Glial differentiation does not require a neural ground state

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SUMMARY

Glial cells differentiate from the neuroepithelium. In flies, gliogenesis depends on the expression of glide/gcm. The phenotype of glide/gcm loss- and gain-of-function mutations suggested that gliogenesis occurs in cells that, by default, would differentiate into neurons. Here we show that glide/gcm is able to induce cells even from a distinct germ layer, the mesoderm, to activate the glial developmental program, which demonstrates that gliogenesis does not require a ground neural state. These findings challenge the common view on the establishment of cell diversity in the nervous system. Strikingly, ectopic glide/gcm overrides positional information by repressing the endogenous developmental program. These findings also indicate that gliogenesstightly depends on glide/gcm transcriptional regulation. It is likely that glide/gcm homologs act similarly during vertebrate gliogenesis.

Key words: glial cell deficient/glial cell missing (glide/gcm), Glial differentiation, Cell fate, Drosophila, Neurogenesis

INTRODUCTION

Nervous system development takes place after positional information has been laid along the dorsoventral axis. In Drosophila, maternal information provided by a gradient of nuclear dorsal protein subdivides the embryo into ventral, lateral and dorsal territories giving rise respectively to mesoderm, neurogenic region and dorsal ectoderm and amnioserosa (Rusch and Levine, 1996). The initial subdivision is then maintained by the expression of zygotic genes. The definition of the neurogenic region relies on decapentaplegic (dpp) and short gastrulation (sog) (Biehs et al., 1996; for a review, see Bier, 1997). dpp in dorsal ectoderm represses genes that induce neural development. sog in lateral stripes blocks dpp and permits neurogenesis. Similarly, dpp homolog BMP4 suppresses neurogenesis in early frog development while chordin, the sog homolog, blocks BMP4 activity (Sasai et al., 1995; Wilson and Hemmati-Brivanlou, 1995).

Importantly, once the neurogenic region is defined, precursor cells located at stereotyped positions proliferate and differentiate into neurons and glial cells. We and others have shown that glide/gcm is required for the differentiation of all glial cells in the peripheral and in the central nervous systems except for midline glial cells (Hosoya et al., 1995; Jones et al., 1995; Vincent et al., 1996; for reviews see Pfrueger and Barres, 1995; Anderson, 1995; Giangrande, 1996). glide/gcm codes for a transcription factor (Schreiber et al., 1997) that is thought to activate the gli differentiation program and to repress the neuronal one (Giesen et al., 1997). In glide/gcm embryos, glial cells do not differentiate due to their transformation into neurons; in addition, ectopic expression of glide/gcm within the nervous system results in ectopic expression of repo, an early glial marker, and repression of neuronal markers, suggesting that neurons are converted into glial cells (Hosoya et al., 1995; Jones et al., 1995). Thus, despite the apparent fixed array of neurons and glial cells, most cells in the nervous system have the competence to take the glial fate if provided with glide/gcm. On the basis of the gain- and loss-of-function phenotypes, it has been proposed that the neuronal pathway constitutes a default route and that glide/gcm dictates the choice between neuronal and glial fate, which implies that gliogenesis can only occur in the neurogenic region (Anderson, 1995).

An alternative model proposes that the competence to adopt the glial fate is common to several cell types and that there is no requirement for a neural ground state, predicting that even cells outside the nervous system may adopt the glial fate if challenged with glide/gcm. To test this model, we used the Gal4 system (Brand and Perrimon, 1993). In this paper we show that ectopic glide/gcm induces expression of glial-specific genes in cells in which dpp normally inhibits neurogenesis and gliogenesis. Importantly, glide/gcm is also able to exert its differentiative function in a different germ layer, the mesoderm, indicating that a single gene is sufficient to activate the glial differentiation program. Mesodermal expression of glide/gcm results in the repression of mesoderm-specific genes and in the loss of muscle morphology, concomitant with ectopic expression of several glial markers, suggesting that glial cells are induced at the expense of mesodermal tissue. Thus, glide/gcm can redirect the differentiation programs induced by maternal and zygotic positional information. Altogether, these data strongly suggest that gliogenesis does not require a ground neural state and that glide/gcm transcription must be tightly regulated in order to ensure correct embryonic development.
MATERIALS AND METHODS

