Glial development in the *Drosophila* CNS requires concomitant activation of glial and repression of neuronal differentiation genes

Kay Giesen1,*,‡, Thomas Hummel1,*,‡, Angelika Stollewerk1, Stephen Harrison2,†, Andrew Travers2 and Christian Klämbt1,‡,§

1Institut für Entwicklungsbiologie, Universität zu Köln, 50923 Köln, Germany
2Laboratory of Molecular Biology, Medical Research Council, Cambridge, CB2 2QH, UK

*Equal contribution to this work
‡Present address: Chiron Corporation, 4560 Horton Street, Emeryville, CA 94608, USA
†Present address: Institut für Neuro- und Verhaltensbiologie, Badestrasse 9, 48149 Münster, Germany
§Author for correspondence (e-mail: klaembt@mail.uni-muenster.de)

**SUMMARY**

Two classes of glial cells are found in the embryonic *Drosophila* CNS, midline glial cells and lateral glial cells. Midline glial development is triggered by EGF-receptor signalling, whereas lateral glial development is controlled by the *gcm* gene. Subsequent glial cell differentiation depends partly on the *pointed* gene. Here we describe a novel component required for all CNS glial development. The *tramtrack* gene encodes two zinc-finger proteins, one of which, ttkp69, is expressed in all non-neuronal CNS cells. We show that ttkp69 is downstream of *gcm* and can repress neuronal differentiation. Double mutant analysis and co-expression experiments indicate that glial cell differentiation may depend on a dual process, requiring the activation of glial differentiation by *pointed* and the concomitant repression of neuronal development by *tramtrack*.

Key words: *Drosophila*, CNS, glial cell, pointed, tramtrack, gcm, neuron

**INTRODUCTION**

Any functional nervous system of vertebrate or invertebrate origin requires the development and correct cellular interplay of numerous neuronal and glial cells. Since the first description of glial cells some 150 years ago, an increasing number of different functions have been attributed to them but relatively little is known about the mechanisms controlling glia development (Anderson, 1995). In insects, a variety of glial cells has been thoroughly described by conventional histological methods (Carlson and Saint Marie, 1990; Hoyle, 1986; Jacobs and Goodman, 1989). An even higher degree of glial diversity has been revealed in *Drosophila* with the help of the enhancer trap technique (O’Kane and Gehring, 1987). Such enhancer trap lines have been used to initiate analyses of glia development and function in some detail (for review see, Klämbt et al., 1996).

Based on position and gene expression, embryonic CNS glial cells can be divided into two major classes, the midline glia and the lateral glia. Four segmental midline glial cells are found in close association with the developing commissures (Bosson and Technau, 1994; Jacobs and Goodman, 1989). They are characterized by the expression of the EGF-receptor, *argos* and *pointedP2* (Freeman et al., 1992; Klämbt, 1993; Zak et al., 1990). The lateral glial cells are a more heterogeneous group of cells, characterised by the expression of *pointedP1*, *repo* and *gcm* (Campbell et al., 1994; Halter et al., 1995; Hosoya et al., 1995; Jones et al., 1995; Klämbt, 1993; Vincent et al., 1996; Xiong et al., 1994).

Glia in both embryos are required for the formation of the axonal scaffold of the ventral cord, which consists of two segmental commissures and longitudinal axon tracts connecting individual segments (Goodman and Doe, 1993). The commissures are brought into their final shape by migration of two of the four segmental midline glial cells in-between anterior and posterior commissure (Klämbt et al., 1991). To date several genes have been described that are required for development of these cells. Mutations in any of these genes generally lead to a characteristic embryonic CNS axon pattern phenotype of fused commissures (Klämbt, 1993; Klämbt et al., 1991; Raz and Shilo, 1992).

