Glide2, a second glial promoting factor in *Drosophila melanogaster*

Martial Kammerer and Angela Giangrande¹

Institut de Génétique et Biologie Moléculaire et Cellulaire, IGBMC/
CNRS/ULP/INSERM – BP 163, 67404 Illkirch, c.u. de Strasbourg,
France

¹Corresponding author
e-mail: angela@titus.u-strasbg.fr

The fly glial cell deficient/glial cell missing (*glide/gcm*) gene codes for a transcription factor that induces gliogenesis. Lack of its product eliminates lateral glial cells in the embryonic nervous system. Here we identify a second gene, *glide2*, that is homologous to *glide/gcm* in the binding domain and that is also necessary and sufficient to promote glial differentiation. *glide2* codes for a transcription factor that displays a weaker and delayed expression compared with *glide/gcm*. The two genes, which are located 27 kb apart and share *cis*-regulatory elements, are able to auto- and cross-regulate, indicating that they form a gene complex. Finally, we show that lack of both products eliminates all lateral glial cells, which means that the two genes contain all the fly lateral glial promoting activity.

Keywords: fly/glial cells/Glide2/Glide/Gcm/transcription factor

Introduction

The *glial cell deficient/glial cell missing* (*glide/gcm*) gene codes for a transcription factor that induces gliogenesis in *Drosophila melanogaster* embryonic and adult nervous system (Hosoya *et al.*, 1995; Jones *et al.*, 1995; Akiyama *et al.*, 1996; Vincent *et al.*, 1996; Bernardoni *et al.*, 1997, 1998, 1999; Schreiber *et al.*, 1997, 1998; Akiyama-Oda *et al.*, 1998, 1999, 2000a,b; Miller *et al.*, 1998; Granderath *et al.*, 2000; Van De Bor *et al.*, 2000; Ragone *et al.*, 2001; Van De Bor and Giangrande, 2001). In the embryonic central nervous system (CNS), it is transiently expressed in all the precursors of lateral glial cells, but not in midline glial cells, for which the promoting factor is still to be determined. Lack of *Glide/Gcm* results in the absence of lateral glial cells, which are transformed into neurons. Conversely, ectopic expression leads to glial differentiation at expense of other cell types, indicating that *glide/gcm* is sufficient to induce the glial fate (Hosoya *et al.*, 1995; Jones *et al.*, 1995; Vincent *et al.*, 1996; Akiyama-Oda *et al.*, 1998; Bernardoni *et al.*, 1998).

The observation that *glide/gcm* does not affect all lateral glial cells, as well as the lack of promoting factors for midline cells, prompted us to ask whether genes homologous to *glide/gcm* are required in these processes. Interestingly, a second fragment containing the so-called *gcm* motif has been identified in the fly genome (Akiyama *et al.*, 1996), suggesting that a *glide/gcm*-like gene might exist. This is

in agreement with the finding that two genes of the *gcm* family have been so far identified in each species, from rodents to humans (Akiyama *et al.*, 1996; Kammerer *et al.*, 1999). The two genes, also called *gcmA* and *gcmB*, display homology only within the *gcm* motif (Akiyama *et al.*, 1996; Kammerer *et al.*, 1999).

Here we show that a *glide/gcm* homologue, *glide2*, is located 27 kb upstream of *glide/gcm* in the 30B region. *glide2* codes for a transcription factor that is able *in vitro* to activate *glide/gcm* as well as its own expression. We also show that *glide/gcm* regulates *glide2* expression *in vivo* and *in vitro*, and that the two genes share regulatory sequences. Like *glide/gcm*, *glide2* is sufficient to induce gliogenesis within and outside the nervous system. Moreover, like *glide/gcm*, it is expressed in lateral but not in midline glial cells, and is required for the differentiation of these cells. Finally, lack of both products eliminates all lateral glial cells, indicating that the 30B locus contains all the information that is necessary to induce the lateral glial fate.

Results

Organization of the 30B locus

The existence in the fly genome of a 141 bp fragment homolog to the *gcm* motif (Akiyama *et al.*, 1996) prompted us to analyze the putative role and profile of expression of the gene containing this sequence. At first we used the fragment to generate probes and screen fly libraries. One genomic clone covering 11 kb of what we called the *glide2* locus, as well as an 863 bp clone containing a partial cDNA, were obtained and characterized. Sequences were then compared with those provided by the Fly Genome Project (Adams *et al.*, 2000). Locus and gene structure, as well as the predicted amino acid sequence, are shown in Figure 1A and B. The *glide2* gene maps at 30B and is located 27 kb 5' to the *glide/gcm* gene, the two genes being disposed head to head (DDBJ/EMBL/GenBank locus accession number AE003625). Some 480 bp downstream of *glide2* is located a gene homologous to *thioredoxin* (M.Kammerer and A.Giangrande, in preparation) whereas two predicted genes are located between *glide/gcm* and *glide2* (Figure 1A). No expressed sequence tags (ESTs) have been recovered for such genes in embryonic libraries. Their predicted open reading frames (ORFs) have some similarity with esterase-like proteins and are not related to the *glide/gcm* family. One member of this protein family, Gliotactin, is required for the formation of the blood–brain barrier, but not to promote gliogenesis (Auld *et al.*, 1995).

Structure of the *glide2* gene

Computational analysis on the *glide2* genomic sequence predicts the existence of a gene containing three exons,

Fig. 1. Organization of the *glide2* locus and comparison amongst *glide/gcm* genes throughout evolution. (A) The *glide2* locus. Line at the top represents the genomic clone (λEMBL3). The 30B locus is shown below. Arrows indicate transcribed sequences. The structure of the *glide2* cDNA clone is indicated below the genomic fragment (λZAP). Open rectangles below the cDNA clone indicate the four exons that constitute the *glide2* transcribed sequences. Arrowheads indicate oligonucleotides used in RT-PCRs. Open rectangles at the bottom indicate the predicted ORF, the black rectangle shows the gcm motif. (B) Predicted amino acid sequence for the *glide2* gene. Double underlining indicates the putative PEST motif. Single underlining depicts the homopolymeric amino acid stretches. (C) Sequence alignment of fly, human, mouse and rat proteins in the gcm motif. Black boxes indicate conserved amino acids, asterisks indicate the conserved histidine and cysteine residues. (D) Phylogenetic tree of Gcm family. Phylogenetic tree according to multiple sequence alignments was determined using PHYLIP calculations (Joe Felsenstein). Bar represents number of substitutions per site.

however, the 5' end of our cDNA clone maps upstream of the predicted gene. To determine the structure and the 5' end of *glide2*, we performed reverse transcription PCR (RT-PCR) on RNA from wild-type embryos using different oligonucleotide sets (see arrowheads in Figure 1A). This revealed the existence of a *glide2* cDNA containing four exons and ~2.4 kb long. A putative Initiator element (Inr) (Hultmark *et al.*, 1986) is present 58 bp 5' to oligonucleotide P1. Such cDNA contains a predicted ORF of 606 amino acids, with a deduced molecular weight of 65 kDa (DDBJ/EMBL/GenBank accession number AF184664). This protein sequence differs from the 613 amino acid protein proposed in the FlyBase Genome Annotation Database of *Drosophila* (GadFly) (DDBJ/EMBL/GenBank accession number AAF52793) in the first four amino acid residues. The first exon contains an untranslated region of 433 bp (relative to the putative start site in the Inr), as well as the first 11 coding nucleotides. The second exon contains the gcm motif as well as the PEST motif, typical of rapidly degraded proteins (Rogers *et al.*, 1986). Interestingly, the latter motif is conserved throughout evolution in the Gcm family even though the position and the number of PEST vary. Stretches of homopolymeric repeats of glutamine, serine, alanine, glycine, tyrosine, histidine and proline are located in the first 60 and in the last 250 amino acids. Surprisingly, while it has been shown that *glide/gcm* codes for a transcription factor (Akiyama *et al.*, 1996; Schreiber *et al.*, 1997, 1998; Miller *et al.*, 1998), no clear nuclear localization signal

(NLS) could be identified in Glide2. The only other member of the Gcm family devoid of NLS is hGcmA.