Stocks
Wild-type Sevelen stock was used as a control. Three independent transgenic UAS-glide/gcm lines, F18A, M21G and M24A (Bernardoni et al., 1997), were crossed with twist-Gal4 (Baylies and Bate, 1996), 24B (Brand and Perrimon, 1993), scs-Gal4 (gift from M. Mlodzik), elav-Gal4 (Luo et al., 1994) or with MD237, which reveals the wild-type pattern of pannier (Heitzler et al., 1996). MD237 expression pattern was followed using a UAS-lacZ line (Brand and Perrimon, 1993). The enhancer trap lines rC56 (Klaes et al., 1994) and AE2 (Auld et al., 1995) were used as markers for lateral glial cells, AA142, as a marker for midline cells (Klaes et al., 1994).

Immunohistochemistry and western blot
Embryos were labelled as described by Vincent et al. (1996). Antibodies against the following proteins were used: repo (1:150); MHC (1:1000); D-MEF2 (1:800); β-gal (Promega, Cappel) (1:1000); elav (1:50); HRP (1:500). Secondaries conjugated with Oregon Green, FITC, Cy3 or biotin were used at 1:500. Biotin-conjugated secondaries were revealed using the ABC kit (Vector) as described by Giangrande et al. (1993). Preparations were analyzed using conventional (Axiophot, Zeiss) or confocal (DMRE, Leica) microscopy.

Anti-glide/gcm was raised, as described by Bernardoni et al. (1997), against a peptide within the C-terminal region of the protein. Western blot was carried out as described by Bernardoni et al. (1997) using a 1:500 dilution. Signal quantitation was performed on a Biorad GS700 imaging densitometer.

RNA analysis
In situ hybridizations were performed on embryos after overnight collection as described by Lehmann and Tautz (1994). The probe was as in Bernardoni et al. (1997).

RESULTS

Expression of glide/gcm in mesoderm induces early and late glial-specific genes
Three UAS-glide/gcm lines (Bernardoni et al., 1997) were crossed with a twist-Gal4 line. The twist promoter drives Gal4 expression in all presumptive mesoderm and in mesodermal tissue (Baylies and Bate, 1996), two tissues in which glide/gcm is not expressed.

Western analyses performed on embryonic extracts with anti-glide/gcm antibodies revealed that the three lines express different levels of glide/gcm, UAS-glideF18A (F18A) and UAS-glideM21G (M21G) and F18A respectively, confirming the difference in transgene expression observed in western analyses. The embryonic progeny of these cross was labelled with a glial marker, anti-repo (Campbell et al., 1994; Halter et al., 1995), which recognizes a nuclear protein that is expressed, like glide/gcm, in all lateral but not in midline glial cells (Scholz et al., 1997) nor in mesoderm. repo expression starts slightly after that of glide/gcm and is abolished in glide/gcm embryos (Hosoya et al., 1995; Jones et al., 1995; Vincent et al., 1996). In addition, the repo promoter contains eleven glide/gcm binding sites (Akiyama et al., 1996). The fact that repo is most likely a target of glide/gcm enabled us to use the anti-repo antibody to trace ectopic glide/gcm activity.

Ectopic glide/gcm expression in the F18A line (Fig. 2A,B) led to repo expression at the position of the heart, a tissue of mesodermal origin (Fig. 2D). Furthermore, anti-repo labelling.
at the position of midline cells, which have a mesectodermal origin, was observed (Fig. 3A,B). The lateral glial identity of repo-positive cells at the position of the midline was confirmed with two enhancer trap lines: AA142, which specifically labels midline cells, and rC56, which labels all central but midline glial cells (Klaes et al., 1994). Indeed, AA142-positive cells are missing at the midline whereas ectopic cells are labelled with rC56 (Fig. 3C-F).

