Development 129, 3295-3309 (2002)
Printed in Great Britain © The Company of Biologists Limited 2002
DEV2836

Gliogenesis in *Drosophila*: genome-wide analysis of downstream genes of *glial cells missing* in the embryonic nervous system

Boris Egger[1], Ronny Leemans[1], Thomas Loop[1], Lars Kammermeier[1], Yun Fan[1], Tanja Radimerski[1], Martin C. Strahm[2], Ulrich Certa[3] and Heinrich Reichert[1,*]

[1]Biozentrum/Pharmazentrum, University of Basel, CH-4056 Basel, Switzerland
[2]Roche Bioinformatics, F. Hoffmann-La Roche, Ltd, CH-4070 Basel, Switzerland
[3]Genetics Pharmaceuticals Division, F. Hoffmann-La Roche, Ltd, CH-4070 Basel, Switzerland
*Author for correspondence (e-mail: heinrich.reichert@unibas.ch)

Accepted 13 April 2002

SUMMARY

In *Drosophila*, the *glial cells missing* (*gcm*) gene encodes a transcription factor that controls the determination of glial versus neuronal fate. In *gcm* mutants, presumptive glial cells are transformed into neurons and, conversely, when *gcm* is ectopically misexpressed, presumptive neurons become glia. Although *gcm* is thought to initiate glial cell development through its action on downstream genes that execute the glial differentiation program, little is known about the identity of these genes. To identify *gcm* downstream genes in a comprehensive manner, we used genome-wide oligonucleotide arrays to analyze differential gene expression in wild-type embryos versus embryos in which *gcm* is misexpressed throughout the neuroectoderm. Transcripts were analyzed at two defined temporal windows during embryogenesis. During the first period of initial *gcm* action on determination of glial cell precursors, over 400 genes were differentially regulated. Among these are numerous genes that encode other transcription factors, which underscores the master regulatory role of *gcm* in gliogenesis. During a second later period, when glial cells had already differentiated, over 1200 genes were differentially regulated. Most of these genes, including many genes for chromatin remodeling factors and cell cycle regulators, were not differentially expressed at the early stage, indicating that the genetic control of glial fate determination is largely different from that involved in maintenance of differentiated cells. At both stages, glial-specific genes were upregulated and neuron-specific genes were downregulated, supporting a model whereby *gcm* promotes glial development by activating glial genes, while simultaneously repressing neuronal genes. In addition, at both stages, numerous genes that were not previously known to be involved in glial development were differentially regulated and, thus, identified as potential new downstream targets of *gcm*. For a subset of the differentially regulated genes, tissue-specific in vivo expression data were obtained that confirmed the transcript profiling results. This first genome-wide analysis of gene expression events downstream of a key developmental transcription factor presents a novel level of insight into the repertoire of genes that initiate and maintain cell fate choices in CNS development.

Key words: *glial cells missing*, Glial cell, Nervous system, Oligonucleotide microarray, Gene expression, *Drosophila*

INTRODUCTION

During CNS development, two major cell types are generated, namely neurons and glial cells. These can be generated either by common precursors (neuroglioblasts) or by precursors that are specialized to produce either neurons (neuroblasts) or glial cells (glioblasts) (Akiyama-Oda et al., 1999; Bernardoni et al., 1999; Gage, 2000; Malatesta et al., 2000; Qian et al., 2000). Neuroglial development has been studied in detail in *Drosophila*, where each embryonic neuromere consists of approximately 60 glial cells and 700 neurons (Klämbt and Goodman, 1991; Ito et al., 1995; Schmidt et al., 1997; Jones, 2001). In *Drosophila*, neuroblasts divide asymmetrically to produce ganglion mother cells, which divide once to produce two neurons (Doe and Skeath, 1996), whereas glioblasts produce only glial cells. Glial and neuronal cell lineages in *Drosophila* also derive from neuroglioblasts, which divide asymmetrically to produce a neuroblast and a glioblast.

In *Drosophila*, the *glial cells missing* (*gcm*) gene encodes a transcription factor that controls the determination of glial versus neuronal fate in neuroectodermally derived cells (Hosoya et al., 1995; Jones et al., 1995; Vincent et al., 1996; Wegner and Riethmacher, 2001). In *gcm* mutants, cells that normally develop into glia enter a neuronal differentiation pathway leading to a loss of glia and a gain of neurons. By contrast, targeted *gcm* misexpression in neural progenitors leads to an increase of glial cells at the expense of neurons. Neither a specific embryonic stage nor a neural ‘ground state’ appears necessary for *gcm* action as misexpression of *gcm* in epidermis or mesoderm suppresses normal cell fate and causes

cells to adopt a glial fate (Akiyama-Oda et al., 1998; Bernardoni et al., 1998). Mesectodermal midline glial cells do not require *gcm* function (Granderath and Klämbt, 1999).

The molecular mechanisms of *gcm* action in glial development are poorly understood. Clearly, *gcm* transcription factor action depends on its target genes; however, relatively few genes, such as the *reversed polarity* (*repo*) gene, are known to act downstream of *gcm* (Akiyama et al., 1996). To identify *gcm* downstream genes in a comprehensive manner, we carried out a novel functional genomic approach using genome-wide oligonucleotide arrays. These arrays are used to analyze the transcripts in wild-type embryos versus embryos in which *gcm* is misexpressed throughout the CNS. Tissue-specific misexpression was achieved by using a *scabrous-GAL4* (*sca-GAL4*) line (Klaes et al., 1994) to drive *gcm* expression throughout the embryonic neuroectoderm. Transcripts were analyzed at two defined temporal windows during embryogenesis. First, during a period of initial *gcm* action on determination of glial cell precursors; and second during a later period when glial cells have already differentiated. In both cases, we found significant changes in transcript abundance for hundreds of identified genes following *gcm* misexpression. Remarkably, over half of these genes have not yet been studied in any in vivo context in *Drosophila*. All of these identified genes are potential direct or indirect downstream targets of *gcm* and may, thus, be involved in regulating glial cell fate.

MATERIALS AND METHODS

Flies

The wild-type was Oregon-R. For targeted misexpression of *gcm*, virgin females from *scabrous-GAL4* (Klaes et al., 1994) were crossed to *yw*; *UAS-gcm*; *UAS-gcm* males (Jones et al., 1995). For *gcm* loss-of-function studies the null allele *gcmΔP1* (Jones et al., 1995) was used balanced over *CyO-wglacZ*. Homozygous mutants were identified by absence of either anti-RK2/REPO or anti-β-gal staining. Stocks were kept on standard medium at 25°C. After a 1 hour pre-collection, wild-type and *sca-gcm* embryos were collected in parallel for 1 hour and staged to 6-7 hours AEL (stage 11) or to 13-14 hours AEL (late stage 15/early stage 16). Stages are according to Campos-Ortega and Hartenstein (Campos-Ortega and Hartenstein, 1997).

Arrays and hybridization

A custom-designed *Drosophila* oligonucleotide array (roDROMEGAa, Affymetrix, Santa Clara, CA) was used (Montalta-He et al., 2002). It contains 14,090 sequences representing 13,369 single transcripts encoding *Drosophila* proteins deposited in SWISS-PROT/TrEMBL databases (Celera Genome/BDGP Release no. 1) (Adams et al., 2000), as well as prokaryotic and custom chosen control sequences. Each sequence is represented on the array by a set of 14 oligonucleotide probes of matching sequence and 14 probes with a single nucleotide mismatch. The average difference (Avg Diff) between the perfect match hybridization signal and the mismatch signal is proportional to the abundance of a given transcript (Lipshutz et al., 1999). RNA was isolated; cDNA was synthesized; and cRNA was labeled and hybridized to the array as described previously (Leemans et al., 2001). Four replicates were performed for each experimental condition.

