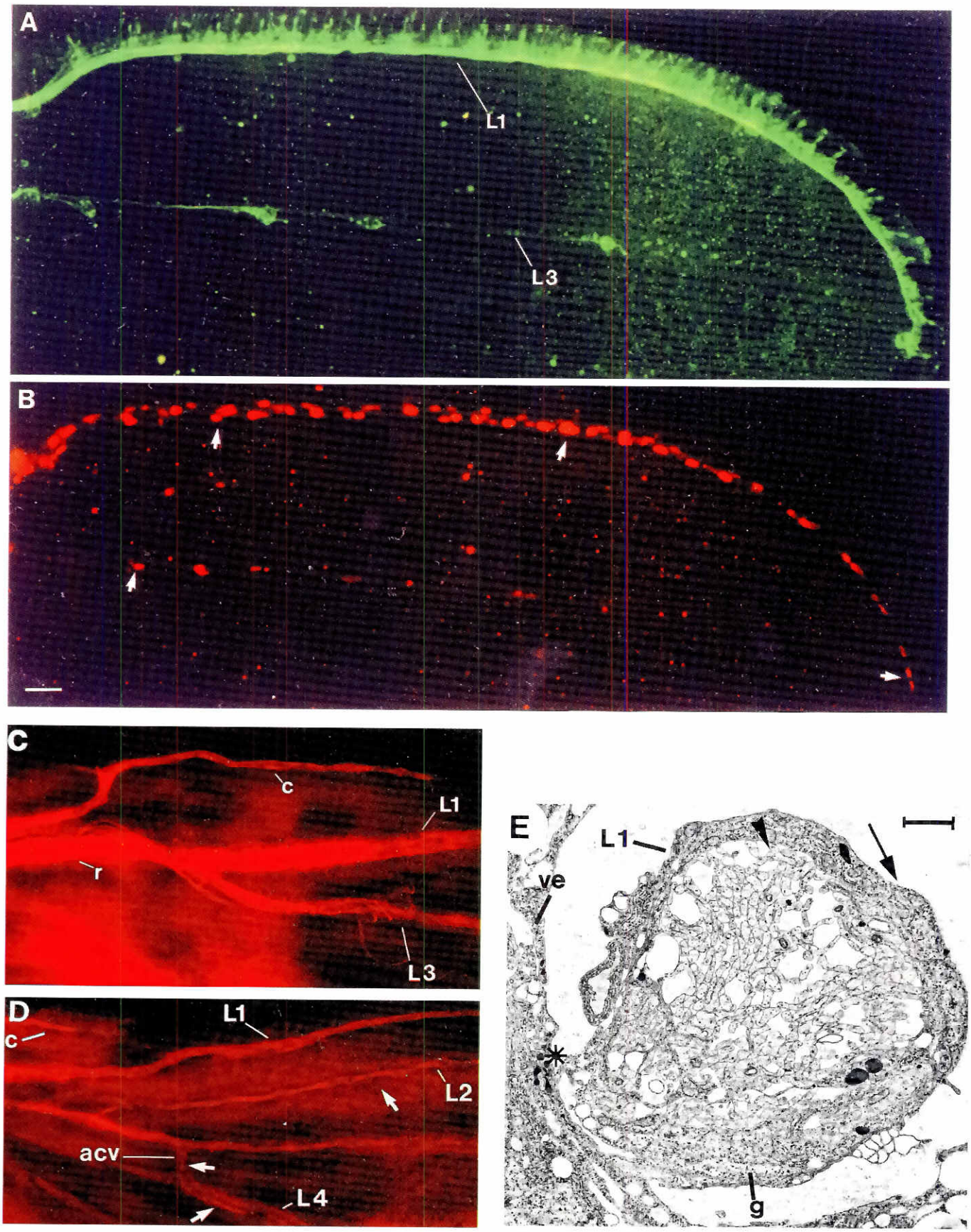


Fig. 1. Glial cell organization in the embryonic nervous system. **(A)** *Ventral view of an embryo labeled with RK2 (Campbell et al., 1994), an antibody against the repo product which recognizes all glial nuclei except those of the midline glial cells. In this and in following figures, anterior is to the left.* **(B)** *The same embryo labeled with Mab 22c10, an antibody that recognizes central and peripheral neurons.* **(C)** *Double exposure of the views shown in* **(A and B)**. *Some of the glial subtypes are indicated: pg, peripheral glial cells; lg, longitudinal glial cells; eg, exit glial cells; sg, subperineural glial cells. pn and vc indicate the peripheral nerves and the ventral cord, respectively.* **(D)** *ß-gal in the rA87 enhancer trap line labels central and peripheral glial nuclei (Giangrande et al., 1993; Klaes et al., 1994; Halter et al., 1995), ventral view of a mature embryo. Bar, 50 μ. Note that labeling in* **(D and A)** *recognize the same cells. Additional cells of unknown identity are labeled in* **(D)**. **(E)** *Schematic representation of glial cells in the embryonic ventral cord (taken from Ito et al., 1995). Interface glial cells, to which longitudinal glial cells belong, are indicated in purple. Nerve root glial cells are checkered. Channel glial cells are located between the anterior and posterior commissures and are indicated by grey ovals (dark grey, dorsal glial cells; light grey, ventral glial cells). Some subperineural glial cells are in red, the others in grey/blue. Cell body glial cells are crosshatched, exit glial cells are hatched and peripheral glial cells are shown by broken hatched nuclei. Exit glial cells, present on peripheral nerves at the border between the ventral cord and the periphery, are considered as peripheral glial cells.*