Strikingly, ectopic expression in the M24A line resulted in the activation of glial markers in many more cells. For example, repo-positive cells were detected at the position of the pharynx and in metameric stripes along the dorsoventral axis, at the position of somatic muscles (Fig. 2C). In order to quantify the effects of mesodermal glide/gcm activity we counted the number of repo-positive nuclei at the position of somatic mesoderm throughout the anteroposterior axis and at the position of pharynx muscles (shown in D). The positions of the heart (H), pharynx (PH) and somatic muscles (SM) are indicated. (E) 24B-Gal4/+; F18A/+ embryo. (F) 24B-Gal4/M24A embryo. Repo-positive cells are fewer in number in E and F compared to those in C and D. Bar 50 μm.

Fig. 2. twi-glide/gcm and 24B-glide/gcm expression induce anti-repo labelling in mesodermal derivatives. (A-C,E,F) Anti-repo labelling of stage 14 embryos; lateral views. (A) Wild type: repo is not expressed in the dorsal-most part of the embryo (asterisks). (B) twi-Gal4/+; F18A/+ embryo. Weak ectopic glide/gcm induces repo expression (open arrows in all panels) dorsally (D), at the position of the heart (shown in D) and laterally (L), in scattered cells. (C) twi-Gal4/M24A embryo. Strong ectopic glide/gcm results in repo expression at the position of somatic mesoderm throughout the anteroposterior axis and at the position of pharynx muscles (shown in D). (D) Anti-D-MEF2 labelling on wild-type stage 14 embryo. The positions of the heart (H), pharynx (PH) and somatic muscles (SM) are indicated. (E) 24B-Gal4/+; F18A/+ embryo. (F) 24B-Gal4/M24A embryo. Repo-positive cells are fewer in number in E and F compared to those in C and D. Bar 50 μm.
the glial program are only reached in subsets of glide/gcm expressing cells. Interestingly, glide/gcm ectopic expression in M21G is variable, some embryos appeared similar to F18A embryos, some to M24A embryos and others displayed intermediate phenotypes (Fig. 1 and data not shown). This is in agreement with the observations that the marker used to trace the insertion of the transgene, white, is also variably expressed in this line and that twi-Gal4/+; M21G/+ embryos display a variable phenotype with respect to ectopic repo expression.

Finally, the number of repo-expressing cells is higher in embryos carrying both M24A and M21G compared to that found in embryos carrying M24A alone (data not shown). In this combination, the repo profile of expression mimicks that of β-gal in twi-Gal4; UAS-lacZ embryos, which also supports the idea that the transgenic lines express threshold levels of glide/gcm (and thus activate repo) only in a subset of mesodermal cells.

Two additional glial-specific enhancer trap lines were used to confirm the results obtained with anti-repo: rC56 and AE2, which labels subsets of peripheral and central glial cells (Auld et al., 1995). The expression pattern of AE2 reflects that of gliotactin, a gene coding for a transmembrane protein expressed at late embryonic stages and required for blood-nerve barrier formation. Indeed, ectopic labelling was observed in twi-Gal4/UAS-glide embryos with both markers (Figs 4, 5). The number of AE2-positive cells is lower than that of repo-positive cells. This might indicate that only a subset of cells attain late differentiation or that, as gliotactin is normally expressed in a sub population of glial cells, only a fraction of ectopic cells takes that identity.

The above results clearly indicate that ectopic glide/gcm is sufficient to induce many aspects of the glial genetic program outside the nervous system, although we cannot formally exclude that ectopic glial cells may not be functional due to the lack of contact with neurons. Indeed, glial and neuronal cells interact with each other during development and ontogenesis.