Data analysis

Data acquisition and processing was as described elsewhere (Leemans et al., 2001). For quantification of relative transcript abundance, the average difference value (Avg Diff) was used. All arrays were normalized against the mean of the total sums of Avg Diff values across all 16 arrays. For differential transcript imaging, only transcripts that had significant changes in Avg Diff ($P \leq 0.01$; unpaired *t*-test) in the 1.5-fold and above range were considered, and then only if the mean Avg Diff for the transcript ≥50 in at least one condition.

In situ hybridization and immunocytochemistry

In situ hybridization was carried out according to Tautz and Pfeifle (Tautz and Pfeifle, 1989). Embryos were mounted in Canada balsam (Serva) and photographed with a Prog/Res/3008 digital camera (Kontron, Zürich) on a Zeiss Axioskop microscope with differential interference contrast optics. Immunocytochemical experiments were carried out as described previously (Therianos et al., 1995; Leemans et al., 2001). The primary antibodies were rat anti-RK2/REPO 1:1000 (Campbell et al., 1994), mouse anti-TEN-M 1:250 (Baumgartner et al., 1994), rabbit anti-EY 1:500 (Kammermeier et al., 2001), mouse anti-WRAPPER 1:5 (Noordermeer et al., 1998) and goat anti-HRP (FITC-conjugated) 1:20 (Jackson Immunoresearch). For fluorescent labeling, secondary antibodies were Alexa568 and Alexa488 conjugated, all 1:150 (Molecular Probes). For laser confocal microscopy, a Leica TCS SP was used.

RESULTS

Targeted misexpression of *gcm* in the embryonic neuroectoderm results in a switch from neuronal to glial fate

For targeted misexpression of *gcm* in the neuroectoderm of *Drosophila* embryos, a *sca–GAL4* enhancer trap line (Klaes et al., 1994) was crossed with an *UAS-gcm* responder line (*sca-gcm*) (Jones et al., 1995). This resulted in ectopic *gcm* expression in the embryonic CNS, starting from embryonic stage 9 and diminishing, similar to endogenous *gcm* expression, at embryonic stage 15. Although misexpression of *gcm* starts at stage 9 in *sca-gcm* embryos, ectopic expression of the *repo* gene, a known direct target of *gcm*, was not seen before stage 11, similar to endogenous *repo* expression.

In order to identify genes that are either direct *gcm* target genes or among the initial set of downstream genes of *gcm*, we carried out a first genome-wide analysis of differential gene expression at embryonic stage 11 when the first glial marker, the direct *gcm* target gene *repo*, is expressed. In the wild type during stages 10-11, two small groups of neuroectodermal cells per hemisegment transiently express *gcm*, and a single *gcm*-expressing glial precursor delaminates from each of these groups and expresses the *repo* gene (Fig. 1A,C) (Hosoya et al., 1995; Jones et al., 1995). By contrast, in *sca-gcm* embryos during stages 10-11, all of the cells in the neuroectoderm express *gcm* (Fig. 1B) and, as a consequence, most of the neural precursor cells become REPO positive (Fig. 1D). With the exception of altered gene expression in cells of the neuroectoderm, neither gene expression changes outside of the neural lineage nor any obvious morphological changes are seen in these stage 11 *sca-gcm* embryos.

In order to identify also additional indirect downstream genes of *gcm* that act further along in the genetic cascade of *gcm* action, we carried out a second genome-wide transcriptional analysis at embryonic stage 15/16 when glial cells are differentiated. In the wild type at stage 15/16, ~700 neurons and 60 glial cells per neuromere have differentiated, and the glial cells (with the exception of midline glia) are REPO positive (Fig. 1E) (Ito et al., 1995). By contrast, in stage

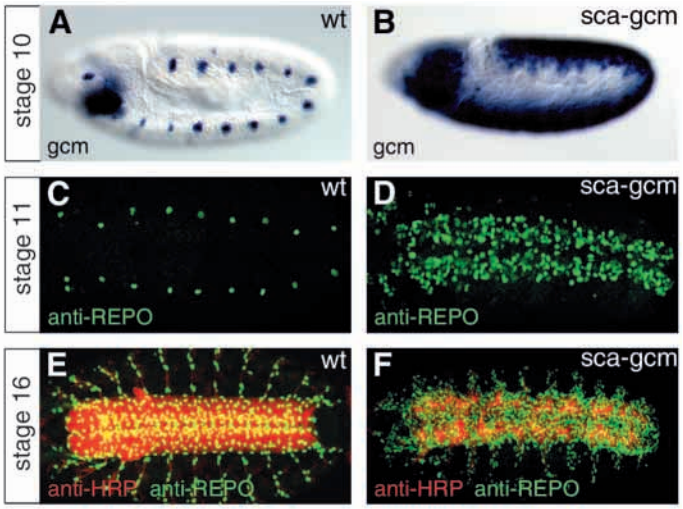


Fig. 1. Targeted misexpression of *gcm* results in gain of glial cells at the expense of neuronal cells. (A,B) In situ hybridization of stage 10 embryos shows *gcm* expression in wild-type (A) and in *sca-gcm* embryos (B); lateral views, anterior towards the left. In the wild type, small clusters of cells in the neuroectoderm express *gcm*; in *sca-gcm* embryos, all cells of the neuroectoderm express *gcm*. (C,D) Immunostaining with anti-REPO in wild-type (C) and in *sca-gcm* (D) embryos; laser confocal microscopy of stage 11, ventral views of the VNC, anterior is towards the left. In the wild-type, single *gcm*-expressing glial precursors in each hemisegment express the *repo* gene. In *sca-gcm* embryos, virtually all of the neuronal and glial precursor cells are REPO positive. (E,F) Double immunostaining with anti-REPO (green) and anti-HRP (red) in wild-type (E) and in *sca-gcm* (F) embryos; laser confocal microscopy of stage 15/16 embryos. In the wild type, neurons and glial cells are differentiated and correctly positioned, and all lateral glial cells express *repo*. In *sca-gcm* embryos, 80%-90% of the cells in the CNS express *repo*.

15/16 sca-*gcm* embryos, 80%-90% of the cells in the CNS express REPO protein (Fig. 1F) and have a glial morphology (Hosoya et al., 1995). Correspondingly, the number of cells expressing the neuronal marker *embryonic lethal abnormal vision* (*elav*) in these *sca-gcm* embryos is reduced by ~90% (data not shown) (Hosoya et al., 1995), and a striking reduction of the CNS axon scaffold is observed. In addition to the pronounced changes in the number of glial versus neuronal cells, stage 15/16 *sca-gcm* embryos also show defects in ventral nerve cord condensation and in peripheral innervation. No other gross morphological changes were seen in these stage 15/16 *sca-gcm* embryos.

Overview of differential gene expression following *gcm* misexpression

Analysis of differential gene expression in stage 11 and stage 15/16 *sca-gcm* versus wild-type embryos was carried out with oligonucleotide arrays representing 13,369 annotated *Drosophila* genes. This corresponds to virtually all of the currently annotated genes of the *Drosophila* genome sequence (Adams et al., 2000). For each embryonic stage, 2×4 replicate oligonucleotide arrays were used to detect transcript levels in *sca-gcm* embryos when compared with wild-type controls. Only transcripts that show an expression level fold change (FC) ≥1.5 or ≤–1.5 at significance values of $P \leq 0.01$ (*t*-test) were considered to be differentially expressed (see Materials and Methods). A complete list of all of these genes, as well as their quantitative fold change values is given at www.ncbi.nlm.nih.gov/geo/.