1993). For these reasons, this review will not discuss the thecogen cell further and will refer to the axon-associated glial cells as peripheral glial cells.

Molecular mechanisms of glial differentiation

In addition to providing glial specific markers, enhancer trap lines allow the cloning of genes close to the transposon using the plasmid rescue technique (Mlodzik and Hiromi, 1992). Importantly, enhancer trap lines have also been extremely useful to generate new mutants. A targeted mutagenesis can be achieved using the transposase recombinase in order to disrupt the gene identified by ß-gal labeling. A number of genes affecting glial cell development have been isolated by this approach. The product of two of them, *reverse polarity (repo)* (Campbell *et al.*, 1994; Xiong *et al.*, 1994; Halter *et al.*, 1995) and *glial cell deficient (glide)* (Vincent *et al.*, 1996), also called *glial cell missing (gcm)* (Hosoya *et al.*, 1995; Jones *et al.*, 1995) label all peripheral glial cells and all central but midline glial cells. This separation between midline glial cells and the other glial cells of the central and the peripheral nervous system is in agreement with previous developmental analyses that set midline glial cells apart from all the other glial cells (Klämbt *et al.*, 1991). Midline glial cells originate from mesectoderm which is constituted by two rows of cells that are brought together after gastrulation. All the other glial cells differentiate from the ectoderm, with the exception of perineural cells, which appear to originate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from the mesoderm (Edwards *et al.*, 1993). Midline cells rely for their development on *slit* and on the genes of the *spitz* group. In some mutants such as *single-minded* and *slit*, midline precursors die or do not differentiate further, whereas in others, such as *Star* and *pointed (pnt)*, they differentiate but fail to migrate (Klämbt *et al.*, 1991).

The two earliest genes expressed specifically in the glial lineage, *glide* and *repo*, encode nuclear factors. Apart from these common features, these genes behave rather differently. *glide* codes for a 504 aa protein with no apparent homology with other known gene families and is expressed only transiently during development (Hosoya *et al.*, 1995; Jones *et al.*, 1995; Vincent *et al.*, 1996; Verdeil, Vivancos and Giangrande, in preparation; see for reviews Anderson, 1995; Pfrieger and Barres, 1995). Its absence leads to embryonic lethality most likely due to the absence of glial cells, the phenotype that gives the name to the gene (Fig. 4). In *glide* homozygous embryos glial cells do not differentiate because they are transformed into neurons indicating that *glide* acts as a molecular switch in the determination of cell fate in the nervous system (Fig. 5). Once a precursor cell in the nervous system expresses *glide* it becomes committed to the glial fate and activates the glial differentiation program. Therefore, the fate of this multipotent precursor depends on the activity of a single gene. In addition, if *glide* is expressed at ectopic positions, it induces glial cell differentiation at those positions. All the glial cells expressing *glide* seem to be affected in the mutant, indicating that *glide* functions as a general glial promoting factor. Putative targets for *glide* have already been identified. In *glide* mutants early glial markers such as *repo* and a highly divergent homeobox protein, *prospero*, expressed at early stages of longitudinal glial cell development (Doe *et al.*, 1991), are not expressed. Molecular analyses now in progress will help us to determine whether indeed these two genes are direct targets of *glide*. *glide* is also expressed in peripheral and central glial cells of the postembryonic nervous system (Giangrande *et al.*, 1993; Giangrande, unpublished results). It will be of great interest to determine its role in these cells.

In vertebrates, glial growth factors (GGFs) seem to play a similar role to *glide*. A single *GGF* gene encodes alternatively spliced ligands for tyrosine kinase receptors, which are expressed on glial cells (Marchionni *et al.*, 1993). Schwann cells, the vertebrate peripheral glial cells, originate from a multipotent precursor present in the neural crest. This transient structure during development gives rise to several cell types (see for reviews McConnell, 1991; Stemple and Anderson, 1993; Le Douarin *et al.*, 1994). The fate of clonal cultures of neural crest cells can be manipulated by treatment with GGF (Lemke and Brockes, 1984; Marchionni *et al.*, 1993; Shah *et al.*, 1994). Under basal growth conditions, most clones, after 16 days of culture, contain both neurons and Schwann cells, whereas only a few contain exclusively Schwann cells. Addition of GGF reverses the clone phenotype: now the majority contain just Schwann cells, while only a few are mixed. These findings indicate that, as for other cell types, the cellular mechanisms of glial development are conserved throughout evolution.