Among other functions, lateral glial cells are involved in the formation of the longitudinal axon tracts. Development of all lateral glial cells is under the control of the gene *gcm*. *gcm* encodes a nuclear protein and acts as a master regulatory gene of glial development. In its absence, glia-specific gene expression is abolished and glial cells are transformed into neuronal cell types (Hosoya et al., 1995; Jones et al., 1995), suggesting that *gcm* acts as a genetic switch between glial and neuronal cell fate. A candidate gene mediating subsequent glial cell differentiation has recently been identified. The two ETS proteins encoded by the gene *pointed* (*pnt*), *pntP1* and *pntP2*, are expressed specifically in glial cells within the developing embryonic CNS (Klämbt, 1993). Here they are necessary and sufficient for several aspects of glial cell differentiation (Klaes et al., 1994).

Glia in the nervous system. They are capable of controlling proliferation rates of
neighboring neuroblasts (Ebens et al., 1993) and are likely to secrete neurotrophic factors (Xiong and Montell, 1995). Furthermore neuron-glia interaction appears to be involved in the guidance of many neuronal growth cones, possibly through the induction of neuronal antigen expression (Klaes et al., 1994).

To understand the genetic network underlying glial cell development in Drosophila, we have taken a genetic approach and have screened about 17 000 EMS and 900 P-element-induced lethal lines (Karpfen and Spradling, 1992) for axon pattern phenotypes indicative of CNS glia defects (T. Hummel, K. Schimmelpfeng and C. Klambt, unpublished data). One complementation group identified by this approach was found to be allelic to the previously described gene tramtrack (ttk). ttk was initially isolated by virtue of its DNA-binding properties to the transcriptional regulatory region of fushi tarazu. tramtrack encodes two different zinc-finger-type transcription factors, ttkp69 and ttkp88, which share a common BTB protein-protein interaction domain and serve as transcriptional repressors (Harrison and Travers, 1990; Brown et al., 1991; Read and Manley, 1992; Bardwell and Treisman, 1994; Zollman et al., 1994). Mutations in ttk were first identified on the basis of their requirement during eye development, where ttk acts as a negative regulator of neuronal development (Xiong and Montell, 1993). During development of the embryonic peripheral nervous system (PNS), ttk is required for lineage decisions (Salzberg et al., 1994; Guo et al., 1995).

Here we describe the function of ttk during embryonic CNS development. Only one of the two ttk proteins, ttkp69, is expressed in the nervous system, where it is found exclusively in all non-neuronal cells. In contrast to its function in the embryonic PNS, ttk does not appear to influence glial cell lineage decisions in the CNS. It is however required for proper glial cell development in the CNS. To determine the relationship of tramtrack and pointed, we analyzed the phenotypes of doubly mutant embryos as well as of embryos ectopically expressing both ttk and pointed. Our data indicate the existence of two independent genetic pathways regulating glial cell development, which in the lateral glia are both downstream of gcm. One, mediated via pointed, results in the activation of glia cell differentiation, whereas the other, mediated via tramtrack, results in the suppression of neuronal differentiation in these cells.

MATERIALS AND METHODS

Genetic analysis

The tramtrack alleles have been identified in a large-scale screen for mutations affecting the development of the embryonic axon pattern. Mutagenized chromosomes were kept over balancer chromosomes, which allowed the identification of homozygous mutant embryos. Details of the screen will be published separately.

Antibodies and immunohistochemistry

Anti-ttkp69 polyclonal antisera were raised in rats. The R113 clone (Harrison and Travers, 1990) contains only sequences that are exclusive to ttkp69 and does not encode an epitope that is common to both the 69 kDa and the 88 kDa ttk protein. E. coli expressed R113 was purified by PAGE and injected every 4 weeks for a total of 12 weeks as an emulsion with Freund’s complete adjuvant. Anti-ttk-p88 antibodies (Read and Manley, 1992) have been used as described. The embryos were fixed for 10 minutes in 4% formaldehyde/PBS/n-heptane for anti-ttk stainings. Other antibody stains were performed as described (Klambt et al., 1991). The following antibodies were used: mAb22C10 (Fujita et al., 1982), BP102 (A. Bieker, N. Patel and C. S. Goodman, personal communication); ID4 (G. Held and C. S. Goodman, personal communication); anti-repo (Halter et al., 1995); anti-β-galactosidase (Cappel, USA). The embryos were mounted and dissected in 70% glycerol. To perform double-labelling experiments, we used alkaline-phosphatase and HRP-conjugated secondary antibodies. Confocal microscopy was done using FITC- and Texas-red-conjugated secondary antibodies (Dianova, Hamburg) on a Zeiss LSM.