**Fig. 3.** twi-glide/gcm expression induces lateral glial-specific genes in the midline. Ventral views of stage 13 embryos. (A,B) Anti-repo labelling in (A) a wild-type embryo, (B) twi-Gal4/+; F18A/+ embryo. In the wild type, repo expression is absent in the midline (indicated by the horizontal lines in the insets on the right, which show a higher magnification of the area above the vertical bars in A and B). MM-CBG indicates a class of lateral glia called medial-most cell body glia labelled by anti-repo: one MM-CBG is present per hemisegment in the abdomen (A1), two cells are present in the thorax (T3). In embryos expressing weak ectopic glide/gcm, repo is induced at the position of midline cells, see arrows. (C,D) Anti-β-gal labelling in (C) AA142/+ embryo; (D) twi-Gal4/+; F18A/AA142 embryo. Midline is indicated by an open arrow. Curved arrows show labelling in the salivary glands, present in both wild-type and mutant embryos, while midline-specific labelling is absent in D. (E,F) Anti-β-gal labelling, in (E) rC56/+ embryo and (F) twi-Gal4/+; F18A/rC56 embryo. (G-I) Anti-HRP labelling of stage 14 embryos, ventral view, showing the axonal scaffold organization. (G) Wild-type; (H) twi-Gal4/+; F18A/+, (I) twi-Gal4/M24A embryos, respectively. The white arrowhead indicates a complete commissure fusion, the black arrowhead indicates the almost complete lack of the posterior commissure; the broad arrows indicates the few commissure fibers left in an embryo expressing twi-glide/gcm strongly. Bars (A,B) 50 μm; (E-I, insets) 25 μm; (C,D) 62.5 μm.
and somatic mesoderm (see Bate, 1993). At the end of stage 10, the third mesodermal division has taken place as mesodermal cells move dorsally to cover the ectodermal territories. At the completion of germ band retraction, the visceral mesoderm is removed and midgut primordia fail to migrate, suggesting that visceral mesoderm is a required substrate for this movement (Azpiazu and Frasch, 1993). Likewise, in twi-glide/gcm embryos, attachment sites and syncitia in gut cells display severe disorganization, most likely as a consequence of defects in visceral mesoderm. Indeed, as the anterior and posterior midgut primordia grow out and migrate toward each other, they move over bands of visceral mesodermal cells and meet before completion of germ band retraction. Subsequently, the endoderm spreads towards the dorsal and the ventral sides to surround the yolk sac as a monolayer epithelium (see Bate, 1993 for a review). The phenotype of tinman embryos, in which the visceral mesoderm is removed and midgut primordia fail to migrate, suggests that visceral mesoderm is a required substrate for this movement (Azpiazu and Frasch, 1993). Likewise, in twi-glide/gcm embryos, gut cells accumulate in clusters instead of surrounding the yolk sac as a monolayer epithelium. Finally, the loss of muscle-specific features (myofilaments, attachment sites and syncitia) in twi-glide/gcm embryos was also confirmed at the ultra-structural level (data not shown).

twi-glide/gcm expression leads to severe defects in the ventral cord

The analysis of cross sections showed that the overall ventral cord morphology is altered in twi-glide/gcm embryos. In order to characterize the nature and the origin of the defects, we used several markers. This confirmed the presence of repo-negative cells at the midline and adjacent to it, most likely due to migration (Fig. 7B,C), demonstrating that a high number of cells do not activate the midline fate, even though we cannot exclude that some of them express an intermediate fate. Anti-elav labelling performed to identify neuronal cell bodies showed that elav expression is restricted to the ventral cord and to the peripheral nervous system, demonstrating that the effects of ectopic glide/gcm expression are limited to the glial lineage and that mesodermally derived cells do not simultaneously express glial and neuronal markers (Fig. 7F,G). The elav labelling also showed that the neurons are not compacted into unfused muscle cells.

To analyze further the mode of action of glide/gcm, we asked whether ectopic expression of glial-specific genes had occurred at the expense of mesodermal-specific genes. To this end, we determined the expression profile of two markers using mesoderm-specific antibodies in twi-Gal4/UAS-glide embryos. D-MEF2 is expressed in all muscle and cardial progenitors as well as in differentiated cells (Bour et al., 1995; Lilly et al., 1995) whereas MHC is only expressed in differentiated cells (Kiehart and Feghali, 1986). In transgenic animals, both antibodies labelled much fewer cells than in the wild type, indicating that mesodermal glide/gcm expression leads to the repression of muscle-specific genes (Figs 5, 6).