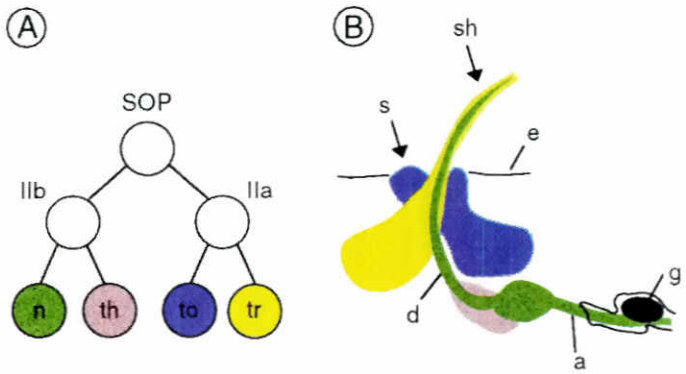

Fig. 3. Schematic representation of the lineage of a sensory organ. (A) *The external sensory organ, which performs mechano- or chemoreceptor function, originates from the sensory organ precursor cell (SOP). The SOP divides and gives rise to two second order precursor cells, PIIa and PIIb, which divide and give the four cells of the sensory organ: n, neuron (green); th, thecogen (pink); to, tormogen (blue); tr, trichogen (yellow).* **(B)** *Tormogen and trichogen secrete the cuticular structures of the sensory organ: the socket (s) and the shaft (sh), respectively. Note that the thecogen cell surrounds the neuronal soma and the dendrite (d) whereas the glial cell (g) is located along the axon(s) (a). (e) indicates the epithelium surface. Chemoreceptors are multiinnervated organs which develop in a similar way but require additional cell divisions to obtain the correct number of neurons (4-5).*

repo is a homeobox-containing gene that starts being expressed at early stages and remains expressed throughout development (Campbell *et al.*, 1994; Xiong *et al.*, 1994; Halter *et al.*, 1995). In *repo* mutants early glial markers such as *glide* are expressed while late markers are not. These features suggest a role in glial fate differentiation rather than determination.

All the other genes so far identified are expressed in subsets of glial cells and/or are expressed at later stages of development relative to *glide*. As mentioned above, *prospero*, a gene required for neuronal identity, is expressed in longitudinal glial cells. In the absence of *prospero*, glial cells differentiate but display an abnormal spatial organization (Doe *et al.*, 1991; Vaessin *et al.*, 1991). Another gene expressed in subsets of glial cells is *pointed (pnt)*, a locus that encodes two proteins by differential splicing: P1, expressed specifically in longitudinal glial cells and P2, expressed in midline glial cells (Klämbt, 1993). These two proteins share a region homologous to the ETS domain, a DNA binding domain present in a family of proteins related to the *ets* oncogene (Karim *et al.*, 1990; Klaes *et al.*, 1994). Interestingly, although the P1 product does not seem to be required for glial determination, its ectopic expression leads to the appearance of glial markers at ectopic positions, suggesting that it is sufficient for the expression of at least some glial properties (Klaes *et al.*, 1994). *glide* seems to

Fig. 2. Glial cell organization in the pupal wing. *Neuronal and glial organization in the rA87 enhancer trap line as detected by the neuronal specific antibody anti-HRP* **(A)** *and by anti-ß-gal* **(B)**. *L1 and L3 indicate the two major wing nerves located in veins L1 and L3. Arrows indicate glial nuclei all along the nerves (taken from Giangrande, 1995). Bar, 50 μ.* **(C)** *Proximal wing region labeled with Mab 5B12, an antibody that recognizes glial cell surfaces. Note the presence of the glial sheath around all wing nerves: L1, L3, and the more proximal costal (c) and radius (r) nerves.* **(D)** *Mab 5B12 labeling realized on a Hairy-wing (Hw) pupal wing. Hw mutation induces ectopic proneural gene expression, which determines glial differentiation at ectopic positions (arrows), see labeling on the anterior cross vein (acv), L2 (L2) and L4 (L4) veins, which do not normally contain glial cells.* **(E)** *EM section of a wildtype pupal wing: L1, L1 nerve; ve, vein epithelium; g, glial cells. The arrowhead indicates an axon. Note the contact between glial and epithelium cells (asterisk) and the basal lamina secreted by the glial cell (arrow). Bar, 1 μ.*