The degree of conservation between Glide/Gcm or Glide2 and the different family members is very similar (Figure 1D), which makes it difficult to assign a specific ancestor for *gcmA* and *gcmB*. The homology between the two fly genes within the gcm motif, however, is stronger (69%) than that found between any of them and the other members of the family (56–64%). These data suggest that independent duplications have taken place in arthropod and vertebrate lineages. Within the motif, Glide2 retains the four cysteines and the seven histidines displaying the same relative spacing H-X₁₁-H-X₈-C-X₅-C-X₃-C-X₁₄-C-X₁₁-C-X_{2,4}-C-X_{8,9}-C-X₂-H-X₂₃-H as that found in Glide/Gcm (Akiyama *et al.*, 1996) (Figure 1C). Cysteines within the gcm motif have been shown to be either essential for DNA binding and transactivation or to play a role in the regulation of redox sensitivity to DNA binding (Miller *et al.*, 1998; Schreiber *et al.*, 1998).

***glide2* is expressed in lateral glial cells of the embryonic CNS**

To understand the mode of action of *glide2*, we analyzed its expression during development. RT-PCRs on total RNA from different stages produced amplification products of the expected size. *glide2* expression is first detected in embryos, it decreases during larval stages and peaks again in 1-day pupae (Figure 2A). Almost no RNA is present in adult flies. We also performed *in situ* hybridiza-

Fig. 2. Comparison of *glide/gcm* and *glide2* profile of expression. (A) *glide2* expression at different stages. RT-PCR was performed on: embryos (E), first to third instar larvae (L1–L3), wandering larvae (WL), 0–24 h pupae (P), adult males (M) and females (F). *rp49* was used to normalize the amount of RNA in each reaction. (B) *glide2* expression during embryogenesis and comparison with *glide/gcm* expression. Ventral views: in this and in following figures anterior is to the left. *In situ* hybridization on wild-type embryos with a (a–f) *glide2*- or a (g and h) *glide/gcm*-specific riboprobe. (a) Expression is first detected at stage 9 in procephalic mesoderm (asterisk) and in one cell of each hemineuromere of the ventral cord (arrow). (b) By stage 10, the levels of expression in the ventral cord have increased. (c) By early stage 12, the expression in the procephalic mesoderm has completely disappeared. The number of *glide2* expressing cells in the ventral cord has increased. (d) At mid-stage 12, *glide2* starts being expressed in lateral ectodermal stripes along the dorso-ventral axis (arrowhead). Expression is also detectable in cells that correspond, by their position, to longitudinal (arrows) and to other lateral glial cells (open arrowheads). A peripheral cell expressing *glide2* is present in each abdominal segment (p). (e) By stage 13, most glial cells have reached their position. *glide2* is expressed in the longitudinal glial cells that start to stretch along the longitudinal connectives (arrows). (f) Stage 15. (g) Early stage 12. (h) Stage 15. Bar: 50 μ m.

tion on wild-type embryos using a *glide2*-specific riboprobe (Figure 2B). Transcripts are first detected at around stage nine in the procephalic mesoderm, the region from which hemocytes take origin, and in one cell per hemisegment, at the position of the neuroglioblast 1–3 lineage (Figure 2B, panel a). The levels of expression, as well as the number of expressing cells in the trunk, increase as development proceeds (Figure 2B, panels b and c). By

stage 12, most transcripts are restricted to the nervous system, at the position of glial lineages (Figure 2B, panels c and d). Some expression is also detectable in ectodermal stripes located laterally at the position of apodemal cells (Figure 2B, panel d). No expression was found in midline cells.

The profiles of *glide2* and *glide/gcm* (Hosoya *et al.*, 1995; Jones *et al.*, 1995; Bernardoni *et al.*, 1997) are very similar, even though *glide2* is expressed at much lower level than *glide/gcm* (Figure 2B, compare panels c and g). Using probes of similar length and the same experimental conditions for the two reactions, we detected *glide2* expression after 4 h, whereas *glide/gcm* expression was detected after a few minutes. This is in agreement with the observation that the *glide2*-specific signal cannot be detected on northern blots containing 10 μ g of poly(A)⁺ RNA (data not shown). Overall, *glide2* expression is delayed compared with that of *glide/gcm*, which is already detected at the end of the blastoderm stage (Jones *et al.*, 1995; Bernardoni *et al.*, 1997) and fades by stage 15 (Figure 2B, compare f with h).

Glide2 is sufficient to induce glial differentiation

glide2 expression profile suggested a role in glial differentiation; we therefore tested whether *glide2* is able to induce gliogenesis. Using *scabrous* (*sca-Gal4*) and *twist* (*twi-Gal4*) drivers, we expressed *glide2* throughout the neurogenic region and in the mesoderm, respectively. In both cases, we observed ectopic gliogenesis, as revealed by labeling with anti-Repo, an antibody that recognizes all lateral embryonic glial cells (Campbell *et al.*, 1994; Xiong *et al.*, 1994; Halter *et al.*, 1995) (Figure 3A, B, E and F). Thus, as for *Glide/Gcm* (Hosoya *et al.*, 1995; Jones *et al.*, 1995; Akiyama-Oda *et al.*, 1998; Bernardoni *et al.*, 1998), *Glide2* is sufficient to induce the glial fate, irrespective of the cell type in which it is expressed.

Given the similar phenotype obtained with the two genes, we asked whether the effects shown in Figure 3 were due to ectopic activation of *glide/gcm*. By using a *glide/gcm*-specific probe we found that *glide2* expression in the neurogenic region does induce *glide/gcm* (Figure 3C and D) whereas mesodermal *glide2* expression does not (Figure 3G and H), indicating that the *Glide2* protein is sufficient to promote gliogenesis. To confirm that *glide2* does not require *glide/gcm*, we also induced ectopic expression in a null mutant, *glide/gcm*^{N7-4}, which misses most lateral glial cells (Figure 4B). Indeed, neuroblast *glide2* expression leads to massive ectopic Repo in the CNS of *glide/gcm*^{N7-4} embryos (Figure 4C). When *glide/gcm* is induced in the same background and using the same driver, ectopic gliogenesis was observed, as expected (Figure 4D). Interestingly, *Glide/Gcm* induces more glial cells than *Glide2*.