At stage 11, we detected 417 transcripts with differential expression values in *sca-gcm* embryos when compared with wild type. This corresponds to ~3% of the transcripts on the array. Approximately the same number of transcripts have increased (n=219) and decreased (n=198) abundance levels, indicating that *gcm* causes both activation and repression of downstream gene transcription. At stage 15/16, we detected 1259 genes with differential expression values in *sca-gcm* embryos compared to wild type. This corresponds to ~9% of the transcripts on the array. Thus, markedly more transcripts are differentially expressed at stage 15/16 than at stage 11. Again, approximately the same number of transcripts are upregulated (n=609) and downregulated (n=650).

For an overview, all differentially expressed genes of known or predicted molecular function were grouped into functional classes. At stage 11, 199 transcripts of known function belonging to 13 functional classes are differentially expressed in *sca-gcm* embryos (Table 1). The two functional classes with the largest number of differentially regulated transcripts are enzymes (78) and nucleic acid-binding proteins (44), including 26 transcription factors. At stage 15/16, 614 transcripts of known function belonging to 15 functional classes are differentially expressed in *sca-gcm* embryos (Table 1). The two functional classes with the largest number of differentially regulated transcripts are again enzymes (249) and nucleic acid-binding proteins (96), including 38 transcription factors. Strikingly, however, at both stages, the majority of the differentially expressed transcripts are of

Table 1. Differential gene expression in functional classes following *gcm* misexpression

Molecular function	Number of transcripts	
	Stage 11	Stage 15/16
Nucleic acid binding	44	96
DNA binding	33	64
Transcription factor	26	38
RNA binding	6	27
Translation factor	5	4
Ribonucleoprotein	0	1
Cell cycle regulator	0	10
Chaperone	7	13
Motor protein	4	7
Defense/immunity protein	4	3
Enzyme	78	249
Kinase/phosphatase	13	59
Enzyme activator	0	3
Enzyme inhibitor	3	7
Apoptosis regulator	0	2
Signal transducer	12	50
Cell adhesion	4	19
Structural protein	3	39
Transporter	25	51
Ligand binding or carrier	13	63
Antioxidant	1	2
Tumor suppressor	1	0
Function unknown	218	645
Total	417	1259

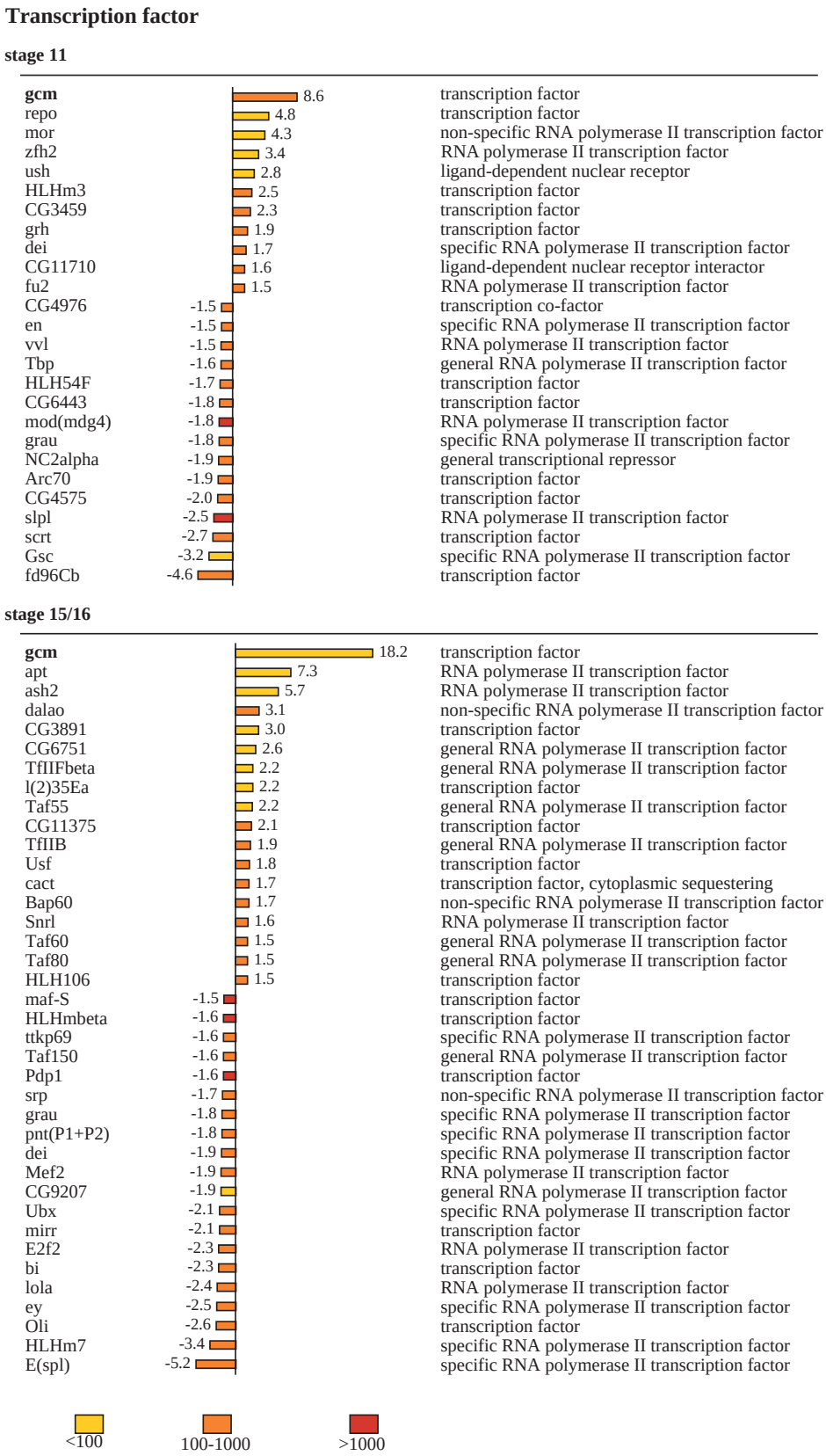


Fig. 2. Changes in transcript levels of the genes encoding transcription factors after *gcm* misexpression. Bars represent the fold changes in gene expression levels between wild-type embryos and *sca-gcm* embryos. Positive values indicate that the relative expression level of a gene is increased (upregulation) and negative values indicate a decrease (downregulation). Normalized average difference values are given for the wild-type condition as follows: yellow bars represent <100; orange bars represent 100-1000; red bars represent >1000.