To determine whether glial-specific and mesodermal-specific genes are co-expressed, double labelling experiments with a glial (rC56) and a mesodermal (D-MEF2) marker were performed. The analysis of single sections revealed the presence of three cell types, those that express either the glial or the mesodermal marker, which constitute the majority of the cells, and those that express both markers (Fig. 5G-I), possibly identifying cells with an intermediate fate. These results strongly suggest that mesodermal cells fail to differentiate because they have been transformed into glial cells and that competition between two fates takes place upon ectopic glide/gcm activity.

A close inspection of mutant embryos revealed that the muscle layer is severely disrupted (Figs 6, 7). At the end of embryogenesis, somatic and visceral muscles can be easily identified at the inner surface of the epidermis and coating the gut, respectively. In wild-type embryos, body wall muscles display a stereotyped pattern along the anteroposterior axis and are organized in longitudinal, oblique and transverse muscles. In twi-glide/gcm embryos, muscle fibers are reduced in number and poorly organized (Fig. 6). Embryos expressing glide/gcm lack most fibers and display a significant number of round, unfused muscle cells.

These results were also confirmed by immunohistochemistry on embryonic sections (Fig. 7). In wild-type embryos, most repo-positive cells are in the central nervous system, with a few cells located along peripheral nerves. In twi-glide/gcm embryos, many repo-positive cells are present at the position of muscles and the body wall muscle layer normally underlying the epithelium is severely disrupted. In addition, gut cells display severe disorganization, most likely as a consequence of defects in visceral mesoderm. Indeed, as the anterior and posterior midgut primordia grow out and migrate toward each other, they move over bands of visceral mesodermal cells and meet before completion of germ band retraction. Subsequently, the endoderm spreads towards the dorsal and the ventral sides to surround the yolk sac as a monolayer epithelium (see Bate, 1993 for a review). The phenotype of tinman embryos, in which the visceral mesoderm is removed and midgut primordia fail to migrate, suggests that visceral mesoderm is a required substrate for this movement (Azpiazu and Frasch, 1993). Likewise, in twi-glide/gcm embryos, gut cells accumulate in clusters instead of surrounding the yolk sac as a monolayer epithelium.

Finally, the loss of muscle-specific features (myofilaments, attachment sites and syncitia) in twi-glide/gcm embryos was also confirmed at the ultra-structural level (data not shown).
laterally symmetric hemineuromeres and that the midline cannot be recognized (Fig. 7F,G). This is most likely the consequence of repo expression in the mesectodermal cells, which are normally required to guide commissural growth cones towards and across the midline. To demonstrate this, we used markers labelling the axonal commissures. Indeed, ectopic glide/gcm results in severe defects: lack of the posterior commissure, fusion of posterior and anterior commissures and lack of both commissures (Fig. 3G-I and data not shown), this last phenotype being observed in embryos expressing high levels of glide/gcm. Interestingly, such defects are similar to those observed in mutants affecting midline cell development. For example, orthodenticle and mutations in the EGF receptor pathway result in absence of specific midline cells and display lack of posterior