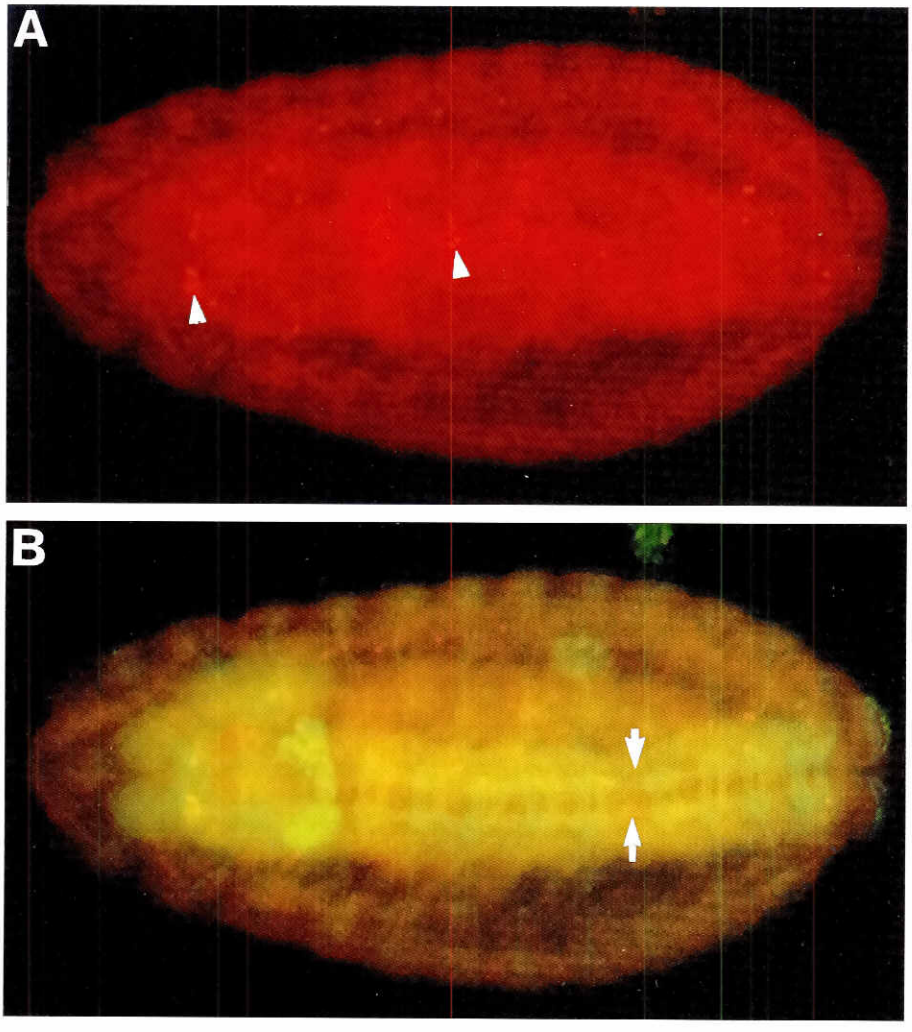

Fig. 4. Glial and neuronal organization in *glide* embryos. *Mutant embryo labeled with the glial marker RK2* **(A)** *or with RK2 and the neuronal marker anti-HRP* **(B)** *(taken from Vincent et al., 1996). Note that only very few RK2 positive cells (arrowheads) are present in the mutant, compared with the wildtype in Figure 2. The allele shown in* **(A)** *is a hypomorph, no glial cells are present in a glide null background. The ventral cord displays breaks in the longitudinal connectives (arrows).*

be upstream of *pnt* in the cascade that leads to glial differentiation because in these ectopic expression experiments, *glide* is not activated ectopically. Finally, it has recently been shown that huckebein, a zinc finger transcription factor (Weigel *et al.*, 1990), is required for the development of two glial cells from the neuroblast 1-1 lineage. In the mutant, additional neurons seem to be produced at the expense of subperineural glial cells A and B (Bossing *et al.*, 1996).

Gliogenesis: crosstalk between glial and neuronal cells

The heterogeneity observed in the morphology and the position of glial cells is also reflected in their mode of differentiation. Some glial cells such as the adult laminal glial cells in the optic lobe (Winberg *et al.*, 1992) as well as the embryonic longitudinal and midline glial cells (Jacobs *et al.*, 1989; Klämbt *et al.*, 1991) seem to originate from a dedicated glial precursor whereas others, such as some subperineural cells, originate from a precursor, neuroblast 1-1, that also gives rise to neurons (Udolph *et al.*, 1993). The existence of a mixed lineage suggests that glial and neuronal differentiation are linked, which is also the case of some glial and neuronal cells in grasshopper (Condron and Zinn, 1994). Condron *et al.* (1994) have shown that the *engrailed* gene is required for the early neuronal to glial transition that takes place in the grasshopper median neuroblast (MNB) lineage whereas activation of protein kinase A is responsible for the glial to neuronal transition that occurs in the late phases of this lineage (Condron and Zinn, 1995).