These results altogether demonstrate that (i) *glide2* is sufficient to induce gliogenesis, (ii) *glide2* can induce *glide/gcm* expression, and (iii) *glide2* can induce gliogenesis directly, without *glide/gcm*.

glide2 codes for a transcription factor

glide/gcm codes for a transcription factor that binds to the *Glide/Gcm* binding site (GBS) and thereby activates target genes (Akiyama *et al.*, 1996; Schreiber *et al.*, 1997, 1998; Miller *et al.*, 1998; Grandérath *et al.*, 2000). Given the

Fig. 3. *glide2* ectopic expression induces glial differentiation. (A–D) Ventral views of stage 15 embryos (E–H). Lateral views of stage 13 embryos. (A, B, E and F) Anti-Repo labeling. (C, D, G and H) *glide/gcm* expression. (A, C, E and G) Wild type (WT). (B and D) *sca-Gal4; UAS-glide2*. (F and H) *twi-Gal4; UAS-glide2*. (A–D) Repo protein and *glide/gcm* RNA are ectopically expressed in the ventral cord of *sca-Gal4; UAS-glide2* compared with wild-type embryos. (E–H) Note that *twi-Gal4; UAS-glide2* induces mesodermal Repo but not *glide/gcm* expression. Arrowheads in (F) show lateral clusters of Repo-positive cells absent in wild type. Bar: 50 μ m.

Fig. 4. *glide2* ectopic expression induces glial differentiation independently of *glide/gcm*. Ventral views of stage 15 embryos, anti-Repo labeling. (A) Wild type (WT), (B) *glide^{N7-4}*, (C) *glide^{N7-4}; 31nb-Gal4/UAS-glide2*, (D) *glide^{N7-4}; 31nb-Gal4/UAS-glide*. Arrowheads indicate the few Repo-positives present in *glide^{N7-4}*. Ectopic expression of *glide2* in all neuroblasts induces massive ectopic Repo labeling in a *glide/gcm* null context (C). Note that ectopic expression of *glide/gcm* in the same context induces more ectopic Repo labeling than that observed with *glide2*. Bar: 50 μ m.

structure of Glide2 and its ability to induce *glide/gcm*, we asked whether it behaves as a transcription factor. To do this, we co-transfected a *glide2* expression vector with a chloramphenicol acetyl transferase (CAT) reporter containing a GBS (5'-ATGCGGGT-3') in front of the thymidine kinase (tk) promoter fused to the CAT coding sequence (Figure 5A). Transactivation was quantified by measuring the amounts of CAT protein. The results were compared with those obtained with an expression vector containing *glide/gcm* coding sequences or with an expression vector that does not contain any insert. Transactivation assays were performed by using different amounts of expression vector. As expected, *glide/gcm* expression results in the activation of transcription, which was arbitrarily given a 100% value. Glide2 also induces transcription, compared with the results obtained with the 'empty' transcription vector. Glide2, however, is weaker compared with Glide/Gcm. Moreover, its transactivation potential decreases as the amount of protein increases:

Fig. 5. *glide2* encodes a transcription factor. (A) The reporter construct pBLCAT5-GBS-C was co-transfected in S2 cells with increasing amount of PAC, PAC-*glide/gcm* (PAC-*glide*) or PAC-*glide2* (PAC-*glide2*). GBS contained in pBLCAT5-GBS-C corresponds to site 1 in (C). Results are given in percentage of CAT amounts. The level of activation observed when the reporter is co-transfected with 5 μ g of PAC-*glide/gcm* was arbitrarily assigned the 100% value. (B) The reporter construct pBLCAT6-1.96 containing 1.96 kb of the *glide2* promoter was co-transfected in S2 cells with increasing amount of PAC, PAC-*glide/gcm* or PAC-*glide2*. Results are given in relative CAT amounts obtained upon comparison with those induced by PAC. (C) Schematic drawing of the 27 kb region contained between *glide/gcm* and *glide2*. Vertical ovals indicate the position of the ten sites corresponding to the GBS consensus. GBS numbers 1, 6 and 8 are in 3'→5' orientation whereas GBS numbers 2, 3, 4, 5, 7, 9 and 10 are in 5'→3' orientation. Site 1 on this map corresponds to site C in Miller et al. (1998).

0.5 μ g of *glide2* expression vector lead to a congruous activation (25%) whereas 5 μ g of the same vector lead to very weak activation (<10%).

In a second co-transfection assay, we used a fragment containing three GBSs (Figure 5B). In this context, Glide2 and Glide/Gcm showed similar transactivation potentials when >2.5 μ g of expression vector were transfected. The 1.96 kb fragment used in this test is located in the *glide2* promoter, suggesting that *glide2* expression depends on a

Fig. 6. *glide/gcm* ectopic expression induces ectopic *glide2* expression. (A) *glide2* and (B) *repo* expression in a *glide/gcm* gain-of-function context. RT-PCRs were performed on wild-type (WT) or *sca-Gal4; UAS-glide* (*sca-glide*) overnight embryos. *rp49* was used as above. (C and D) Stage 13, ventral views: *glide2* expression detected by *in situ* hybridization. (C) Wild type (WT), (D) *sca-Gal4; UAS-glide*. *glide2* RNA is ectopically expressed in the ventral cord of *sca-Gal4; UAS-glide* embryo. Bar: 50 μm.

feedback loop and/or on cross-regulation from Glide/Gcm. Indeed, the 27 kb fragment separating the two genes contains many GBS consensus [5'-AT(G/A)CGGG(T/C)-3'], most of which are located closer to *glide2* than *glide/gcm* transcription start site. The map in Figure 5C shows the position of the predicted GBS consensus sequences as well as the 1.96 kb fragment used for CAT assays. It should be noted that this map only shows the sites that are homologous to either of the four consensus sequences or those that carry a mismatch in the first or in the last position, which have been shown to affect binding very weakly (Akiyama *et al.*, 1996; Schreiber *et al.*, 1998). These are the most effective GBSs. For the sake of simplicity, sites containing mismatches at other positions were omitted, even though previous work from our and others' laboratories have shown that they induce moderate but consistent gene expression (Miller *et al.*, 1998; Schreiber *et al.*, 1998). The *in vitro* and *in vivo* data indicate that Glide2 is a transcription factor and that it activates *glide/gcm* expression. They also indicate that Glide/Gcm is a more potent transcription factor than Glide2, thus explaining the quantitative differences observed in ectopic expression experiments (Figure 5).

Although the presence of binding sites cannot be taken as an evidence for direct regulation, it is interesting to note that the two predicted genes are located between sites 3 and 5 and are separated by site 4. Further analyses will be required to define the profile of expression of such genes and to determine whether they are targets of Glide-like molecules.