DNA constructs and transgenic flies

To obtain UAS-ttkp69 and UAS-ttkp88 flies, full-length cDNA clones were inserted into the pUAST vector (Brand and Perrimon, 1993) and used for germ-line transformation. Several independent transformant lines were established for both constructs. The following GAL4 activator lines were used. The line sl-GAL confers specific expression predominantly in the midline glial cells (Scholz et al., 1997). To ectopically express the different proteins in the entire neuroectoderm, we used a GAL4 enhancer trap insertion into the scabrous gene (kindly provided by Dr Hinz, Köln). The Krüppel-GAL4 driver line was kindly provided by M. Leptin, Köln.

Electron microscopic analysis

Fixation, staining and sectioning was performed as described (Stollewerk et al., 1996).

RESULTS

Identification of a complementation group affecting CNS glia development

In wild-type embryos, most CNS axons are organized in a highly characteristic arrangement consisting of two segmental commissures and two lateral longitudinal axon bundles connecting the individual segments (Fig. 1A). To identify new components involved in the control of glia development, we screened a large number of EMS and P-element-induced mutant lines for alterations in the organisation of the axonal scaffold in the embryonic ventral nerve cord indicative of glial cell defects. In this way, we identified a complementation group consisting of 1 EMS and 5 P-element-induced alleles, we initially termed schmal. Subsequent meiotic mapping of the EMS mutation and the cytological position of the P-element insertions placed schmal alleles at 100D1-2, a position identical to that previously described for the tramtrack (ttk) gene. Complementation analysis confirmed that schmal and ttk are allelic. The organization of the tramtrack gene is shown in Fig. 2. tramtrack embryos are characterized by an embryonic CNS axon pattern phenotype of fused segmental commissures, indicating a requirement of tramtrack during midline glia development (Fig. 1B). The CNS phenotype of homozygous ttkD2-50 embryos is identical to the CNS phenotype of homozygous ttkB330 embryos (Salzberg et al., 1994). However, ttkD2-50 mutant embryos show a stronger increase in the level of 22C10 expression in the lateral body wall (see Fig. 2 for details).

In addition, in ttk embryos, longitudinal axon tract formation is impaired and the connectives appear thinner. This phenotype is indicative of a defect in the longitudinal glia (Jacobs, 1993; Klaes et al., 1994). Using mAb ID4 directed against fasciclinII (G. Held and C. S. Goodman, personal communication, kindly provided by C. S. Goodman) we further observed that, whereas
in the wild-type CNS, fasciclinII expression is confined to three fascicles in each longitudinal tract (Fig. 1C) (Grenningloh et al., 1991), in ttk embryos, a partial defasciculation of the fasciclinII-positive axon bundles is apparent (Fig. 1D). In wild-type embryos, mAb 22C10 staining can be detected in the PNS neurons and their axons. In addition, low levels of 22C10 expression are observed in a single muscle fibre (Fig. 1E). Mutant ttk embryos are characterized by highly elevated levels of 22C10 in all somatic muscles and many derivatives of the visceral mesoderm (Fig. 1F). By contrast, no increase in the level of 22C10 expression is found in the embryonic CNS of the mutants (Fig. 1G,H).

**ttkp69 is expressed in CNS glial cells**

The phenotypic analyses presented above suggest a requirement of ttk during glial cell development. To assay the expression of ttk within the developing CNS, we first analyzed the β-galactosidase expression pattern associated with the P-element-induced ttk alleles. The β-galactosidase expression pattern found in such enhancer trap insertion lines often mimics endogenous gene expression. β-galactosidase expression associated with the ttk10556 allele is found in the ectoderm, the trachea and the mesoderm (data not shown) but, in addition, is detected in the ventral nerve cord, where it is confined to lateral and midline glial cells (Fig. 3A).