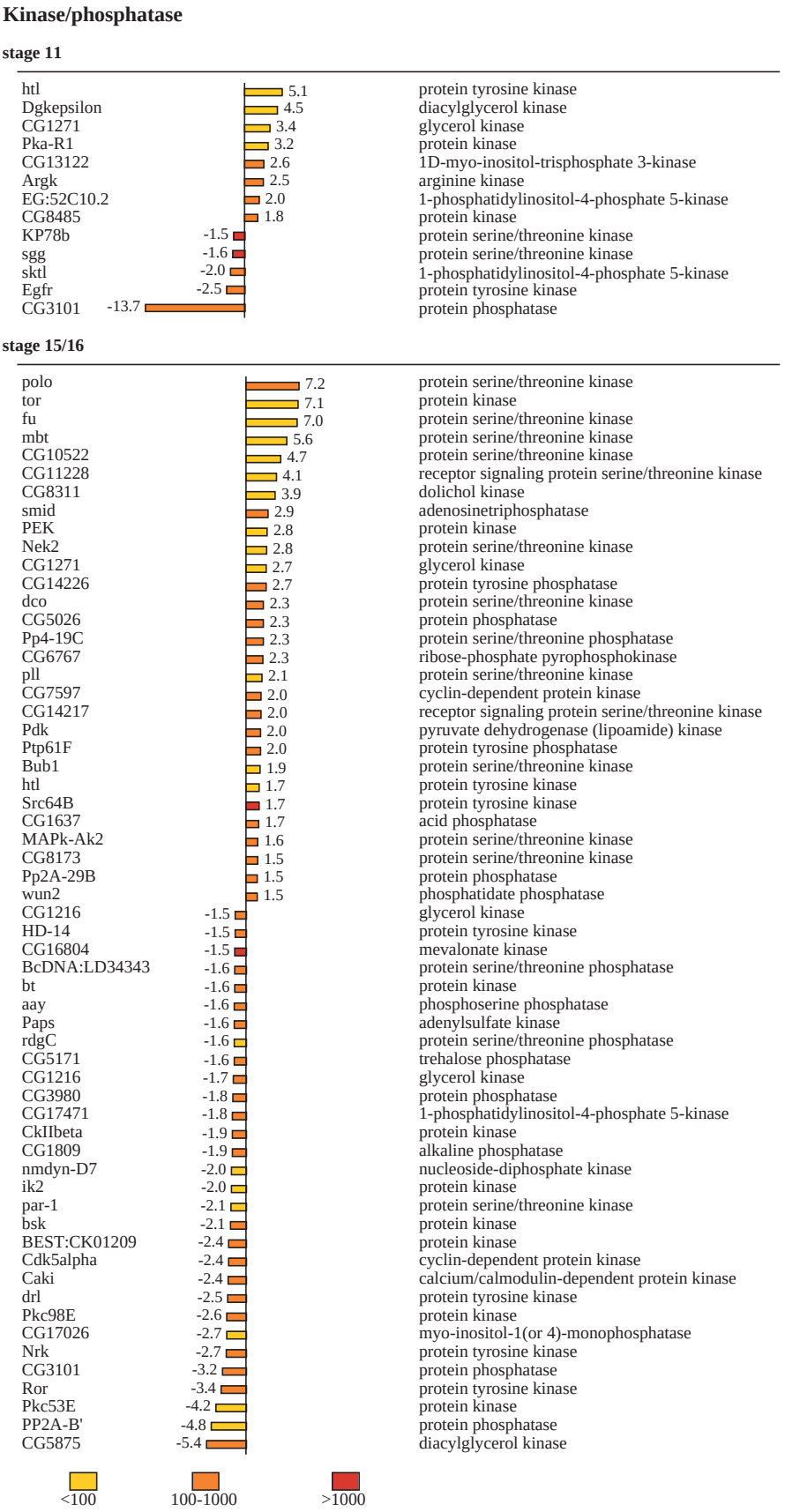


Fig. 3. Changes in transcript levels for the genes encoding protein kinases and phosphatases following *gcm* misexpression. Data are presented as in Fig. 2.

(currently) unknown function; 218 (52%) at stage 11 and 645 (51%) at stage 15/16.

Differential expression of genes encoding transcription factors

The fact that *gcm* acts as a fate switch and key regulator of gliogenesis suggests that *gcm* might control several other transcription factors that, in turn, would regulate the expression of their own downstream genes. Transcript profiling of *gcm* misexpression indicated that *gcm* does indeed control the expression of numerous other transcription factors. These transcription factor-encoding genes and a quantification of their changes in expression level are shown in Fig. 2.

In stage 11 embryos, 26 genes encoding transcription factors are differentially regulated by targeted *gcm* misexpression (11 upregulated, 15 downregulated). The *gcm* gene has the highest expression level increase (8.6-fold), in accordance with our experimental procedure. (The *gcm* gene also has a high absolute level of expression at this stage; see color coding in Fig. 2.) The *repo* gene, a known direct target of *gcm* (Akiyama et al., 1996), has the second highest increase in expression level (4.8-fold). Many of the other upregulated transcription factor genes such as *zinc finger homeobox-2* (*zhf-2*), *u-shaped* (*ush*) and the Enhancer of split complex-member *HLHm3* are known to act in different aspects of embryonic nervous system development (Delidakis and Artavanis-Tsakonas, 1992; Lundell and Hirsh, 1992; Cubadda et al., 1997). Genes of the Enhancer of split complex, for example, act during neural versus epidermal cell fate decision (Jennings et al., 1994), and in the mouse, Enhancer of split members *Hes1* and *Hes5* have been shown to enhance glial cell fate (Furukawa et al., 2000; Hojo et al., 2000). Among the transcription factors with decreased expression levels are *engrailed* (*en*) and *ventral veins lacking/drifter* (*vvl/drf*), which are expressed in a subset of neuronal precursor cells and are also involved in midline glial cell development, but not in lateral glial cell development (Condron et al., 1994; Anderson et al., 1995). Other genes encoding transcription factors with decreased expression levels are *sloppy paired 1* (*slp1*), *goosecoid* (*gsc*) and *forkhead domain 96Cb* (*fd96Cb)*, which are expressed in subsets of neural precursor cells (Hacker et al., 1992; Hahn and Jäckle, 1996; Bhat et al., 2000). Moreover, the *scratch* (*scrt*) transcription factor, a pan-neuronal gene encoding a zinc-finger protein that promotes neuronal development and can induce additional neurons when ectopically expressed (Roark et al., 1995), also shows decreased expression levels.

In stage 15/16 embryos, 38 genes encoding transcription factors are differentially regulated by targeted *gcm* misexpression (18 upregulated, 20 downregulated). As expected, *gcm* has the highest expression level increase (18.2-fold). (The absolute level of expression of the *gcm* gene is now an order of magnitude lower at this stage than at stage 11; see color coding in Fig. 2.) In contrast to high REPO protein levels in stage 15/16 *sca-gcm* embryos (Fig. 1F), significant expression of *repo* transcripts is not detected at this stage. Several genes encoding transcription factors, which are expressed in specific neurons, such as *eyeless* (*ey*) and *Ultrabithorax* (*Ubx*) (Hirth et al., 1998; Kammermeier et al., 2001), are downregulated. Moreover, several members of the Enhancer of split complex such as *HLHmbeta*, *HLHm7*, and *E(spl)*, are downregulated at stage 15/16, in contrast to stage 11; in addition to a role in early neurogenesis, these genes continue to be expressed in the normal developing nervous system of the wild type at later embryonic stages (Wech et al., 1999).

The marked increase in the number of affected transcripts at stage 15/16 is due in part to the fact that numerous genes encoding transcription factors belonging to the basal transcription machinery are differentially regulated at this stage. Among these are *TfIIFbeta*, *Taf55*, *TfIIB*, *Taf60*, *Taf80* and *Taf150* (Frank et al., 1995; Lee et al., 1997; Aoyagi and Wassarman, 2000). Moreover, among the upregulated genes encoding transcription factors, several are involved in chromatin remodeling, such as the brahma complex or associated genes [*dalao* (*dalao*), *Brahma associated protein 60 kp* (*Bap60*), *Snf5-related 1* (*Snr1*) and *absent, small or homeotic disc 2* (*ash2*) (Francis and Kingston, 2001)]. This suggests that the maintenance of glial cell differentiation at later embryonic stages involves chromatin remodeling as well as the regulation of global transcriptional processes.

In addition to the above mentioned genes for transcription factors involved in chromatin remodeling, several genes encoding other proteins that bind to DNA/chromatin are influenced by *gcm* misexpression. These genes and a quantification of their expression level changes are shown in Fig. 4. In stage 11 embryos, seven genes encoding chromatin-binding proteins are differentially regulated (three are upregulated, four are downregulated), and at stage 15/16 embryos, 26 genes encoding chromatin-binding proteins are differentially regulated (17 are upregulated, nine are downregulated). Prominent among the upregulated genes thought to be involved in chromatin condensation and segregation are *gluon* (*glu*) (Steffensen et al., 2001) and the two DNA replication factor genes *Mini chromosome maintenance 6* and *Mini chromosome maintenance 7* (*Mcm6* and *Mcm7*) (Ohno et al., 1998). Among the genes with downregulated expression are the three Sox-related genes *sox-like* (*sox-like*), *Sox box protein 14 (Sox 14)* and *Dichaete* (*D*), which encode DNA-bending proteins. *D* is known to be expressed in neural precursor cells and in midline glial cells (Soriano and Russell, 1998; Sanchez-Soriano and Russell, 2000).