Regulation of *glide2* by *glide/gcm*

The observation that *glide2* regulates *glide/gcm* expression, as well as the localization of the two genes in the same region, prompted us to ask whether *glide/gcm* regulates *glide2* *in vivo*. In order to answer this question,

we analyzed the effects of loss- and gain-of-function *glide/gcm* mutations on the expression of *glide2*. By performing RT-PCR, we found an increase of *glide2* expression in embryos that express *glide/gcm* throughout the neurogenic region (Figure 6A). The increase of *glide2* expression is comparable with that observed with *repo* (Figure 6B). The activation of *glide2* by *glide/gcm* was further confirmed by *in situ* experiments with *glide2*-specific probes on wild-type and *sca-glide/gcm* embryos (Figure 6C and D). Massive ectopic *glide2* expression is present in *sca-glide/gcm* embryos, the labeling being limited to the neurogenic region, in which *glide/gcm* has been induced. These results, together with the transfection assays, strongly suggest that *glide/gcm* directly induces *glide2* expression. They also explain why *glide2* expression is slightly delayed compared with that of *glide/gcm*.

We also analyzed *glide2* expression in two *glide/gcm* null mutations, *glide/gcm*^{N7-4} and *glide/gcm*²⁶, which were generated by DEB (Lane and Kalderon, 1993) and P element excision, respectively (Vincent *et al.*, 1996) (Figure 7A and B). The insertion in the line used for P mutagenesis, *glide/gcm*^{rA87}, was defined by genomic DNA sequencing as being at -82 bp from the transcription start site, which was identified by S1 mapping (A.A.Miller and A.Giangrande, unpublished data). *glide/gcm*^{N7-4} is a point mutation that eliminates DNA binding, whereas *glide/gcm*²⁶ contains a deletion covering all transcribed sequences as well as the first 82 bp in the 5' promoter and 1020 bp 3' to the gene. Individual embryos were selected by using blue balancers and submitted to RT-PCR. Interestingly, we found that *glide2* expression is not severely affected in *glide/gcm*^{N7-4} embryos, while it is increased (4-fold induction) in *glide/gcm*²⁶. These intriguing results prompted us to analyze the spatial distribution of *glide/gcm* transcripts by *in situ* hybridization. Much to our surprise, we found that most of the *glide2*-specific signal is absent in the ventral cord of *glide/gcm*^{N7-4} embryos, whereas it is less affected in *glide/gcm*²⁶. In *glide/gcm*^{N7-4}, very few cells still express *glide2*, the level being much lower than in the wild type (Figure 7E and F). In *glide/gcm*²⁶ embryos, more cells express it; in addition, the level of *glide2* expression is rather high, as confirmed by the fact that some of them enter the glial pathway and also express *repo* (Figure 7G). Our *in situ* results also indicate that *glide2* regulation is tissue-specific, since the expression in the lateral stripes is not induced by *glide/gcm* (Figure 7C and D). Indeed, lateral stripes show stronger labeling in *glide/gcm*²⁶ than in wild-type embryos, which explains why *glide2* RNA levels are increased in RT-PCR experiments. Thus, *glide/gcm* at least partially controls *glide2* expression.

Glide2 is required for glial differentiation

By performing PCR on genomic DNA, we established that *glide2* resides within the *Df(2L)132* (Lane and Kalderon, 1993). We found that this deficiency spans ~115 kb and covers two lethal complementation groups, *glide/gcm*^{N7-4} and, more proximally, *l(2)DA2*. No known genes affecting glial differentiation are contained within the deficiency. *Df(2L)132* completely lacks Repo labeling in embryonic PNS and CNS (Figure 8A, panel d) whereas *glide/gcm* null mutants, which partially affect *glide2* expression, still retain some Repo labeling (see panels b and c in Figure

Fig. 7. *glide2* expression is impaired in *glide/gcm* null mutants. (A) *glide2* expression in *glide/gcm* loss-of-function mutations. RT-PCRs were performed on RNA from single wild-type (WT) or *glide/gcm* stage 12 embryos. Two null alleles were analyzed, *glide²⁶* (26) or *glide^{N7-4}* (N7-4). The levels of expression in mutant embryos were compared with those observed in WT embryos, which were arbitrarily assigned the value of one. *n* indicates the number of embryos analyzed for each genotype. (B) Schematic drawing showing the mutations at the *glide/gcm* locus. Horizontal arrow indicates the transcribed sequences. *glide^{rA87}* P element insertion site is shown by the inverted triangle. Deletion in *glide²⁶* is indicated by the interrupted line. Open rectangles indicate the predicted ORF and black boxes show the gcm motif. Vertical arrowhead indicates the substitution occurring in *Glide^{N7-4}*. (C and D) Lateral views of late stage 12 embryos, *in situ* hybridization with a *glide2*-specific riboprobe. (C) Wild-type (WT), (D) *glide²⁶*. Note the increased expression of *glide2* in lateral stripes in *glide²⁶* compared with wild type (see arrows). (E–G) Ventral views of abdominal segments, confocal images. In red, anti-Repo labeling, in green, *glide2* riboprobe. (E) Wild type (WT), (F) *glide^{N7-4}*, (G) *glide²⁶*. Lines indicate the position of the midline. Empty arrowheads indicate the position of lateral stripes. Arrows indicate cells expressing *glide2* in the ventral cord. White arrowheads indicate cells co-expressing *glide2* and Repo. (H) Model of *glide2* regulation in a wild-type background. In (I and J), models of *glide2* regulation in *glide^{N7-4}* and *glide²⁶*, respectively. Horizontal line represents the genomic region containing *glide/gcm* and *glide2*. Black arrows indicate transcribed sequences. Blue box represents the enhancer element common to *glide/gcm* and *glide2*. Blue arrows indicate relative contribution of this element to *glide/gcm* and *glide2* expression. Red oval represents the *Glide/Gcm* protein, red arrow the contribution of *Glide/Gcm* to *glide2* transcription. Interrupted line in (J) represents the deleted sequences in *glide²⁶*. The red cross in (I) indicates that the *Glide^{N7-4}* protein is unable to activate *glide2* expression. Bars: (C and D) 50 μ m, (E–G) 10 μ m.

Fig. 8. *glide2* is required for glial differentiation. (A) Stage 15 embryos from the following genotypes labeled with an anti-Repo antibody: (a) wild type (WT), (b) *glide*²⁶, (c) *glide*^{N7-4}, (d) *Df(2L)132*. Note that in (b) the number of Repo-positive nuclei is dramatically decreased, but glial cells are still present in the head and in the ventral cord (arrowheads). (c) A few glial cells are detectable in the head (arrowheads). (d) No Repo-positive cells are detectable in the CNS of this embryo. The lateral bipolar dendritic cell normally present in all segments, see asterisk in (a–c), is also missing in the double mutant. (B) Stage 15 embryos from the following genotypes labeled with an anti-BP102 antibody: (a) wild type (WT), (b) *glide*²⁶, (c) *glide*^{N7-4}, (d) *Df(2L)132*. Arrowheads indicate longitudinal connective breaks. (C) Mutations in the region containing *glide/gcm* and *glide2*. Breakpoints for *Df(2L)132* were determined by PCR. Boxes show lethal complementation groups. Interrupted line shows the deletion in *Df(2L)132*. Arrows show predicted genes. Names show ESTs or transcripts found in the embryo. Vertical arrows show P element insertion close to *glide2*, EP(2)2252 (A), EP(2)2018 (B), EP(2)2433 (C) and EP(2)0361 (D). Bar: 50 μ m.