The existence of a multipotent precursor in the *Drosophila* nervous system is also confirmed by the glial to neuronal fate switch observed in *glide* embryos (Fig. 5) (Hosoya *et al.*, 1995; Jones *et al.*, 1995; Vincent *et al.*, 1996). Mixed lineages have also been observed in vertebrates. When *in vivo* or *in vitro* lineage analyses are performed on cells that are derived from the neural crest, cerebral cortex or the spinal cord, mixed as well as pure clones have been observed, implying the existence of a common precursor for glial and neuronal cells (see for reviews McConnell, 1991; Jessen and Mirsky, 1992; Stemple and Anderson, 1993; Le Douarin *et al.*, 1994; Miller, 1996).

Similarly to what has been observed in the embryo, tight association between neurons and glial cells has been observed in the development of adult peripheral glial cells. Neuronal differentiation is under the control of two classes of genes, the proneural and the neurogenic genes (Giangrande and Palka, 1990; Campos-Ortega, 1994) whose mutations display opposite phenotypes. Loss of function proneural mutations lead to loss of neural tissue due to the lack of neural precursor development while gain of function mutations inducing ectopic expression of proneural genes

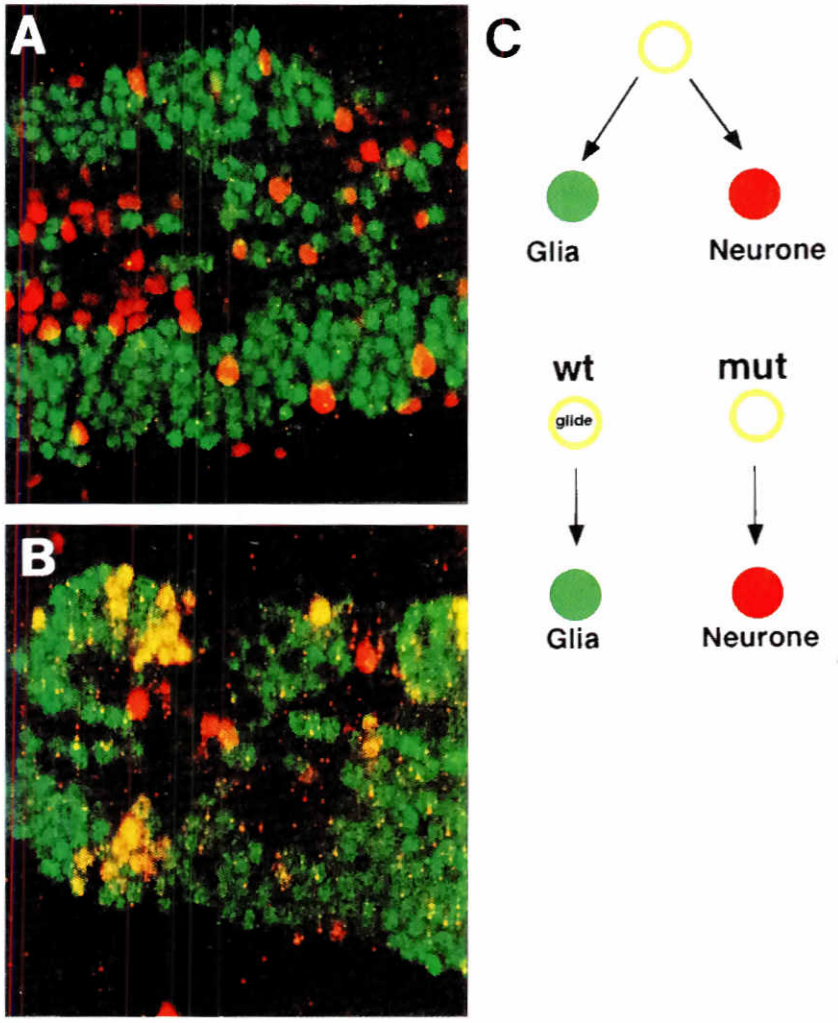

Fig. 5. Glial cells are transformed into neurons in *glide* embryos. (A and B) *Optical sections taken along the ventral cord of a mature embryo labeled with the neuronal marker anti-elav (green) and with anti-ß-gal (red). Note that both antigens are nuclear.* **(A)** *Labeling profile in the glial specific enhancer trap line rA87. Glial cells do not express the neuronal marker.* **(B)** *Labeling profile in a glide loss of function mutant. In this allele, obtained by imprecise excision of the P element contained in rA87, ß-gal is still expressed, even though glial cells do not differentiate. ß-gal positive cells now express the neuronal marker (orange nuclei), indicating that a cell fate switch has occurred in the absence of the glide product.* **(C)** *Schematic representation of the role played by glide in the establishment of the glial fate. A multipotent cell in the nervous system gives rise to a neural or to a glial cell depending on the activity of glide. In the mutant, the absence of the glide product leads to the inactivation of the glial developmental program and to the activation of the neuronal program.*

lead to ectopic sensory organ development. Loss of function neurogenic mutations lead to neural hyperplasia, due to an increase in precursor number. Glial cell development also requires the activity of these two classes of genes and the effects of the two classes of mutations on glial cells parallel those observed for neurons (Fig. 2D) (Hartenstein *et al.*, 1992; Nelson and Laughon, 1994; Giangrande, 1995; Giangrande, unpublished observations). However, the mechanism of action on the two cell types seems to be different. While neural precursors express the proneural genes, glial precursors do not accumulate the products of these genes at detectable levels (Giangrande, 1995). This suggests that the role of proneural genes in gliogenesis is indirect. Specifically, in the case of proneural mutations, glial cells do not differentiate because the neural lineage is affected, which implies that the development of glial cells depends on that of sensory organs.