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**Fig. 5.** Expression of muscle- and glial-specific genes in the mesoderm of twi-glide/gcm embryos. Lateral views of stage 14 embryos, confocal images. (A,D,G) β-gal labelling of rC56-positive cells is shown in red, (C,F,I-K) D-MEF2 labelling of mesodermally derived cells is shown in green, (B,E,H) double labelling. (A-F,J,K) Total projections obtained by superposition of optical sections. (A-C) wt (rC56/+) , same embryo. D-MEF2 heart labelling is indicated by an asterisk. (D-I) twi-Gal4/M24A; rC56/+, same embryo. White lines in D indicate the region displayed in (G-I), which show a single optical section to allow a colocalization analysis. Open arrows in G indicate cells expressing only β-gal, arrows in I indicate cells expressing only D-MEF2, arrowheads in (G-I) indicate cells that express both markers. (J,K) twi-Gal4/+: F18A/+ , white lines in K indicate the region shown in J. Note that some labelling at the position of the heart is missing (star). The number of D-MEF2-positive cells decreases as the amount of glide/gcm increases (compare K and F). Bars (A-F,K) 50 μm; (G-J) 25 μm.
the effects of ectopic glide/gcm activity in another cell type. Dorsal ectoderm is subdivided into two lineages, the amnioserosa, which maps to the dorsal-most region of the embryo, and, adjacent to it, the dorsal epidermis. Both lineages are specified by the activity of dpp, which suppresses neurogenesis and gliogenesis. We used a panner-Gal4 (pan-Gal4) line (Heitzler et al., 1996), which drives expression in the dorsal epidermis and in the amnioserosa at late stages of embryogenesis (β-gal starts being detected at stage 12). Upon weak expression, ectopic anti-repo labelling was observed in several cell clusters of the dorsal epidermis (Fig. 8A,C,F). Upon strong expression, anti-repo labelling was observed in many more dorsal epidermal cells (Fig. 8E). Dorsal closure did not occur in many panner-glide/gcm embryos, resulting in swelling of all internal organs (data not shown), which most likely accounts for the lethality observed in such a cross and argues for the presence of defects in dorsal epidermis differentiation. In order to look at the morphology of the ectopic repo-positive cells, the labelling was performed simultaneously with anti-repo and with anti-β-gal antibodies in embryos carrying both UAS-glide and a UAS-lacZ transgene in which the β-gal has a cytoplasmic localization (Brand and Perrimon, 1993). This showed that, indeed, repo-positive cells display a typical elongated glial cell morphology (Fig. 8B,D,G). Note that, due to the uniform β-gal expression in dorsal ectoderm, morphology can only be scored in ectopic repo-positive cells that migrate out of this territory. Finally, the glial identity was also confirmed using the late AE2 marker (Fig. 8H,I). These results show that glide/gcm expression in non-neurogenic regions is sufficient to induce many aspects of glial differentiation.

The fact that repo-positive cells were not observed in the amnioserosa of most embryos (Fig. 8) may have several explanations. First, amnioserosa is the region of highest expression of dpp, therefore we may need more glide/gcm to activate glial-specific genes than that provided by the transgene. Second, the panner-Gal4 construct induces expression rather late in development, which may limit the responsiveness to glide/gcm, as already seen in the mesoderm. Finally, amnioserosa is made up of cells that do not divide. We have observed that ectopic activation of glial-specific genes driven by glide/gcm expression in post-mitotic cells is less efficient than expression in pre-mitotic cells of the neurogenic region. Indeed, embryos expressing glide/gcm early in the neurogenic region or in neurons express ectopic repo at the expense of the elav neuronal marker (Jones et al., 1995; Hosoya et al., 1995). However, early expression in the neurogenic region induces the fate change in many more cells than expression in post-mitotic cells (compare repo and elav labelling between sca-glide/gcm and elav-glide/gcm embryos in Fig. 9).