8A). In particular, 36 and 40 repo-positive cells were found, respectively, in the cord and in the head of *glide/gcm*²⁶ embryos ($n = 10$) whereas three and seven were found in *glide/gcm*^{N7-4} embryos ($n = 10$). This further confirms that *glide2* is required for gliogenesis. Interestingly, the amount of Repo-positive cells directly correlates with the levels of *glide2* expression previously observed in the mutants, the lowest value being in the deficiency, the highest in *glide/gcm*²⁶. Moreover, *glide/gcm*²⁶ and *glide/gcm*^{N7-4} embryos also display weaker axonal defects compared with those observed in the

deficiency, as shown by labeling with several axon-specific antibodies. Indeed, breaks in the longitudinal commissures, as well as abnormal fasciculation in the intersegmental and segmental nerves, were more often seen in the deficiency than in *glide/gcm* mutants (Figure 8B and data not shown). This substantiates the role of *glide2* in the differentiation of glial cells, which are necessary to maintain the axon scaffold (Hosoya *et al.*, 1995; Vincent *et al.*, 1996). Although we cannot formally exclude the possibility that genes contained in the deficiency might somehow act in glial differentiation and axonal navigation, the results obtained with this mutant are in agreement with all the other *in vivo* and *in vitro* data and support the hypothesis that *glide2* and *glide/gcm* act in concert to promote glial differentiation.

Since *glide2* is located proximal to *glide/gcm*, we asked whether *l(2)DA2* corresponds to the *glide2* allele. By labeling with the anti-Repo antibody, however, we did not find any mutant phenotype (data not shown). Since *Df(2L)132* also lacks *thioredoxin*, the gene that is proximal to *glide2* (see Figure 1A), as verified by PCR with specific primers (data not shown), this means that either the *glide2* mutation is not lethal or that previous mutagenesis screens have not saturated the region. Finally, two EP lines (EP(2)2252 and EP(2)2018), three bases apart, are located downstream of *glide2*. The elements reside in the 5' untranslated region of *thioredoxin*, 227 and 224 bp from the coding region. It has previously been shown that antisense transcripts can be generated if an EP element inserts into the transcribed sequence of a gene in an antisense orientation (Rorth *et al.*, 1998). Since the two lines display opposite orientation of the P element, we asked whether any of them had a mutant glial phenotype due to expression of an antisense *glide2* RNA. However, no defects were observed in the two lines.

Discussion

Glide2 is a glial promoting factor

Previous studies demonstrated that *glide/gcm* plays a major role in fly glial differentiation (Hosoya *et al.*, 1995; Jones *et al.*, 1995; Vincent *et al.*, 1996; Akiyama-Oda *et al.*, 1998, 1999, 2000a,b; Bernardoni *et al.*, 1998, 1999; Miller *et al.*, 1998; Granderath *et al.*, 2000; Van De Bor *et al.*, 2000; Ragone *et al.*, 2001; Van De Bor and Giangrande, 2001), however, the presence of some glial cells in the CNS of null mutants suggested that additional gene(s) contribute(s) to gliogenesis. The present study shows that a second fly gene, *glide2*, codes for a glial promoting factor. Several lines of evidence indicate that *Glide/Gcm* and *Glide2* work together to promote gliogenesis: (i) their similar profile of expression; (ii) the ability of *Glide2* to promote gliogenesis when ectopically expressed *in vivo*; (iii) *Glide2* requirement for glial differentiation and its ability to activate transcription through GBSs; and (iv) *glide/gcm* and *glide2* auto- and cross-regulation *in vivo* and *in vitro*. When both genes are eliminated, all lateral glial cells are missing, suggesting that the *glide/gcm* cluster accounts for all lateral glial promoting activity in the fly. The present study also clearly demonstrates that *Glide*-like activity is not necessary to trigger gliogenesis in the midline.

The functional significance of gene duplication is in many cases still unclear. It is likely that duplicated, dispensable genes are in the process of acquiring novel functions. Interestingly, *engrailed* mutations are lethal whereas *invected* mutations are not (Gustavson *et al.*, 1996). Moreover, Engrailed and Invected have distinguishable functions in anterior–posterior patterning of the wing (Simmonds *et al.*, 1995). It is unlikely that *glide2* is specifically required in a subtype of glial cells of the embryonic ventral cord, since the glial cells still present in *glide/gcm* mutants do not show a stereotyped arrangement. This favors the hypothesis that *glide2* expression guarantees sufficient levels of Glide-like activity. *glide2* could also play a role at later stages in glial proliferation, migration and/or other processes. However, these phenotypes cannot be scored in the absence of glial cells produced by the *glide/gcm* mutation. For this reason, it will be useful to generate conditional *glide/gcm* mutants that make it possible to bypass the early requirement for this product during glial differentiation. Finally, *glide2* might play a role in the differentiation of the adult central nervous system, where it is also expressed (V. Van De Bor and A. Giangrande, unpublished data).

The observation that *glide2* is sufficient to induce gliogenesis raises the question of why this gene is not able to rescue the *glide/gcm* phenotype. This situation has also been observed in other pairs of genes. For example, the segmentation genes *engrailed* and *invected* code for very similar homeobox-containing proteins and show similar profiles of expression (Gustavson *et al.*, 1996). Even though both proteins display comparable repressor activities in transfection assays, Invected is not able to compensate for the lack of Engrailed, most likely due to the fact that its expression is delayed compared with that of *engrailed* (Gustavson *et al.*, 1996). The expression of *glide2* is induced later than that of *glide/gcm* and therefore it may not be able to compensate for the defect. Moreover, *in vitro* and *in vivo* results indicate that *glide2* is a less potent activator and a less potent glial promoting factor. Finally, *glide2* is expressed at lower levels than *glide*. This quantitative and qualitative regulation of expression may account for the different behavior.

While it is likely that *glide2* does not code for the major glial promoting factor, the present study shows that this gene plays an important role in gliogenesis. Moreover, *glide2* contribution to glial differentiation is certainly underestimated, due to the fact that *glide/gcm* controls at least in part the expression of *glide2*. Indeed, we have demonstrated that mutants so far considered as being null for *glide* are in fact also affecting *glide2*. It will be important to produce mutants that specifically affect *glide2*. In an effort to generate such mutants we tried to induce local hops using the EP line EP(2)2252 inserted downstream of *glide2*. Unfortunately, no mutations could be recovered at the *glide2* locus, as verified by PCR.

The *glide/gcm* gene complex

Gene duplication is of central interest to evolutionary developmental biology since it has been implicated in evolutionary increase in complexity (Holland, 1999). A number of duplicated genes in *Drosophila* are characterized by features that are also shared by *glide2* and *glide/gcm* [*engrailed* and *invected* (*en/inv*; Gustavson *et al.*,

1996), *knirps* and *knirps-related* (Rothe *et al.*, 1989; Chen *et al.*, 1998), *zerknüllt 1* and 2 (Rushlow *et al.*, 1987), *sloppy-paired 1* and 2 (*slp1/2*; Grossniklaus *et al.*, 1992), *gooseberry-distal* and *gooseberry-proximal* (Baumgartner *et al.*, 1987)].