Only four genes encoding DNA-binding proteins, including two that encode transcription factors, are differentially expressed in both early and late stage *sca-gcm* embryos. This represents only 4% of the genes for DNA-binding proteins that are differentially expressed in these embryos. This finding, which in qualitative terms holds for all other functional classes of differentially expressed genes, indicates that the molecular genetic mechanisms of early glial fate determination are largely different from those involved in the later maintenance of differentiated glial cells.

Differential expression of genes encoding kinases and phosphatases

Cell-cell interactions between neuronal and glial cells are crucial for key cellular processes such as metabolic exchange, extrinsic signaling and electrical insulation. The switch from neuronal to glial fate caused by *gcm* misexpression is, therefore, likely to affect genes that encode proteins involved in cell-cell signaling. Transcript imaging analysis of *gcm* misexpression indicates that *gcm* does indeed control numerous genes that encode kinases

and phosphatases involved in signaling pathways. A list of these genes, as well as a quantitative representation of their changes in expression levels, is shown in Fig. 3. Once again, a marked increase in the number of affected transcripts was observed at stage 15/16 as compared with stage 11.

In stage 11 embryos, 13 genes encoding kinases or phosphatases are differentially regulated by *gcm* misexpression (eight are upregulated, five are downregulated). Among the genes with increases in transcript abundance is *heartless* (*htl*), which encodes a fibroblast growth factor (FGF) receptor expressed in lateral glial cells (Shishido et al., 1997). Conversely the *Epidermal growth factor receptor* (*Egfr*) shows a decrease in transcript abundance; the *Egfr* pathway is implicated in midline glial cell development (Scholz et al., 1997). Decreased expression is also observed for *shaggy* (*sgg*), which encodes a protein kinase, and for *skittles* (*sktl*), which encodes a putative phosphatidylinositol-4-phosphate 5-kinase. Cells in *sgg* mutant embryos cannot adopt early epidermal fates and instead develop characteristics of CNS cells (Bourouis et al., 1989). Mutations in *sktl* cause abnormal development in the PNS (Prokopenko et al., 2000).

In stage 15/16 embryos, 59 genes encoding kinases or phosphatases are differentially regulated by *gcm* misexpression (29 are upregulated, 30 are downregulated). Several genes involved in cell proliferation and mitotic division are upregulated. These included *polo* (*polo*), *discs overgrown* (*dco*), *smallminded* (*smid*) and *Nek2* (*Nek2*) (Llamazares et al., 1991; Schultz et al., 1994; Long et al., 1998; Zilian et al., 1999). By contrast, genes involved in aspects of neuronal development, such as axogenesis and synaptogenesis are downregulated. Among these are *derailed* (*drl*), *Neuron-specific kinase* (*Nrk*) and *Cdk5 activator-like protein* (*Cdk5alpha*). The *drl* gene is involved in axonal guidance including routing across the midline (Bonkowsky et al., 1999). *Nrk* is specifically expressed in the embryonic CNS (Oishi et al., 1997). *Cdk5alpha* controls multiple aspects of axon patterning (Connell-Crowley et al., 2000). The only gene in this class that is known to be involved in glial differentiation is *htl*, which is upregulated at stage 11 and remains upregulated in stage 15/16 embryos, albeit at a lower level.

Differential expression of genes involved in cell cycle regulation

As mentioned above, several chromatin-binding protein and kinase/phosphatase-encoding genes involved in cellular proliferation and in mitotic division are upregulated by *gcm* misexpression. This suggests that other genes involved in proliferation and division may also be affected by *gcm* misexpression. Transcript profiling of *gcm* misexpression indicates that *gcm* does indeed influence genes that encode cell cycle regulators. These genes and a quantitative representation of their changes in expression levels are shown in Fig. 4B.

Ten genes encoding cell cycle regulators are differentially regulated by *gcm* misexpression (seven are upregulated, three are downregulated) in stage 15/16 embryos. For example, increases in transcript abundance are found for *Cyclin B* (*CycB*), *Cyclin A* (*CycA*) and *Cyclin D* (*CycD*). These genes encode regulators of cyclin-dependent kinases that act in different phases during mitotic cell cycles (Follette and O'Farrell, 1997). By contrast, and rather unexpectedly, a marked decrease in transcript abundance is found for *Cyclin E* (*CycE*). *CycE* is essential for S-phase progression and its downregulation leads to the arrest of cell proliferation (Knoblich et al., 1994). Remarkably, in the earlier embryonic stage 11, none of the genes in the class of cell cycle regulators is influenced by *gcm* misexpression.

Differential expression of genes encoding cell adhesion molecules

Several cases for *gcm*-dependent regulation of genes encoding cell adhesion molecules were observed. These genes, as well as a quantitative representation of their expression level changes is shown in Fig. 4C. At stage 11, four genes encoding cell adhesion molecules are differentially regulated by *gcm* misexpression (two are upregulated, two are downregulated). At stage 15/16, 19 genes encoding cell adhesion molecules are differentially regulated by *gcm* misexpression (four are upregulated, 15 are downregulated).

A striking example for a gene with a marked increased transcript level (13.6 fold) in stage 15/16 embryos is *wrapper*, which encodes a cell adhesion molecule that is expressed in midline glial cells and in late stages also in lateral glial cells (Noordermeer et al., 1998). Genes with decreased transcript levels in stage 15/16 embryos that are mainly expressed in neurons are *Tenascin major* (*Ten-m*), *Cadherin-N* (*CadN*) and *neuromusculin* (*nrm*). All three act during axogenesis and synaptogenesis (Kania et al., 1993; Baumgartner et al., 1994; Levine et al., 1994; Iwai et al., 1997). The fact that most of the affected genes in the cell adhesion class show *gcm*-dependent decreased transcript levels could reflect the large diversity of cell adhesion molecules expressed by neurons.

gcm misexpression may influence genes that act in the hemocyte lineage

In addition to its key role in gliogenesis, *gcm* also functions in a mesodermal lineage that gives rise to hematopoietic cells (Bernardoni et al., 1997; Lebestky et al., 2000). When ectopically expressed in the early mesoderm, *gcm* can induce expression of *Peroxidasin* (*Pxn*), which is a marker for macrophage cells. Misexpression of *gcm* in cells of the neural lineage also gives rise to a few cells that express hemocyte markers, although most cells differentiate into glia (Bernardoni et al., 1997). In accordance with these findings, transcript profiling of *gcm* misexpression embryos indicates that several genes encoding marker proteins for cells of the hemocyte lineage are differentially regulated.

In stage 15/16 embryos, differential expression levels are detected for *Pxn*, *serpent* (*srp*) and *the Scavenger receptor class C* (*type I*) gene (Pearson et al., 1995), all of which are expressed in hemocytes. Scavenger receptors play a crucial role in the phagocytosis of apoptotic cells and might also be able to mediate the direct recognition of microbial pathogens (Platt et al., 1998). It is noteworthy that the genes encoding Lysozyme B, Lysozyme C, Lysozyme D and Lysozyme E are all upregulated by *gcm* misexpression in stage 11 embryos. These four closely related lysozyme genes, clustered at locus 61F on the third chromosome, function as part of a system of inducible antibacterial immunity (Daffre et al., 1994). These findings support the notion that the glial cell lineage and the hemocyte lineage, which give rise to cells involved in defense and immunity, may be molecularly related (Bernardoni et al., 1997).