**DISCUSSION**

**Glial differentiation does not require a neural ground state**

Evidence from several sources suggest that a neural ground state is a prerequisite for gliogenesis. First, glial cells, which constitute the majority of cells in the vertebrate nervous system, arise from the neuroepithelium, a territory defined through the combined activity of neural inhibitory and
permissive factors (for a review, see Bier, 1997). Indeed, only the ventral region of the vertebrate neural tube gives rise to astrocytes and oligodendrocytes, suggesting that the competence to adopt the glial fate is restricted to a subset of cells of the developing embryo (Warf et al., 1991). Similarly, fly glial cells differentiate within the ventral cord at stereotyped positions. Second, gliogenesis and neurogenesis are tightly linked during development: glial and neuronal cells are controlled by the same genes and in many cases have a common precursor (flies: Condron and Zinn, 1994; Nelson and Laughon, 1994; Giangrande, 1994, 1995; Bossing et al., 1996; Schmidt et al., 1997; vertebrates: see for reviews, Jessen and Mirsky, 1992; Stemple and Anderson, 1993; Le Douarin et al., 1994; Miller, 1996). Finally, lack of glide/gcm activity in fly glial precursor cells results in a switch from the glial to the neuronal fate (Hosoya et al., 1995; Jones et al., 1995; Vincent et al., 1996). On the basis of these results, it was thought that glial determinants impose the glial fate on cells displaying a neural default state (Anderson, 1995), implying that glide/gcm would only promote gliogenesis in the neuroepithelium.

In this study we show that glide/gcm expression outside the nervous system activates the glial differentiation program. Cells from both dorsal ectoderm and mesoderm, two regions in which neuro- and gliogenesis are normally inhibited, express early and late glial markers when challenged with glide/gcm. These cells exhibit the glial phenotype without going through a neural state, since they never express neural-specific markers. Importantly, ectopic glide/gcm entails repression of the endogenous fate, suggesting that competition takes place between the local and the glial developmental programs. In addition, we have observed that even temporal cues are bypassed by glide/gcm, since ectopic glial cells differentiate earlier than the endogenous ones (data not shown). Our data clearly show that (i) the competence to adopt the glial fate is not restricted to cells of the neurogenic region, (ii) a neural ground state is not a prerequisite for the activation of the glial differentiation program, (iii) glide/gcm is able to redirect the local differentiation programs induced by positional information, (iv) glide/gcm is necessary and sufficient to activate the glial differentiation program, even though glial cells may require additional cues, such as the presence of nearby neurons, to be functional and/or to survive.

Finally, our results establish that glial differentiation tightly depends on the precise spatial and temporal regulation of glide/gcm transcription and explain the glide/gcm phenotype. During embryogenesis glide/gcm is expressed in glial precursors after the neurogenic region has been defined (Hosoya et al., 1995; Jones et al., 1995; Vincent et al., 1996).
In *glide/gcm* embryos, glial precursors adopt the neuronal fate because this is the only other fate they can take, due to their state of determination.

*glide/gcm* homologs have been identified in vertebrates (Akiyama et al., 1996; Altshuller et al., 1996; Kammerer and A. G., unpublished). Given the conservation of the basic developmental pathways throughout evolution, it is tempting to speculate that the mechanism of action of these genes is similar to that observed in *Drosophila melanogaster*.

**A single gene controls glial cell differentiation**

The fact that *glide/gcm* is sufficient to promote the glial
differentiation program ectopically and that the lack of its product removes all glial precursor cells indicates that a single gene controls gliogenesis. Indeed, when pointed, a gene expressed in a subset of lateral glial cells, is induced early throughout the nervous system, it induces a glial-specific marker in some cells, however the overall number of glial cells appears unaffected at the end of embryogenesis (Klaes et al., 1994). Furthermore, the mutant phenotype and genetic analyses indicate that pointed is downstream of glide/gcm (Klaes et al., 1994). This suggests that several genes are activated by glide/gcm and that only the simultaneous activation of all its targets, including repo and pointed, is able to induce gliogenesis. It is worth noting that neuronal differentiation seems to be regulated in a different way. Neurogenesis is under the control of a family of related genes, the helix-loop-helix transcription factors of the achaete-scute complex (ASC). Indeed, although a single member of the family is sufficient to induce ectopic neurons, loss-of-function mutations of any member do not eliminate all neural precursor cells, fostering the idea that cooperativity is required between several members of the family (Campuzano and Modolell, 1992).