First, duplicated genes display similar or overlapping expression profiles, indicating a functional redundancy in the gene complex (Rushlow *et al.*, 1987; Rothe *et al.*, 1989; Grossniklaus *et al.*, 1992; Gustavson *et al.*, 1996). This is confirmed by the fact that double mutants are stronger than single mutants and that ectopic expression of any of the two genes display similar phenotypes (Grossniklaus *et al.*, 1992; Gustavson *et al.*, 1996; Chen *et al.*, 1998). Secondly, in most cases one gene is expressed earlier than the other (Rothe *et al.*, 1989; Grossniklaus *et al.*, 1992; Gustavson *et al.*, 1996). It has been proposed that, because the embryo develops rapidly, genes required at specific stages must be compact and small. For example, *invected* is expressed later than *engrailed*, most likely due to the length of its transcript and to the presence of a 19 kb intron (Gustavson *et al.*, 1996). Similarly, *glide/gcm*, the expression of which precedes that of *glide2*, shows a more compact organization and a shorter intron than *glide2*.

Thirdly, pairs of genes constitute a complex in the sense that expression depends on shared regulatory sequences, as shown for the *slp1/2* and the *en/inv* pairs (Grossniklaus *et al.*, 1992; Gustavson *et al.*, 1996). In the nervous system, the patterning of sensory organs depends on the pattern of proneural genes, *achaete* and *scute*, which are simultaneously expressed by groups of cells (the proneural clusters). The complex profile of expression is acquired through shared enhancer-like elements distributed along the gene complex (~90 kb) (Gomez-Skarmeta *et al.*, 1995). The expression profile, the organization of the two genes and the mutant phenotypes do suggest that shared regulatory sequences are present in the 27 kb that separate *glide2* from *glide/gcm*. Both genes at 30B are able to activate their own transcription and to cross-activate. We previously showed that Glide/Gcm autoregulates *in vitro* and *in vivo* (Miller *et al.*, 1998). In the present study we have found that Glide/Gcm also activates *glide2* expression and vice versa. Moreover, Glide2 also activates its own expression. Sharing of regulatory sequences takes place no matter how the genes are arranged and how far they are located from one another. *glide/gcm* and *glide2* are organized head to head, *engrailed* and *invected* tail to tail, *achaete* and *scute* or *slp1* and 2 show the same orientation. The *achaete/scute* and the *en/inv* genes are separated by several dozen kilobases (Gomez-Skarmeta *et al.*, 1995; Gustavson *et al.*, 1996).

Promoter competition at the *glide* locus

The characterization of two null mutations, *glide/gcm*²⁶ and *glide/gcm*^{N7-4}, has allowed us to clarify the nature of promoter interactions at 30B and also to explain the different behavior of the two alleles. The *glide/gcm*^{N7-4} mutation produces an inactive protein, as a consequence, *glide2* transcription is severely impaired. On the other hand, deletion of *glide/gcm* promoter and transcribed sequences (as seen in *glide/gcm*²⁶) has a milder effect on *glide2*. The most likely interpretation for these results is that *glide2* expression is activated by *glide/gcm* via the

glide binding sites and through a regulatory element that is common to *glide/gcm* and *glide2* (see model in Figure 7). In a wild-type embryo, this element preferentially activates *glide/gcm* by interacting with its proximal promoter. In *glide/gcm*²⁶ embryos, however, the proximal promoter of *glide/gcm* is not available. In its absence, the common regulatory element can only work on the *glide2* promoter and activates it more efficiently. This is why *glide2* transcription is less affected in *glide/gcm*²⁶ than in *glide/gcm*^{N7-4}. Such promoter competition is even more evident outside the nervous system. In the lateral stripes of cells, *glide2* expression is higher in *glide/gcm*²⁶ than in wild-type embryos. Promoter competition also explains why *glide2* expression increases as *glide/gcm* expression declines.

In gene complexes, promoter competition seems to control the spatio-temporal shift of expression, thereby allowing different gene members to acquire specific roles. It can be generated at least in two ways (Ohtsuki *et al.*, 1998). An insulator DNA can specifically block the interaction of a shared enhancer with one gene and not the other. Alternatively, the shared enhancer is equally accessible for the two genes, but prefers the promoter region associated with one of them (Ohtsuki *et al.*, 1998). To understand how promoter competition works in the case of *glide/gcm* and *glide2*, we are currently defining the shared enhancer sequences using transgenic lines. It is interesting to notice that, while promoter competition has so far been described for promoters that harbor different core elements, no striking difference as observed between *glide/gcm* and *glide2* promoters, in that they are both devoid of TATA box and downstream promoter element (Dpe) and they both contain an Inr.

Thus, the *glide-gcm/glide2* pair constitutes a very interesting model system to study complex interactions of *cis*-regulatory sequences during the differentiation of the nervous system.

Materials and methods

Isolation and analysis of *glide2*

Two degenerate oligonucleotides [TGGGCCATGCGCAA(T/C)ACCAA as forward primer and TC(T/G)G(G/T)C(T/C)TTGTACACATGGC as reverse primer] in the conserved region were used to amplify a 141 bp fragment specific for *glide2* from genomic DNA. This fragment was used to screen a genomic library of an isogenized strain of *D.melanogaster* (iso1), which is cloned into a λ EMBL3 vector (provided by J.Tamkun), as well as a 0–24 h embryonic cDNA λ ZAPII library (provided by C.S.Thummel). A genomic clone covering 11 kb of the *glide2* locus and a single cDNA clone of 863 bp were obtained. Computational analysis on the *glide2* locus was realized using the GENE-FINDER program from the CGG at the Sanger Center. Finally, RT-PCRs using oligonucleotides P1 (TCTGCTGCTGCCGCTGCTGCTCTT), P2 (GCCCAGTGGATCAAGTGTCGCCT), P3 (CGTCTTGAAGCTGTAGCCATT) and P4 (GGTGTCGGATTGGTTAAGTACTA) were performed to characterize *glide2* cDNA organization. Homology searches were carried out using the BLAST program (Altschul *et al.*, 1997). Searches for transcription factor binding sites were carried out using the MatInspector program against the TRANSFAC database (Heinemeyer *et al.*, 1999). Phylogenetic analysis was performed using the PHYLIP package (Joe Felsenstein, University of Washington).

RNA preparation and quantitative RT-PCR

RNA was prepared using TRIzol Reagent, according to the manufacturer's protocol. RT-PCR was performed as described in Huang *et al.* (1996).

glide2-specific oligonucleotides were GCGTGC GCAATTCAGTTCA-ATCCA as forward primer and GATCCGTTGGCTGACTTTCCAGCC as reverse primer.

LacZ-specific oligonucleotides were GGCGTTACCCAACTTAATCG as forward primer and TCGCGGAAACCGACATGGC as reverse primer.

repo-specific oligonucleotides were CGGTGGTTAATGGCACCA-CCA as forward primer and GTTCCGCCGTCTGATAGCTGC as reverse primer.

rp49-specific oligonucleotides were TCCTACCAGCTTCAAGAT-GAC as forward primer and GTGTATTCCGACCACGTTACA as reverse primer.