To verify the glia-specific expression of ttk and to determine which of the two ttk proteins is expressed in the CNS, we used specific RNA in situ probes as well as antibodies directed against the ttkp88 (Read and Manley, 1992) and ttkp69 protein forms (see Materials and Methods, for details). Both tramtrack proteins are expressed in ectodermal, mesodermal and tracheal cells (Brown and Wu, 1993; Read et al., 1992). However neither RNA in situ probes nor specific antibodies detected ttkp88 expression within the ventral nerve cord. In addition, no embryonic CNS phenotype is associated with the ttk1 mutation, which specifically affects only ttkp88 expression (Xiong and Montell, 1993). This indicates that ttkp88 is not required for normal CNS development.

In contrast to ttkp88, ttkp69 expression in the developing embryonic CNS is observed from stage 12 onwards. In the midline, this expression is restricted to cells that occupy the characteristic positions of the midline glia. This identification was confirmed by the colocalisation of ttkp69 and β-galactosidase driven by the AA142 enhancer trap insertion, which directs β-galactosidase expression specifically to the midline glia (Fig. 4C, Klämbt et al., 1991). By stage 15, many cells in the lateral CNS express ttkp69. Based on their position within the developing nervous system, most of these cells appear to be glial (Fig. 3). Indeed, confocal microscopy demonstrated that all cells that express the glia marker repo (Halter et al., 1995), also express ttkp69 (Fig. 3C-E). In addition, the transverse nerve exit glial cells or DM cells (Chiang et al., 1994; Gorczyca et al., 1994) express ttkp69. Some CNS cells that express ttkp69 but do not express repo (Fig. 3C-E, arrowheads).
The observed increase of 22C10 expression in the lateral body wall of mutant ttk embryos suggested that ttk might act as a negative regulator of neuronal antigen expression. To test whether loss of ttk function had a similar effect in the CNS we analysed the expression of both 22C10 and elav, an early neuronal antigen (Robinow and White, 1988), in the glial cells by confocal microscopy. Neither 22C10 nor elav were expressed ectopically in glial cells (data not shown).

**Ultrastructural analysis of glial cells in mutant tramtrack embryos**

The above results indicated that ttk is expressed in CNS glial cells and is required for their development. To further characterize the glial cell phenotype, we analyzed different glial cells on a ultrastructural level. In wild-type stage 16 embryos, midline glial cells can be easily identified based on their ultrastructural phenotype. The number of ttk mutants, as in pnt embryos, is reduced in point mutants, which leads to glial cell defects

To follow glial cell development in mutant tramtrack embryos, we employed molecular markers specific for different CNS glial cell types. To assay the development of lateral glial cells, we have used anti-repo antibodies and the rA87 enhancer trap insertion into the gene glial cells missing (gcm), which leads to β-galactosidase expression in all lateral glial cells (Fig. 4A, Jones et al., 1995). Both probes gave identical results. Lateral glial cells are present in mutant ttk embryos, but reduced in number, and the normally stereotyped glia pattern is disturbed (Fig. 4B). The CNS of stage 16 ttk embryos contains about 20% less lateral glial cells than a wild-type CNS (4 wild-type neuronomers with an average number of 43 rA87-positive cells and 7 mutant ttk neuronomers with an average number of 34 rA87-positive cells were counted). To monitor midline glial cell development, we used the AA42 enhancer trap insertion line. In mutant ttk embryos, although the midline glial cells are initially present in normal number and position, they fail to perform their normal migration. By stage 16 only 1-2 AA42-positive cells can be detected in most segments in mutant ttk embryos. These cells are found displaced dorsally anterior to the anterior commissure (Fig. 4D). However, some neuronomers lack midline glial cells.

The ultrastructural phenotype of glial cells in ttk mutants is, however, different to the one observed in ttk mutants, as in pnt embryos exit glial cells are unable to properly ensheathe the axon bundles of both peripheral nerves (Fig. 5C).
We conclude from these observations that ttkp69 is required for the maintenance of glial development in the CNS but, in contrast to pnt, ttk neither determines glial differentiation, since many glial cells can still differentiate relatively normally in mutant ttk embryos, nor does it appear to influence lineage decisions in the CNS as it does in the developing PNS (Guo et al., 1995). Thus a possible role for tramtrack might be to block the execution of inappropriate differentiation pathways during glial development.