The requirement for cell-cell communication and the activity of growth factors in vertebrate glial proliferation has already been documented (see for reviews Reynolds and Woolf, 1993; Barres and Raff, 1994). Indeed, oligodendrocyte precursor cells proliferate in response to the growth factors PDGF, bFGF, NT-3 and IGF-1 expressed by astrocytes. In the peripheral nervous system, Schwann cell proliferation is regulated by PDGF, bFGF, TGFß and by GGFs, which are mitogens of neuronal origin.

Neuron-glial cell interactions must also occur at later stages during glial development. The evidence for this comes from the observation that, as in vertebrates, fly glial cells are able to migrate. Glial migration in the peripheral nervous system has been investigated in lineage analyses using genetically labeled mosaics. This approach combines the use of germ line transformation via the P element and the FLPase system. Golic and Lindquist (1989) have shown that, as in yeast, the FLPase recombinase induces recombination between its target sites termed FRTs (Fig. 6). In the "FLP out" construct devised by Struhl and Basler (1993), a P element contains the gene coding for ß-gal downstream of FRTs. The P element has been engineered so that ß-gal is expressed only in cells in which recombination occurs between the two FRTs as well as in their progeny. Therefore, ß-gal provides a clonal marker that can be used in lineage analyses. This genetic approach has made it possible to follow the origin and the pattern of glial migration in the wing, a tissue that is not easily accessible to *in vivo* lineage analysis using HRP or fluorescent markers because of the pupal cuticle. The clonal analysis has demonstrated that wing glial cells originate from the underlying epithelium and migrate along the sensory nerve (Giangrande, 1994). Although it seems that only some cells migrate and that the extent of migration is variable, the direction taken by glial cells is always that taken by the growing axons.