Specific conditions for quantitative RT-PCR included an initial denaturing step of 1 min at 95°C followed by incubation at 94°C for 10 s, at 65°C for 15 s and at 72°C for 30 s. After 23 cycles, extension was at 72°C for 10 min followed by a 4°C soak. Primer concentration in the multiplex PCR was 1 μ M for *glide2* or *repo* and 0.2 μ M for *rp49*. RT-PCR products were separated on 1.2% agarose gel and transferred on nylon membranes. Blots were hybridized with randomly primed ³²P-labeled probes. Signal quantitation was performed on a Fujix Bas 2000.

Cell transfection and CAT ELISA assay

A 1558 bp *HindIII*–*PstI* fragment from the λ 1 recombinant phage was subcloned in pBLCAT6 (pBLCAT6-1.96), a CAT reporter devoid of tk promoter sequences. This fragment contains both *glide2* proximal promoter sequences and a transcription start site. Reporter vector pBLCAT5-GBS-C as well as expression vectors pPAC5C (gift from C.Thummel, which we refer to as pPAC) and pPAC-*glide/gcm* are described in Miller *et al.* (1998).

Transient transfection of the *Drosophila* cell line S2 (Schneider, 1972) was performed according to Di Nocera and Dawid (1983) with 15 μ g DNA containing the following: 1 μ g pCMV-*lacZ*, 500 ng pBLCAT6-1.96 or 1 μ g pBLCAT5-GBS-C, 0–5 μ g pPAC, pPAC-*glide/gcm* or pPAC-*glide2* and SK as carrier DNA. Cells were harvested 48 h after transfection and normalized for β -Gal activity. CAT levels were determined using the CAT ELISA kit (Boehringer).

Stocks

Wild-type stock was Sevelen. *glide/gcm*²⁶ and *glide/gcm*^{N7-4} were described in Vincent *et al.* (1996). *Df(2L)132* was provided by D.Kalderon. *l(2)DA2* was described in Lane and Kalderon (1993). The *UAS-glide/gcm* transgenic line *M24A/CyO twi-lacZ*; *M21G/M21G* is described in Bernardoni *et al.* (1998). The *UAS-glide2* plasmid was obtained by inserting a 4.1 kb fragment of the *glide2* locus into pCasper UAST. Transgenic flies were obtained by injecting the plasmid into *w¹¹¹⁸* animals. *UAS-glide/gcm* or *UAS-glide2* were crossed with *twi-Gal4* (Baylies and Bate, 1996), *sca-Gal4* (M.Mlodzik's gift) or the neuroblast specific driver *31nb-Gal4* (L.Seugnet's gift). Genotypes were recognized by using blue balancers.

In situ hybridization and immunohistochemistry

In situ hybridization was performed as in Bernardoni *et al.* (1999). Digoxigenin-labeled riboprobes were prepared using a 1.6 kb fragment in the 5' region of the *glide2* cDNA and the full-length *glide/gcm* cDNA (Bernardoni *et al.*, 1999). Signal was detected using an anti-DIG antibody conjugated to the Alkaline Phosphatase and revealed with BCIP/NBT (Boehringer) or the TSA Fluorescein system (NEN).

Embryos were immunolabeled as described in Vincent *et al.* (1996). Antibodies against Repo (A.Travers's gift) and β -Gal (Sigma) were used at 1:1000 and BP102 (DSHB) at 1:100. Secondaries conjugated with Cy3 or FITC (Jackson) were used at 1:400.

Preparations were analyzed using conventional (Axiophot2, Zeiss) or confocal (DMRE, Leica) microscopes.

Molecular characterization of *glide*^{A87}, *glide*²⁶ and *Df(2L)132*

*glide*²⁶ was obtained by imprecise excision from the *glide*^{A87} line (Vincent *et al.*, 1996), which contains an insertion at –82 bp from the transcription start site, which was defined by S1 mapping. Deletion extent in *glide*²⁶ and *Df(2L)132*, as well as the position of the P element, were assessed by PCR, as in Miller *et al.* (1998).

Acknowledgements

We thank A.Travers, J.Tamkun, C.S.Thummel, D.Kalderon, M.Bates, M.Mlodzik, L.Seugnet and the Bloomington Center for antibody, genomic and cDNA libraries and flies. We thank R.Walther for the molecular characterization of *glide*²⁶ and for excellent technical

assistance and A. Miller for mapping the *glide/gcm* start site. Thanks to J.L. Vonesh and D. Hentsch for help with confocal microscope and imaging. Confocal microscopy was developed with the aid of a subvention from the French MESR (95.V.0015). We thank all the members of the group for critical reading of the manuscript. This work was supported by the Institut National de la Santé et de la Recherche Médicale, the Centre National de la Recherche Scientifique, the Hôpital Universitaire de Strasbourg, the Human Frontier Science Program and the Association pour la Recherche contre le Cancer. M.K. was supported by BDI (CNRS) and European Leucodystrophies Association fellowships.