Ectopic ttkp69 expression blocks neuronal development

To analyze further the biological functions of tramtrack in differentiation of glial cells, we ectopically expressed the different ttk RNAs using the GAL4 system (Brand and Perrimon, 1993). Expression of UAS-ttkp88 in the neuroectoderm leads to a partial block of neuronal development in the PNS but not to any obvious defects in CNS development. Weak ttkp69 expression in the neuroectoderm results in similar
lesions in the PNS but, in addition, we always observed concomitant CNS defects, indicating functional specificity between the two ttk proteins (data not shown). Expression of higher levels of ttkp69 in the developing nervous system substantially reduced expression of the neuronal elav antigen. Since the expression of all other neuronal markers tested (fasciclinII, 22C10 and BP102) was similarly reduced, we concluded that ttk69 blocks neuronal development. This can be most clearly seen when ttkp69 is expressed only in the middle of the embryo using a Krüppel-GAL4 driver line (Fig. 6A). In contrast, expression of the glial cell-specific marker r150 (Klaes et al., 1994) did not appear to be blocked following ectopic ttkp69 expression in the entire neuroectoderm using the scta-GAL4 driver line (Fig. 6C,D). Segregation of neuroblasts and the production of ganglion mother cells appeared normal, as judged by the expression of snail or prospero (Alberga et al., 1991; Doe et al., 1991) (data not shown). Thus ttkp69 may act to block the competence of cells to differentiate as neurons within the CNS. In stage 17 embryos, expression of ttkp69 results in cell death within the nervous system.

A few neurons still differentiate following ttkp69 expression in the scabrous pattern, which, based on their position, appear to be MP2-like cells. Interestingly these cells express both the r150 glia marker and the 22C10 antigen (Fig. 6D, asterisk).

To address the question whether the ectopically expressed ttkp69 protein exerts its wild-type function, we expressed the ttkp69 protein in midline glial cells of homozygous mutant ttkB330 embryos using the sli-GAL4 driver line. Mutant ttkB330 embryos show a strong tramtrack CNS phenotype (Figs 1, 2). No rescue of the mutant tramtrack phenotype was observed using one copy of the sli-GAL4 driver line. However, with two copies of the sli-GAL4 driver line, partial rescue of the fused commissure phenotype of homozygous mutant ttk embryos was observed (Fig. 6B). This indicates that ectopically expressed ttkp69 protein presumably performs its normal functions and that the lack of complete phenotypic rescue can be attributed to low levels of directed ttkp69 expression in mutant tramtrack embryos or possibly to low levels of ttk expression in other midline cells.

gcm governs divergent pathways that control glia differentiation

In Drosophila, development of PNS glia as well as of all lateral glia depends on the function of the gene glial cells missing (gcm). In mutant gcm embryos, no glial cells except the midline glia develop. Expression of glia-specific genes like repo is abolished and glial cells are transformed into neuronal cells (Hosoya et al., 1995; Jones et al., 1995). gcm encodes a nuclear protein and exerts its function presumably through a direct regulation of glia-specific gene expression (Jones et al., 1995; Akiyama et al., 1996). One such target gene might be pnt and loss of pnt function leads to maldifferentiation of glial cells. Conversely, ectopic expression of pnt leads to the formation of extra glia-like cells in the CNS (Fig. 6E, Klaes et al., 1994).

In situ hybridization experiments showed that pointedP1 expression is under the control of gcm (data not shown). To determine whether ttk expression depends on gcm function as well, we have analyzed ttkp69 expression in gcm mutant embryos. In such embryos, ttkp69 protein expression appears unchanged in the midline cells (Fig. 3B). This is not surprising since gcm is not expressed in these cells (Hosoya et al., 1995; Jones et al., 1995; Vincent et al., 1996). However, although most of the lateral cells no longer express ttkp69, a few ttkp69-positive nuclei still remain in homozygous gcm mutant embryos (Fig. 3B). Since no lateral CNS cells express ttkp69 in embryos mutant for gcm and tracheless (which specifically removes the tracheal system (Jürgens et al., 1984, data not shown), we conclude that ttkp69 is expressed in all