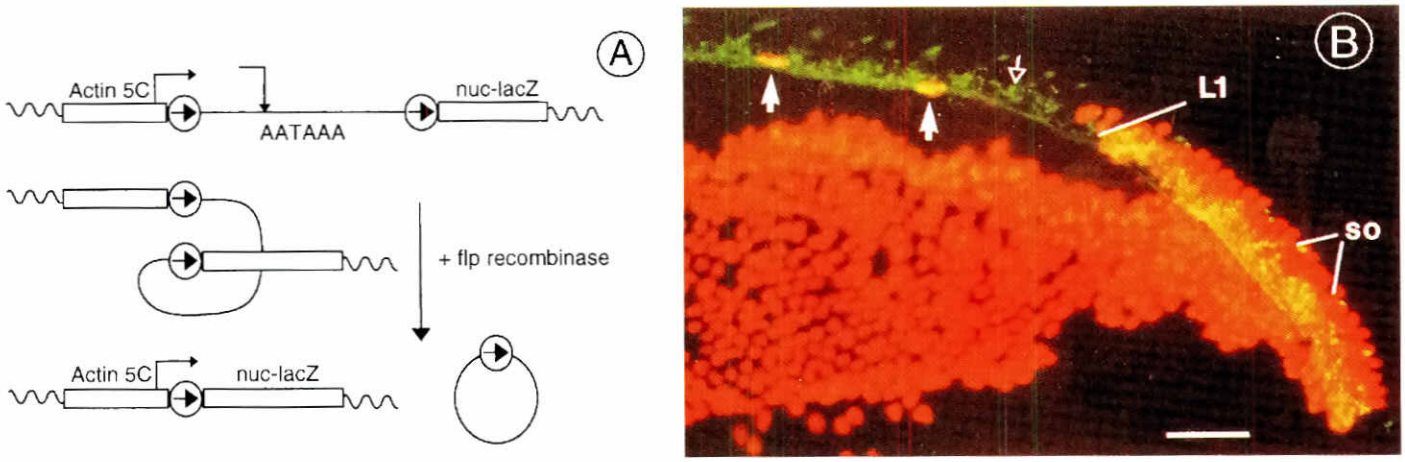

Fig. 6. Clonal analysis using the FLPase recombinase. (A) *The "FLP out" transposon harboring the FLPase target sites (FRTs) is integrated in the genome. FRTs (→) separate the LacZ gene from an Actin5C ubiquitous promoter and hence prevents LacZ transcription. Sequences between FRTs contain a transcription termination site which eliminates the possibility of readthrough of the polymerase. The LacZ gene carries sequences that localize the ß-gal product in the nucleus (nuc-lacZ). On another chromosome is integrated a P element carrying a heat shock promoter fused with the FLPase coding sequences. Heat induction leads to intrachromosomal recombination between the FRTs. As a consequence, LacZ is expressed in the cell in which recombination has taken place as well as in its progeny.* **(B)** *Example of a clonal analysis in the wing. A clone of ß-gal expressing cells (red) can be seen in the distal part of a pupal wing. Two glial nuclei (filled arrows) expressing ß-gal and therefore belonging to the clone are found outside of the clone, along the L1 nerve (green) (see empty arrow). This indicates that glial cells originate from wing epithelial cells and that they are able to migrate during development. In this protocol, recombination is induced at early stages of development, at a stage at which the cells that will give rise to the wing are actively proliferating. The fate of wing cells is analyzed at later stages, when all the cell types have differentiated. (so) indicates cells of the sensory organs. Bar, 25 µ.*

Moreover, mutations that alter axonal navigation also induce defects in glial migration. The association between glial migration and axonal navigation has also been observed in the case of retinal basal glial cells, a population of cells that seems to migrate from the optic stalk into the eye disc, the tissue that will give rise to the adult eye (Choi and Benzer, 1994). Although migration has been demonstrated in vertebrate glial cells, the mechanisms that regulate it are still poorly understood. Thus, it will now be possible to use fly glial cells in order to dissect the mechanisms that regulate cell migration.

A remarkable contribution to the understanding of glial migration comes from the analysis of midline glial cells which undergo a complex and stereotyped pattern of migration (Klämbt *et al.*, 1991). Several genes seem to be necessary for proper midline glial cell migration: *orthodenticle, pointed, spitz, drifter and breathless.* Interestingly, *drifter* and *breathless*, encoding respectively a POU-domain transcription factor and a FGF receptor homolog, are also required for tracheal cell migration, suggesting that common mechanisms control migration of different cell types (Klämbt *et al.*, 1992; Anderson *et al.*, 1995).

Finally, it has been proposed that cell-cell interactions prevent programmed cell death in the nervous system (Raff *et al.*, 1993). In *Drosophila*, half of the midline glial cell population dies by apoptosis after separation of the commissural axon tracts (Sonnenfeld and Jacobs, 1995). A number of observations suggest that axon-glial cell contacts prevent this death. Similarly, oligodendrocyte degeneration in transected rat optic nerves can be rescued by treatment with growth factors such as PDGF, IGF-1 and NT-3. Furthermore, embryonic Schwann cell precursors die *in vitro* unless exposed to bFGF which is normally secreted by axons (Barres *et al.*, 1992, 1993; Jessen *et al.*, 1994; see for reviews Barres and Raff, 1994). It has been suggested that programmed cell death regulates cell number and eliminates inappropriately placed cells. In the nervous system it may serve to conserve the proper ratio between glial cells and neurons so that the number of glial cells matches the length of the axons to be wrapped (Raff *et al.*, 1993).

Supportive and active roles of glial cells

The name "glia" has a Greek origin and means glue or cement. Glial cells originally were thought to play a passive role by providing a compact structure to the nervous system. Considerable evidence now points to glial cells playing a number of active roles. In vertebrates, the insulating sheath provided by glial cells ensures a correct transmission of the electrical signal along neurons. Some central glial cells are required during development for neuronal migration while Schwann cells are necessary for axonal regeneration upon wounding. Furthermore, the presence of different types of ion channels on glial cell membranes (for reviews see Barres, 1991; Ritchie, 1992) might also suggest additional regulatory roles. The increasing number of fly mutations affecting glial cell development and organization should make it possible to assess unambiguously the roles of these cells and to identify glial regulatory genes. As in vertebrates, fly glial cells provide electrical insulation. Gliotactin, a protein expressed in peripheral glial cells, belongs to a family of transmembrane proteins that mediate cell-cell or cell-matrix interactions (Auld *et al.*, 1995). Embryos mutant for the gliotactin product are paralyzed due to the presence of gaps and openings in the glial wrappings. Glial cells form an incomplete sheath around the axons, which are thus exposed to high K^+ extracellular environment. As a result, action potentials cannot propagate. Defects in electrical insulation and K^+ buffering have also been proposed to explain the reversed polarity of the

electrophysiological response to light observed in a viable *repo* allele (Xiong *et al.*, 1994). The loss of electrical insulation between retina and lamina normally provided by satellite and subretinal glial cells as well as the failure of laminal glial cells to buffer K^+ provided by laminal neurons could account for the abnormal electroretinogram in this mutant.