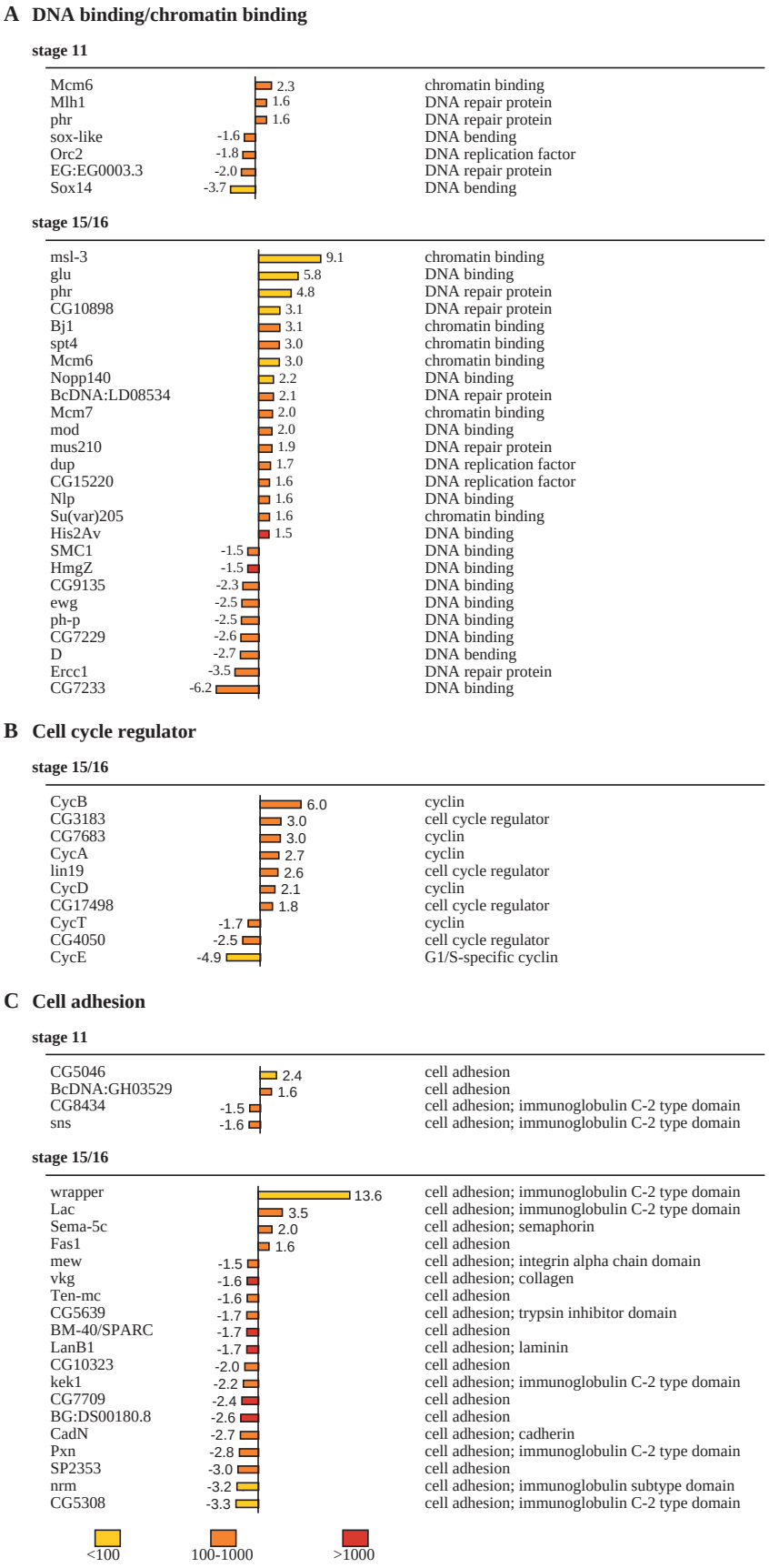
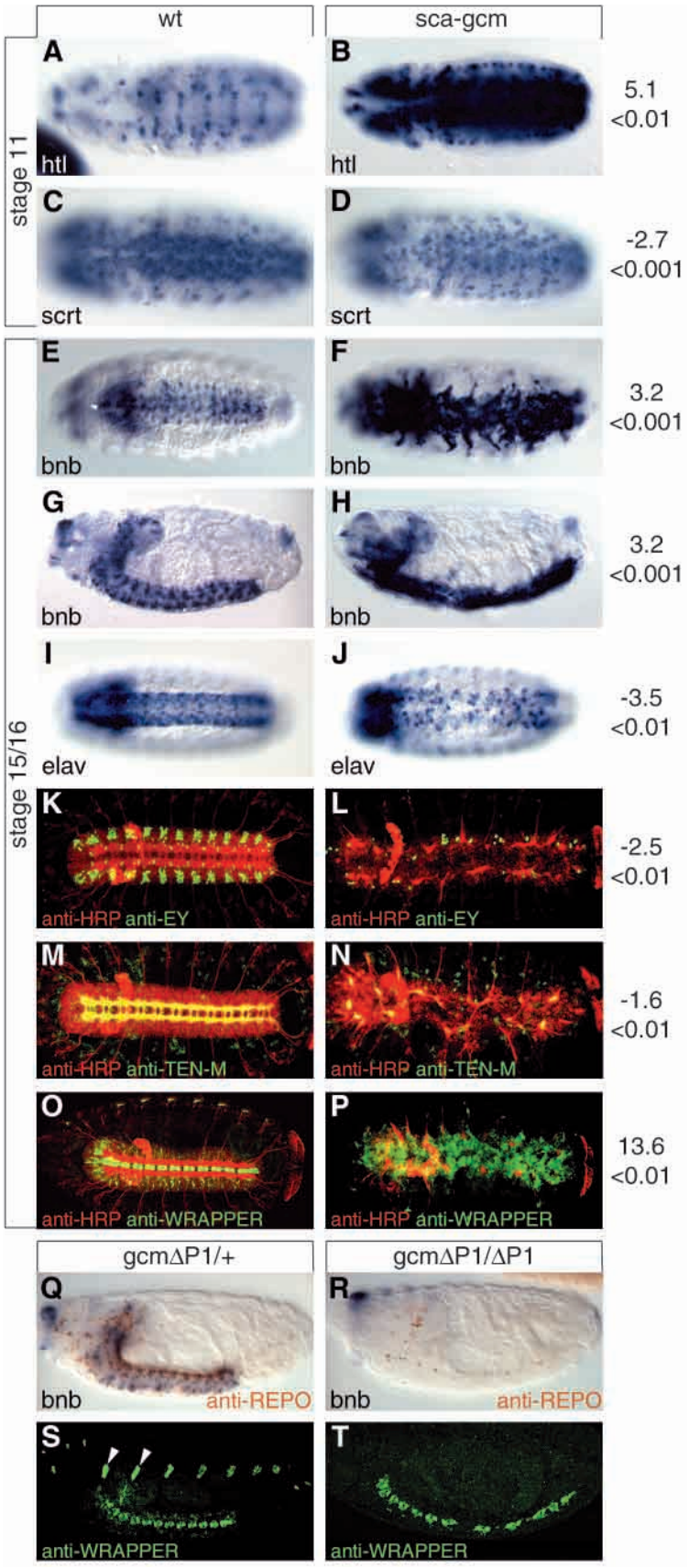


Fig. 4. Changes in transcript levels for the genes encoding DNA/chromatin-binding proteins (A), cell cycle regulators (B) or cell adhesion molecules (C) after *gcm* misexpression. Data are presented as in Fig. 2.



Analysis of spatial expression of candidate *gcm* downstream genes by in situ hybridization and immunocytochemistry

To complement the quantitative transcript profiling analysis with tissue-specific spatial expression data, in situ hybridization and immunostaining was carried out on a subset of the genes that are differentially regulated by *gcm* misexpression (Fig. 5). In all cases, the qualitative changes in tissue-specific gene expression revealed by in situ hybridization and immunocytochemistry reflect and confirm the changes in gene expression determined by transcript profiling.