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**Fig. 5.** Ultrastructural analysis of glial cells in mutant tramtrack embryos. Ultrastructural analysis of exit glial cells in tramtrackB330 and pointed88 mutant embryos. All embryos are stage 16. (A) Cross section through a wild-type CNS at the position where the segmental nerve exits the CNS. Arrow indicates the approximate plane of section in B-D). (B) Sagittal section of a wild-type embryo showing the intersegmental (IS) and segmental nerve (S) ensheathed by exit glial cells (eg). (C) Comparable sagittal section of homozygous pointed88 mutant embryo. The exit glial cells (eg) fail to extend cellular processes to properly ensheath the two nerve bundles. In the section shown, the segmental nerve lacks a glial sheath and contacts the hemolymph. (D) In mutant tramtrackB330 embryos, the exit glial cells form cellular processes that ensheath both the segmental and the intersegmental nerve.
Fig. 6. Genetic interaction of pointed and tramtrack revealed by ectopic expression and double mutant analysis.

(A-I) Frontal views of dissected stage 16 embryonic CNS preparations. The axonal scaffold is labelled using (B,G-I) mAb BP102 or (C-F) mAb 22C10 and subsequent HRP immunohistochemistry. (C-F) CNS glial cells are labelled by β-galactosidase expression conferred by the rI50 enhancer trap insertion. To achieve ectopic expression of the ttk proteins we used (A) a Krüppel-GAL4, (B) a sli-GAL4 or (D-F) a scabrous-GAL4 driver line.

(A) Lateral view of a stage 12 embryo expressing the ttk69 protein using the Krüppel GAL4 driver line stained for the expression of the elav protein. In wild-type embryos, all neuronal cells express the elav protein. Upon expression of ttk69 in the Krüppel domain (parasegments T2 to A4), neuronal differentiation was efficiently blocked. Only a few cells are still capable of expressing the elav protein (arrowheads). (B) To prove that we expressed the wild-type ttk69 function, we rescued the mutant ttk commissure phenotype, by expressing ttk69 specifically in the midline glial cells, using the sli-GAL4 driver line. Whereas mutant ttk B330 embryos display a typical fused commissure phenotype (see G), mutant ttk69 embryos expressing the ttk69 protein in the midline glial cells display separated anterior and posterior commissures (arrow heads denote separated commissures). The phenotypic rescue, however, is not complete. (C) A wild-type embryo carrying the rI50 enhancer trap insertion, stained for β-galactosidase expression (blue) and the presence of 22C10 antigen (brown). The rI50 enhancer trap insertion directly β-galactosidase expression to a subset of lateral glial cells (arrowheads). (D) Following expression of ttk69 in the entire neuroectoderm using the scabrous-GAL4 driver line, neuronal differentiation is impaired. Only few cells express the 22C10 antigen. Glial cell differentiation, as revealed by the β-galactosidase expression conferred by the rI50 enhancer trap line, appears relatively unaffected. Note that the few 22C10-positive cells also express the rI50 enhancer as well (asterisk). In late stage 16/17 embryos, cell death is observed. (E) Expression of pntP1 in the entire neuroectoderm results in the appearance of many ectopic rI50-positive cells. (F) Coexpression of both, pointedP1 and ttk69, similarly directs rI50 expression in many cells of the neuroectoderm. (G) Homozygous mutant ttk D2-50 embryos display a typical axonal CNS phenotype. In particular, the connectives are found closer to the midline. (H) Embryos homozygous for the amorphic pnt88 allele show a CNS phenotype of fused commissures and reduced longitudinal connectives. (I) Embryos homozygous mutant for pointed and ttk display a CNS axon pattern phenotype combining phenotypic traits of both individual mutations.