References

- Adams, M.D. *et al.* (2000) The genome of *Drosophila melanogaster*. *Science*, **287**, 2185–2195.
- Akiyama, Y., Hosoya, T., Poole, A.M. and Hotta, Y. (1996) The *gcm*-motif: A novel DNA-binding motif conserved in *Drosophila* and mammals. *Proc. Natl Acad. Sci. USA*, **93**, 14912–14916.
- Akiyama-Oda, Y., Hosoya, T. and Hotta, Y. (1998) Alteration of cell fate by ectopic expression of *Drosophila glial cell missing* in non-neural cells. *Dev. Genes Evol.*, **208**, 578–585.
- Akiyama-Oda, Y., Hosoya, T. and Hotta, Y. (1999) Asymmetric cell division of thoracic neuroblast 6-4 to bifurcate glial and neuronal lineage in *Drosophila*. *Development*, **126**, 1967–1974.
- Akiyama-Oda, Y., Hotta, Y., Tsukita, S. and Oda, H. (2000a) Mechanism of glia-neuron cell-fate switch in the *Drosophila* thoracic neuroblast 6-4 lineage. *Development*, **127**, 3513–3522.
- Akiyama-Oda, Y., Hotta, Y., Tsukita, S. and Oda, H. (2000b) Distinct mechanisms triggering glial differentiation in *Drosophila* thoracic and abdominal neuroblast 6-4. *Dev. Biol.*, **222**, 429–439.
- Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D.J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.*, **25**, 3389–3402.
- Auld, V.J., Fetter, R.D., Broadie, K. and Goodman, C.S. (1995) Gliotactin, a novel transmembrane protein on peripheral glia, is required to form the blood-nerve barrier in *Drosophila*. *Cell*, **81**, 757–767.
- Baumgartner, S., Bopp, D., Burri, M. and Noll, M. (1987) Structure of two genes at the *gooseberry* locus related to the *paired* gene and their spatial expression during *Drosophila* embryogenesis. *Genes Dev.*, **1**, 1247–1267.
- Baylies, M.K. and Bate, M. (1996) *twist*: a myogenic switch in *Drosophila*. *Science*, **272**, 1481–1484.
- Bernardoni, R., Vivancos, V. and Giangrande, A. (1997) *glide/gcm* is expressed and required in the scavenger cell lineage. *Dev. Biol.*, **191**, 118–130.
- Bernardoni, R., Miller, A.A. and Giangrande, A. (1998) Glial differentiation does not require a neural ground state. *Development*, **125**, 3189–3200.
- Bernardoni, R., Kammerer, M., Vonesh, J.L. and Giangrande, A. (1999) Gliogenesis depends on *glide/gcm* through asymmetric division of neuroglioblasts. *Dev. Biol.*, **216**, 265–275.
- Campbell, G., Göring, H., Lin, T., Spana, E., Andersson, S. and Doe, C.Q. (1994) RK2, a glial-specific homeodomain protein required for embryonic nerve cord condensation and viability in *Drosophila*. *Development*, **120**, 2957–2966.
- Chen, C.-K., Kühnlein, R.P., Eulenberg, K.G., Vincent, S., Affolter, M. and Schuh, R. (1998) The transcription factors KNIRPS and KNIRPS RELATED control cell migration and branch morphogenesis during *Drosophila* tracheal development. *Development*, **125**, 4959–4968.
- Di Nocera, P.P. and Dawid, I.B. (1983) Transient expression of genes introduced in cultured cells of *Drosophila*. *Proc. Natl Acad. Sci. USA*, **80**, 7095–7098.
- Gomez-Skarmeta, J.L., Rodriguez, I., Martinez, C., Culi, J., Ferres-Marco, D., Beamonte, D. and Modolell, J. (1995) *Cis*-regulation of *achaete* and *scute*: shared enhancer-like elements drive their coexpression in proneural clusters of the imaginal discs. *Genes Dev.*, **9**, 1869–1882.
- Granderath, S., Bunse, I. and Klämbt, C. (2000) *gcm* and pointed synergistically control glial transcription of the *Drosophila* gene *loco*. *Mech. Dev.*, **91**, 197–208.
- Grossniklaus, U., Kurth Pearson, R. and Gehring, W.J. (1992) The *Drosophila sloppy paired* locus encodes two proteins involved in segmentation that show homology to mammalian transcription factors. *Genes Dev.*, **6**, 1030–1051.
- Gustavson, E., Goldsborough, A.S., Ali, Z. and Kornberg, T.B. (1996) The *Drosophila engrailed* and *invected* genes: partners in regulation, expression and function. *Genetics*, **142**, 893–906.
- Halter, D.A., Urban, J., Rickert, C., Ner, S.S., Ito, K., Travers, A.A. and Technau, G.M. (1995) The homeobox gene *repo* is required for the differentiation and maintenance of glia function in the embryonic nervous system of *Drosophila melanogaster*. *Development*, **121**, 317–332.
- Heinemeyer, T. *et al.* (1999) Expanding the TRANSFAC database towards an expert system of regulatory molecular mechanisms. *Nucleic Acids Res.*, **27**, 318–322.
- Holland, P.W.H. (1999) Gene duplication: past, present and future. *Semin. Cell Dev. Biol.*, **10**, 541–547.
- Hosoya, T., Takizawa, K., Nitta, K. and Hotta, Y. (1995) *glial cell missing*: a binary switch between neuronal and glial determination in *Drosophila*. *Cell*, **82**, 1025–1036.
- Huang, Z., Fasco, M.J. and Kaminsky, L.S. (1996) Optimization of DNaseI removal of contaminating DNA from RNA for use in quantitative RNA-PCR. *Biotechniques*, **20**, 1012–1020.
- Hultmark, D., Klemenz, R. and Gehring, W.J. (1986) Translational and transcriptional control elements in the untranslated leader of the heat-shock gene *hsp22*. *Cell*, **44**, 429–438.
- Jones, B.W., Fetter, R.D., Tear, G. and Goodman, C.S. (1995) *glial cell missing*: a genetic switch that controls glial versus neuronal fate. *Cell*, **82**, 1013–1023.
- Kammerer, M., Pirola, B., Giglio, S. and Giangrande, A. (1999) GCMB, a second human homolog of the fly *glide/gcm* gene. *Cytogenet. Cell Genet.*, **84**, 43–47.
- Lane, M.E. and Kalderon, D. (1993) Genetic investigation of cAMP-dependant protein kinase function in *Drosophila* development. *Genes Dev.*, **7**, 1229–1243.
- Miller, A.A., Bernardoni, R. and Giangrande, A. (1998) Positive autoregulation of the glial promoting factor *glide/gcm*. *EMBO J.*, **17**, 6316–6326.
- Ohtsuki, S., Levine, M. and Cai, H.N. (1998) Different core promoters possess distinct regulatory activities in the *Drosophila* embryo. *Genes Dev.*, **12**, 547–556.
- Ragone, G., Bernardoni, R. and Giangrande, A. (2001) A novel mode of asymmetric division identifies the fly neuroglioblast 6-4T. *Dev. Biol.*, **235**, 74–85.
- Rorth, P. *et al.* (1998) Systematic gain-of-function genetics in *Drosophila*. *Development*, **125**, 1049–1057.
- Rogers, S., Wells, R. and Rechsteiner, M. (1986) Amino acid sequences common to rapidly degraded proteins: the PEST hypothesis. *Science*, **234**, 364–368.
- Rothe, M., Nauber, U. and Jäckle, H. (1989) Three hormone receptor-like *Drosophila* genes encode an identical DNA-binding finger. *EMBO J.*, **8**, 3087–3094.
- Rushlow, C., Doyle, H., Hoey, T. and Levine, M. (1987) Molecular characterization of the *zernüß* region of Antennapedia gene complex in *Drosophila*. *Genes Dev.*, **1**, 1268–1279.
- Schneider, I. (1972) Cell lines derived from late embryonic stages of *Drosophila melanogaster*. *J. Embryol. Exp. Morphol.*, **27**, 353–365.
- Schreiber, J., Sock, E. and Wegner, M. (1997) The regulator of early gliogenesis *glial cells missing* is a transcription factor with a novel type of DNA-binding domain. *Proc. Natl Acad. Sci. USA*, **94**, 4739–4744.
- Schreiber, J., Enderich, J. and Wegner, M. (1998) Structural requirements for DNA binding of GCM proteins. *Nucleic Acids Res.*, **26**, 2337–2343.
- Simmonds, A.J., Brook, W.J., Cohen, S.M. and Bell, J.B. (1995) Distinguishable functions for *engrailed* and *invected* in anterior-posterior patterning in the *Drosophila* wing. *Nature*, **376**, 424–427.
- Van De Bor, V., Walther, R. and Giangrande, A. (2000) Some fly sensory organs are gliogenic and require *glide/gcm* in a precursor that divides symmetrically and produces glial cells. *Development*, **127**, 3735–3743.
- Van De Bor, V. and Giangrande, A. (2001) Notch signaling represses the glial fate in fly PNS. *Development*, **128**, 1381–1390.
- Vincent, S., Vonesh, J.L. and Giangrande, A. (1996) *glide* directs glial fate commitment and cell fate switch between neurones and glia. *Development*, **122**, 131–139.
- Xiong, W.C., Okano, H., Patel, N.H., Blendy, J.A. and Montell, C. (1994) *repo* codes a glial-specific homeodomain protein required in the *Drosophila* nervous system. *Genes Dev.*, **8**, 981–994.

Received February 1, 2001; revised July 3, 2001;
accepted July 4, 2001