Despite the differential responsiveness of tissues to glide/gcm, the most responsive one being the nervous system, it is clear that even cells from mesoderm, a different germ layer, can adopt the glial fate. It will be interesting to determine whether other genes inducing a cell-specific fate such as eyeless (Halder et al., 1995) and MyoD (Weintraub et al., 1991) behave similarly and whether the pan-neural genes (Bier et al., 1992; Brand et al., 1993; Roark et al., 1995), which are expressed in all neuronal precursors, convert cell types as divergent as mesoderm into neurons.

The precise role of glide/gcm in the repression of the mesodermal fate is still to be elucidated. The mechanisms that activate twi most likely still operate in the mesoderm, since in twi-glide/gcm embryos twi expression is not modified at detectable levels (R. B. and A. G., unpublished results). Based on the recent finding that D-MEF2, a direct target of twist (Cripps et al., 1998), is repressed in twi-glide/gcm embryos, we propose that ectopic glide/gcm interferes with the endogenous developmental program by competing with twist activity. This is also in agreement with the observations that twi expression is modulated in the mesoderm (Baylies and Bate, 1996) and that the competence of the different mesodermal derivatives to repress the endogenous program upon glide/gcm expression depends on the levels of twi present in the cell. For example, heart cells, which contain low amounts of twi, are more apt to repress the endogenous fate and to express repo compared to somatic muscle cells, which contain high amounts of twi and require high amounts of glide/gcm to repress the endogenous fate.

The finding that ectopic glide/gcm triggers transcription of downstream glial-specific targets raises an important question about the mechanisms governing cell-specific transcription. How can genes that are normally silent in mesoderm be switched on by the activity of a single transcription factor? An appealing explanation is that, like other activators (see for reviews Sauer and Tijan, 1997; Tsukiyama and Wu, 1997; Wade et al., 1997), glide/gcm may recruit coactivators that remodel nucleosomes in the regions containing glide/gcm targets and activate transcription of such genes. Interestingly, glide/gcm is less efficient when induced in mesoderm at late stages, which may be due to the reduced accessibility of the chromatin of glide/gcm targets over development. We propose that, as the mesodermal program is activated, a more stable repressive state in those chromatin regions is established and propagated throughout the cell cycle. The establishment of a stable structural difference on chromosomal domains has already been observed during vertebrate development (Wade et al., 1997). A role of the proliferative state in the establishment of a repressed chromatin state is also in agreement with the different degree of glial fate induction observed upon glide/gcm expression in pre- versus post-mitotic cells of the nervous system. Thus, understanding the glide/gcm mode of

![Fig. 9. glide/gcm expression in the neurogenic region and in neurons. Stage 15, ventral view embryos.](image)

(A,B) Wild-type, (C,D) sca-Gal4/M24A, (E,F) M24A/+; elav-Gal4/+ embryos. Embryos were double-labelled with antibodies against repo (A,C,E) and elav (B,D,F) to recognize glial and neuronal cells, respectively. Bar 50 μm.
action represents an extremely important step towards unravelling the general mechanisms of cell differentiation at a molecular level.

**glide/gcm and the choice of the cell fate**

Many genes inducing a specific developmental program are expressed and required in more than one cell type (Carmena et al., 1995; Halder et al., 1995; Callaerts et al., 1997). Similarly, glide/gcm is also expressed and required in the presumptive territory of hemocytes, insect scavenger cells that phagocytose dying cells (Bernardoni et al., 1997). A null glide/gcm mutation entails the loss of a subset of these cells. These observations clearly indicate that genes playing an instructive role can affect different cell fates, suggesting a context dependency. Preliminary results suggest that mesodermal expression of glide/gcm does not have the same effects with respect to glial and hemocyte differentiation (R. B. and A. G., unpublished results). In the future, it will be interesting to assess the precise role of glide/gcm in each differentiation program and to determine the molecular cues that dictate the choice between the possible fates.

The observation that the glial differentiation program can be activated outside the nervous system and that glide/gcm overrides the developmental programs induced by positional information clearly challenges the common view on the establishment of cell diversity in the nervous system. These results also open new perspectives on the understanding of the molecular mechanisms governing cell differentiation.

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