Although the expression of both pntP1 and ttk69 in the lateral glial cells is under the control of gcm, the expression of these genes appears to be independent of each other. Thus ttk69 expression could still be detected in all CNS glia of mutant pnt88 embryos and pointedP1 RNA is expressed normally in ttk B330 mutant embryos (data not shown). To further analyze the relationship of pnt and ttk, we generated homozygous double mutant embryos. ttk mutations lead to a fused commissure phenotype and connectives located closer to the midline (Fig. 6G). Mutant pnt88 (Scholz et al., 1993) embryos also display a fused commissure phenotype, as well as a prominent thinning of the longitudinal connectives, which are not as close to midline as in ttk mutant embryos (Fig. 6H). Thus, although both mutants display a fused commissure phenotype, their phenotypes are distinguishable. Embryos doubly mutant for both ttk and pnt display an additive CNS phenotype combining characteristics of both individual phenotypes (Fig. 6I), indicating that pointed and tramtrack act in parallel.

To further investigate this possibility, we coexpressed both ttk69 and pntP1 in the neuroectoderm using the scabrous-GAL4 driver line. Expression of ttk69 does not influence the expression level of the rI50 glia marker, but does lead to cell death in older embryos (Fig. 6D). Expression of pntP1 leads to ectopic expression of the rI50 marker (Fig. 6E). Embryos expressing both, pntP1 and ttk69, show a similar increase in the level of rI50 glia marker as well as some cell death in older embryos (Fig. 6F), again suggesting that tramtrack and pointed act in parallel.

Since the pnt-ttk double mutant phenotype is different to the
 Activation of glial cell master regulatory genes

 Neuroblasts

 Glial cell differentiation

**Fig. 7.** Model of *Drosophila* glial cell differentiation. Within the neuroectoderm, individual glioblasts or neuroglioblasts are singled out by as yet unknown positional cues. In the progenitors of the lateral glial cells, this leads to the activation of the glial master regulatory gene, *gcm*. *gcm* in turn controls glial cell differentiation by a concomitant activation of glial differentiation and suppression of neuronal differentiation. Activation of a glial cell differentiation program in the glial progenitor cells requires the function of genes as *pointed* and *repo*. The existence of an element that suppresses neuronal development has been first suggested by the analysis of the mutant *gcm* phenotype (Anderson, 1995). This function is mediated by the p69 Zn-finger protein encoded by the gene *tramtrack*.

**DISCUSSION**

Based on our interest in mechanisms controlling glial cell development in *Drosophila*, we have conducted a large-scale mutagenesis experiment, screening for mutations that affect the formation of the axonal scaffold indicative for glial cell defects. Here we report that one of the complementation groups identified by this approach corresponds to the *tramtrack* gene. We have shown that the zinc-finger-type DNA-binding protein ttkp69 is expressed in and required for all CNS glia development. In addition, ttkp69 is able to suppress neuronal differentiation. We suggest that the protein may act by suppressing inappropriate differentiation of CNS glial cells.

**tramtrack as a negative regulator of neuronal development**

During larval development, *ttk* is required in the developing eye (Xiong and Montell, 1993). The *Drosophila* eye consists of 800 ommatidia, each of which contains 8 photoreceptor cells, R1-R8. The development of the R7 photoreceptor cell has been extensively studied (Zipursky and Rubin, 1994). R7 development is induced by activation of the sevenless receptor tyrosine kinase (RTK), which subsequently transduces a signal to the nucleus via a conserved ras/raf signal transduction cascade (Wassarman et al., 1995). Nuclear targets of this signal transduction cascade are the ETS transcription factors encoded by *pointed* and *yan* (Brunner et al., 1994; O’Neill et al., 1994). Another nuclear protein required for R7 development is encoded by *sina* (Carthew and Rubin, 1990). In its absence, no R7 cell develops. In contrast to *sina*, *ttk* acts as a negative regulator of R7 development and loss of ttkp88 alone leads to the appearance of extra R7 cells (Xiong and Montell, 1993). Interestingly the development of extra R7 cells provoked by the loss of ttkp88 also occurs in the absence of *sina* (Lai et al., 1996; Yamamoto et al., 1996). However, the formation of extra R7 cells cannot be seen in the developing eye disc but rather it appears that ttk represses the perception of additional inductive signals during pupal development (Lai et al., 1996).