The analysis of embryos mutant for glial specific genes has shown that glial cells are necessary for several aspects of nervous system organization such as axonal fasciculation and nerve cord condensation (Campbell *et al.*, 1994; Xiong *et al.*, 1994; Halter *et al.*, 1995; Hosoya *et al.*, 1995; Jones *et al.*, 1995; Vincent *et al.*, 1996). Longitudinal glial cells prefigure the nervous system scaffold before axonal navigation (Jacobs and Goodman, 1989). In *glide* mutants, the longitudinal connectives display extensive breaks and axons do not leave the CNS at their normal positions (Vincent *et al.*, 1996) (Fig. 4). The penetrance of this defect is not complete which may be interpreted in at least two ways. One possibility is that glial cells are necessary but not sufficient for the correct formation of axonal tracts. Alternatively, in the mutants, even though transformed into neurons, these cells provide the axons with the right guiding cues. In this respect, another powerful approach to the analysis of fly development is genetic cell ablation induced by toxins or cell death genes (Brand and Perrimon, 1993). This method, also based upon germline transformation with engineered transposons, does not require the use of lasers or other invasive treatments. Hidalgo *et al.* (1995) have used this approach to investigate the role of interface glial cells on axon tract formation. They generated a line carrying a transposon which incorporates the UAS (the binding site of the GAL4 transcriptional activator), fused upstream of the coding sequences for ricin A toxin. Ricin toxin inactivates eukaryotic ribosomes through depurination of 28S ribosomal RNA, which thereby irreversibly inhibits protein translation (Endo and Tsurugi, 1988). A second line is an enhancer trap line in which the GAL4 protein is specifically expressed in interface glial cells (which include longitudinal glial cells). Death of interface glial cells obtained by crossing the two lines leads to selective loss of the longitudinal tracts confirming the essential role of glial cells in the formation of axonal tracts.

Glial cells are also required for neuronal survival. In vertebrates it has been shown that Schwann cells secrete BDNF, a growth factor that promotes the survival of motoneurons and placode-derived sensory neurons (Sendtner *et al.*, 1992). In addition, GDNF, a distant member of the transforming growth factor-ß family purified from a glial cell line, acts as a potent survival factor for motoneurons (Henderson *et al.*, 1994). Finally, astrocytes and Schwann cells produce CNTF, a cytosolic molecule that prevents motor neuron degeneration (Masu *et al.*, 1993). Similarly to vertebrates, fly glial cells appear to be necessary for the maintenance of the nervous system. In a *repo* mutant, neurons in the laminal region of the optic lobe undergo cell death (Xiong and Montell, 1995). Brain degeneration has also been observed in another mutant, *dropdead*, in which the primary defect seems to reside in glial cells (Buchanan and Benzer, 1993). A role in neuronal survival has also been observed in the ventral cord of *glide/gcm* mutants (Jones *et al.*, 1995; Vincent *et al.*, 1996; Giangrande, unpublished observations).

Finally, it has been proposed that anachronism, a glycoprotein produced by central glial cells after embryonic neuroblast proliferation, regulates postembryonic development of neuroblasts in the brain and in optic lobes (Ebens *et al.*, 1993). In *anachronism* mutants, the neuroblast number and identity are not affected, but neuroblasts proliferate precociously.

Concluding remarks

The availability of glial specific markers and mutations has proved crucial for the understanding of fly glial cell organization and development. It has also been possible to establish parallels between insects and vertebrates and to show that the mechanisms of glial development are conserved throughout evolution. *Drosophila* provides a unique model system in which to study the cellular and molecular biology of glial differentiation and function. The results obtained so far have also raised a number of questions with respect to the roles of cell-cell interactions and to the function of different genes in gliogenesis. Also, it will be extremely important to determine whether fly glial cells also play a role in neurite regeneration as has been shown in the mammalian peripheral nervous system and, more recently, in larger insects (see for reviews Bunge, 1993; Smith *et al.*, 1991). Finally, defects in glial development are implicated in a number of diseases. Amongst these are gliomas, the most frequent and aggressive forms of cancer in the nervous system. Thus, understanding the molecular cascade of events involved in glial development will help to answer some fundamental questions of developmental biology but will also shed some light on the fundamental mechanisms that lead to abnormal glial proliferation and differentiation underlying disease.

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Summary

Glial cells constitute a crucial component of the nervous system. They wrap the neuronal somata and axons and play a number of roles during normal neuronal development and activity as well as during axonal regeneration after wounding. The availability of cellular markers and genetic tools have made it possible in *Drosophila* to start identifying the genes and the cell-cell interactions leading to glial cell differentiation. The existence of multipotent precursor cells in the nervous system, the requirement for master genes determining the glial cell fate, the migratory abilities of fly glial cells and the existence of neuron-glial cell interactions during development are some of the features revealed by these approaches. These findings also indicate an evolutionary conservation in the developmental mechanisms between invertebrates and vertebrates. Finally, *Drosophila* is an ideal model system to determine *in vivo* the precise roles of glial cells and to study the etiology of pathologies associated with abnormal glial differentiation.

KEY WORDS: *glial cells, gliogenesis, Drosophila melanogaster, neural development, glide/gcm*

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