Expression of the transcripts for *htl*, *scrt*, *bangles and beads* (*bnb*) and *elav* was examined by in situ hybridization. In stage 11 wild-type embryos, the *htl* gene is expressed in a distinct set of neural precursors in the CNS (Fig. 5A). Outside of the CNS, *htl* is also expressed in elements of the mesodermal lineage (Shishido et al., 1997). After targeted misexpression of *gcm* in cells of the neuroectoderm in stage 11 *sca-gcm* embryos, the expression of *htl* is expanded in the CNS

Fig. 5. Spatial expression of selected candidate *gcm* downstream genes by in situ hybridization and immunocytochemistry. Whole-mount in situ hybridization (A-J,Q,R) and immunostaining (K-T) show expression of differentially regulated genes in wild-type, *sca-gcm* and *gcm* mutant embryos. Ventral views of stage 11 (A-D) and stage 15/16 (E,F,I,J,K-P) embryos, and lateral views of stage 15/16 embryos (G,H,Q-T), anterior is towards the left. Fold changes and *P* values are indicated on the right. (A,B) Expression of *htl* in stage 11 wild-type embryos is visible in a distinct set of neural precursors; in sca-*gcm* embryos, *htl* is expressed throughout the neurogenic region. (C,D) In stage 11 embryos, the *scrt* gene is expressed in neural precursors; in stage 11 *sca-gcm* embryos, the expression of *scrt* is diminished in most of the neural precursors, but is still apparent in a subset of these cells. (E-H) In stage 15/16 wild-type embryos, *bnb* gene is expressed in lateral glial cells; in stage 15/16 *sca-gcm* embryos, the expression of *bnb* increases markedly and appears virtually in all of the cells of the nervous system. (I,J) In stage 15/16 wild-type embryos, the *elav* gene is expressed in all neurons; in stage 15/16 in *sca-gcm* embryos, expression of *elav* is strongly reduced in most of the neurons. (K,L) In stage 15/16 wild-type embryos, the EY protein is expressed in a segmentally reiterated subset of neurons in the CNS; in stage 15/16 *sca-gcm* embryos, the number of EY-expressing cells in the CNS is dramatically reduced. (M,N) In stage 15/16 wild-type embryos, the TEN-M protein is expressed on the axons that make up the longitudinal and commissural tracts of the CNS; this axonal expression of TEN-M is virtually abolished in stage 15/16 *sca-gcm* embryos. (O,P) In stage 15/16 wild-type embryos, the WRAPPER protein is expressed in midline glial cells, in some lateral glial cells and in glial cells supporting the chordotonal sensory organs; this expression has spread to the complete CNS region in stage 15/16 *sca-gcm* embryos. (Q,R) In late stage embryos REPO (brown) is expressed in all and *bnb* (blue) is expressed in a subset of lateral glial cells; in *gcm* mutants REPO expression is reduced to a few cells, and *bnb* expression is completely absent in the CNS. (S,T) In late stage embryos, WRAPPER is expressed in midline glial cells, in some lateral glial cells and in glial cells supporting chordotonal sensory organs (arrowheads); in *gcm* mutant embryos WRAPPER expression in lateral glia (CNS) and in chordotonal sensory organs (PNS) is absent, whereas expression in midline glial cells remains.

region to include virtually all neural precursors (Fig. 5B). No changes in the expression of *htl* are seen outside of the CNS in these embryos. In stage 11 embryos, the pan-neural *scrt* gene is expressed in most or all neural precursors (Fig. 5C) (Roark et al., 1995). After targeted misexpression of *gcm* in cells of the neuroectoderm in stage 11 *sca-gcm* embryos, the expression of *scrt* is diminished in most of the neural precursors, but is still apparent in a subset of these cells (Fig. 5D).

In stage 15/16 wild-type embryos, the *bnb* gene is expressed in lateral glial cells (Ng et al., 1989) (Fig. 5E,G). With the exception of a small group of cells near the anterior and posterior ends of the embryo, no other *bnb* expression is seen outside of the nervous system at this stage. In stage 15/16 *sca-gcm* embryos, the expression of *bnb* increases markedly and appears in virtually all of the cells of the nervous system (Fig. 5F,H). Expression of *bnb* outside of the nervous system does not appear to be influenced in these *sca-gcm* embryos. In stage 15/16 wild-type embryos, the *elav* gene is expressed in all neurons (Fig. 5I). In stage 15/16 in *sca-gcm* embryos, expression of *elav* is strongly reduced, but is still visible in some neurons of the brain as well as in some of the neurons that occupy the ventral-most cell layer in the ventral nerve cord (Fig. 5J).

Given that transcript abundance is not always reflected on the protein level (Keene, 2001), expression of three further candidate *gcm* downstream genes, *ey*, *Ten-m* and *wrapper* was investigated at the protein level by immunostaining in wild-type and *sca-gcm* embryos. In stage 15/16 wild-type embryos, the EY protein is expressed in a segmentally reiterated subset of neurons in the CNS (Fig. 5K). In stage 15/16 *sca-gcm* embryos the number of cells in the CNS that express the EY protein is dramatically reduced (Fig. 5L). In stage 15/16 wild-type embryos, the TEN-M protein is expressed on the axons that make up the longitudinal and commissural tracts of the CNS (Fig. 5M). This well defined axonal expression pattern of TEN-M protein is virtually abolished in stage 15/16 *sca-gcm* embryos (Fig. 5N). The TEN-M protein is also expressed outside the nervous system (Baumgartner et al., 1994), but there is no obvious change in this non-neuronal expression of TEN-M in *sca-gcm* when compared with wild-type embryos. In stage 15/16 wild-type embryos, the WRAPPER protein is expressed in the midline and in some of the lateral glial cells, as well as in glial cells that support the chordotonal sensory organs in the PNS (Fig. 5O) (Noordermeer et al., 1998). In stage 15/16 *sca-gcm* embryos, a substantial increase of WRAPPER expression is seen in the CNS (Fig. 5P).

To control for possible effects of transgene insertion or of differences in genetic background, we repeated the tissue-specific spatial expression analysis for all of the above mentioned genes on embryos that contain either only the *sca-GAL4* construct or only the *UAS-gcm* constructs. In all cases, in situ hybridization and immunostaining results on these embryos were indistinguishable from results obtained on wild-type embryos (data not shown).

To determine if genes that are influenced by *gcm* gain-of-function might be influenced in an inverse way in *gcm* loss-of-function mutants, we studied tissue-specific spatial expression data of the candidate *gcm* downstream genes *repo*, *bnb*, *wrapper*, *elav*, *ey* and *Ten-m* in *gcm* null mutants using in situ hybridization and immunostaining. Expression of the genes *repo*, *bnb* and *wrapper* is upregulated in *gcm* gain-of-function embryos. In stage 15/16 *gcm* null mutant embryos, the expression of the *repo* gene in lateral glial cells, which is seen in the wild-type CNS, is strongly reduced (Fig. 5Q,R) (Hosoya et al., 1995; Jones et al., 1995; Vincent et al., 1996). Comparable findings are obtained for *bnb;* the expression of the *bnb* gene in lateral glial cells, which is seen in the wild-type CNS, disappears (Fig. 5Q,R). These findings contrast with stage 15/16 *sca-gcm* embryos, where the expression of *repo* and *bnb* appears in virtually all of the cells of the CNS. In stage 15/16 *gcm* null mutant embryos, the expression of *wrapper* in the lateral glial cells and in PNS glial cells (but not the midline glial cell expression), which is observed in wild-type embryos, disappears (Fig. 5S,T). This contrasts with stage 15/16 *sca-gcm* embryos, where the expression of *wrapper* becomes more widespread in the CNS. Expression of the genes *elav*, *ey* and *Ten-m* is downregulated in *gcm* gain-of-function embryos. Expression of *elav* in *gcm* null mutants is seen in additional neuronal cells when compared with the wild-type (data not shown) (Hosoya et al., 1995; Jones et al., 1995; Vincent et al., 1996). By contrast, the number of CNS cells that express either *ey* or *Ten-m* is not altered in *gcm* null mutants when compared with the wild type (data not shown). This is not unexpected because *ey* and *Ten-m* are not pan-neuronally expressed in the same way as *elav*, but are expressed only in a subset of neuronal cells in the wild-type CNS (Baumgartner et al., 1994; Kammermeier et al., 2001).