**tramtrack acts as a transcriptional repressor**

The tramtrack protein was first identified on the basis of its ability to bind to regulatory elements of the fushi tarazu gene (Brown et al., 1991; Harrison and Travers, 1990). Several studies have indicated that both zinc-finger proteins encoded by *ttk* suppress transcription. Thus ttkp69 binds to regulatory sequences of certain pair-rule genes (Brown et al., 1991; Brown and Wu, 1993; Harrison and Travers, 1990; Read and Manley, 1992), whereas ttkp88 binds to the *enlarged* gene (Read and Manley, 1992). Ectopic expression of ttkp69, but not ttkp88, results repression of pair rule gene expression, indicating that maternally deposited tramtrack protein may function to establish the correct timing of the onset of zygotic pair rule gene expression (Brown and Wu, 1993; Read et al., 1992).

The observed ectopic expression of the neuronal antigen 22C10 in all mesodermal derivatives supports the idea that *ttk* functions as a transcriptional repressor. However, we can not exclude the possibility that *ttk* activates a repressor of 22C10 expression. In addition, tramtrack might mediate both, transcriptional activation as well as transcriptional repression, as it has been observed for other transcription factors (Seto et al., 1991; Shi et al., 1991).

**Function of tramtrack in glia development**

Development of lateral glia is controlled by the gene *gcm*, which acts as a genetic switch that controls glial versus neuronal fate (Hosoya et al., 1995; Jones et al., 1995; Vincent et al., 1996). In mutant *gcm* embryos, glial cells are transformed into neuronal cell types. Based on the results presented in this paper, we propose that *gcm* controls glial cell fate by regulating two different and independent pathways (Fig. 7). The first directs the actual differentiation of glia and leads to the activation of glia-specific gene expression. This part in glia development appears to be mediated by the transcription factors encoded by *pnt* and *repo*. Loss of *pnt* or *repo* function leads to defects in glia differentiation whereas ectopic
expression of pnt directs several aspects of glial development in otherwise neuronal cells (Klaes et al., 1994; Campbell et al., 1994; Halter et al., 1995; Xiong et al., 1994).

A second important facet of proper glia development could be the repression of inappropriate differentiation, mediated, at least in part, by the ttkp69 protein. Embryos mutant for ttk have reduced numbers of CNS glial cells compared to wild type. The remaining glia however differentiate relatively normally as they are still able to ensheath axon bundles, indicating that tramtrack does not affect actual glial differentiation. Suppression of inappropriate developmental programs in glial cells may be necessary because many glial cells originate from the same precursor cell as neurons (Condron and Zinn, 1994; Soula et al., 1993; Udolph et al., 1993). In this scenario, the default state of cells in a neural lineage is neuronal. Hence any cell routed into glial fate would have to repress inappropriate neuronal differentiation as well as activating glial differentiation genes. An example for such a type of regulatory mechanism is seen in the repression of synapsin I expression in non-neuronal vertebrate cells (Li et al., 1993). Here a transcription factor mediating such repression has been identified (Schoenherr and Anderson, 1995). This factor, neuron-restrictive silencer factor (NRSF), acts as a coordinate repressor of multiple neuron-specific genes in non-neuronal tissues. -like ttk, NRSF encodes a zinc-finger protein and binds to a DNA sequence element that represses the transcription of many neuronal genes in non-neuronal cells (Chong et al., 1995; Schoenherr and Anderson, 1995). Similarly, the C. elegans Zn-finger protein lin-26 is believed to prevent glial and other non-neuronal cells from expressing neuronal fates (Labouesse et al., 1996).

**CONCLUSION**

Based on the analyses of the functions of the genes gcm, pointed and tramtrack, we propose the following model underlying glial development in Drosophila. Early positional cues lead to the activation of master regulatory genes for lateral and midline glial cell development. The master regulator for lateral glia development is encoded by gcm, whereas the master regulator for midline glia development has yet to be identified. These genes act as genetic switches between neuronal and glial development. They direct glial differentiation in a dual mode, by activation of glial differentiation and concomitant repression of neuronal differentiation. The gene tramtrack (and possibly others genes) mediates repression of neuronal differentiation, whereas genes such as pointed and repo are required to activate glial cell differentiation (Fig. 7).

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