DISCUSSION

Candidate gene identification through genome-wide transcript imaging

By analyzing gene expression profiles following *gcm* misexpression in the embryonic CNS, genome-wide transcript images were obtained for two phases of glial development. The first transcript image reflects an embryonic CNS in which precursor cells that normally give rise to neurons have been genetically reprogrammed to give rise to glial cells. It was obtained at an early embryonic stage when the first glial-specific genes, such as the *repo* gene (which is a direct target of *gcm*), start to become expressed. This transcript image is therefore likely to identify genes that act in CNS precursors and are involved in the determination of glial versus neuronal cell lineage. Approximately 400 genes were found to be differentially expressed at this developmental stage, corresponding to 3% of the annotated genes in the fly genome. We posit that the genes that are differentially regulated at this early stage are either direct *gcm* target genes, such as *repo*, or among the initial set of genes downstream of *gcm*.

The second transcript image, obtained at a later embryonic stage when glial and neuronal cells are normally differentiated, reflects an embryonic CNS in which 80-90% of the normal number of neuronal cells have been genetically replaced by glial cells because of *gcm* action (Hosoya et al., 1995). This transcript image is therefore likely to identify genes that are involved in the maintenance of differentiated glial versus neuronal cells. Approximately 1300 genes were differentially expressed at this stage, corresponding to 9% of the annotated genes in the fly genome. We postulate that most of these differentially expressed genes are no longer direct or initial

downstream targets of *gcm*, but are rather indirect downstream genes that act further along in the genetic cascade of *gcm* action.

The difference in total number of differentially expressed genes at the early stage versus the late stage is striking and, in qualitative terms, also holds for each of the major functional gene classes. Moreover, the overlap between the genes that are expressed at the two stages is restricted; only 93 (7%) of the 1259 genes that are differentially expressed at the late stage, are also differentially expressed at the early embryonic stage (Fig. 6). This suggests that the gene regulatory elements that control determination of glial cell fate are largely different from those required for maintenance of glial cell differentiation.

The expression profiles presented here derive from gain-of-function experiments in which the *gcm* gene is misexpressed in the embryonic CNS. A comparison of these findings with expression profiles derived from loss-of-function experiments involving *gcm* null mutants will be an important step in the further analysis of *gcm* downstream genes. However, in *gcm* null mutants only about 60 presumptive glial cells per segment are transformed into neurons; the 700 neurons of each segment are not affected. With the current sensitivity of oligonucleotide microarrays, it is unlikely that significant measurements of gene expression changes in such a small number of cells can be obtained using whole-mount embryos. Thus, these complementary *gcm* loss-of-function experiments must await the development of single-cell isolation techniques for the embryonic nervous system of *Drosophila*.

Candidate genes implicated in the determination of glial versus neuronal cell lineage

Although *gcm* expression is necessary and sufficient to induce glial cell fate in and outside of the nervous system, it normally acts in glial precursors in the wild type (Hosoya et al., 1995; Jones et al., 1995; Vincent et al., 1996; Akiyama-Oda et al., 1998; Bernardoni et al., 1998). A current model of this action is that *gcm* controls the determination of glial cell fate in glial precursors by activating both genes that promote glial differentiation and genes that repress neuronal differentiation (Giesen et al., 1997). Application of this model to our experimental paradigm leads to the prediction that expression of glial precursor-specific genes should be upregulated and expression of neuron precursor-specific genes should be downregulated in stage 11 *sca-gcm* embryos. Our genome-wide expression data verifies this prediction.

We find upregulation of genes that are known to be expressed in glial precursor cells. Prominent among these is *repo*, which contains 11 GCM consensus-binding sites in its upstream regulatory region and is the first identified direct target of *gcm* (Akiyama et al., 1996). Another upregulated gene that is first expressed in the CNS in glial precursors is *htl*, which encodes a FGF receptor (Shishido et al., 1997). Downregulation is found for several genes that are known to be expressed in neuronal precursors. Prominent among these is the pan-neuronal gene *scratch* (*scrt*), which promotes neurogenesis and can induce additional neurons when ectopically expressed (Roark et al., 1995). Interestingly, we also observe downregulation for several genes that are involved in midline glial cell development, such as *Egfr, vvl, en* and *D* (Condron et al., 1994; Anderson et al., 1995; Scholz et al., 1997; Soriano and Russell, 1998). This suggests that midline glial development may be suppressed in *sca-gcm* embryos and might also explain the otherwise unexpected downregulation of *tramtrack* (*ttk*) and *pointed* (*pnt*), as these genes are not only expressed in lateral glial cells but also in midline glial cells (Granderath and Klämbt, 1999).

In addition to genes that are known to be involved in the gliogenesis/neurogenesis decision, we find a large number of genes that have not previously been implicated in this aspect of CNS development. Indeed, for the majority of the known differentially regulated genes identified here, this report represents the first indication for an involvement in gliogenesis and/or neurogenesis. This is also the case for the annotated genes of unknown function, which have not been studied in any in vivo context and make up the majority of the differentially expressed genes identified.

The effects of targeted misexpression of *gcm* in stage 11 *sca-gcm* embryos appear to be restricted to cells of the neuroectoderm. Moreover, these effects manifest themselves primarily in altered gene expression in cells of the neuroectoderm. No morphological changes are seen in stage 11 *sca-gcm* embryos when compared with wild type, and nonspecific side effects of *gcm* misexpression, such as growth abnormalities, defective morphogenesis or increased apoptosis, are not observed in these embryos. We therefore assume that the observed differential gene expression specifically reflects activation or repression of *gcm* downstream genes. It is, nevertheless, unlikely that our study uncovers all the genes that act downstream of *gcm* to induce glial cell fate. This is because our early transcript image is restricted to a specific time point in early gliogenesis development, and *gcm* may influence other targets at other stages. Moreover, the genetic overexpression of *gcm* may create an artificial situation in vivo, in which not all of the candidate downstream genes show changes in magnitude and direction of expression that correspond to their responses to *gcm* action under normal conditions. For example, whereas *gcm* expression in a mesodermal lineage induces genes involved in hemocyte cell development, overexpression of *gcm* in neuroectodermal cells causes a downregulation of the hemocyte marker genes *Pxn* and *srp*. (Downregulation of *Pxn* may, however, also be due to the fact that this gene is also expressed in the nervous system.) Finally, it is conceivable that some of the gene expression changes seen in *sca-gcm* embryos when compared with wild type are due to insertional effects of the transgenes or to differences in genetic background. Although we find no evidence for such effects among the 10 *gcm* candidate genes that we characterized by in situ and immunocytochemical experiments, we cannot rule out such effects for all of the candidate genes identified in this report. Consequently, a full appreciation and verification of all of these candidate *gcm* downstream genes and a comprehensive understanding of their roles in determination of glial versus neuronal cell lineage will require a careful gene-by-gene analysis in mutant embryos. This also applies to the genes that are differentially expressed in stage 15/16 embryos.

Candidate genes implicated in the maintenance of differentiated glial versus neuronal cells

In stage 15/16 *sca-gcm* embryos most of the neurons in the embryonic nervous system are genetically replaced by glial

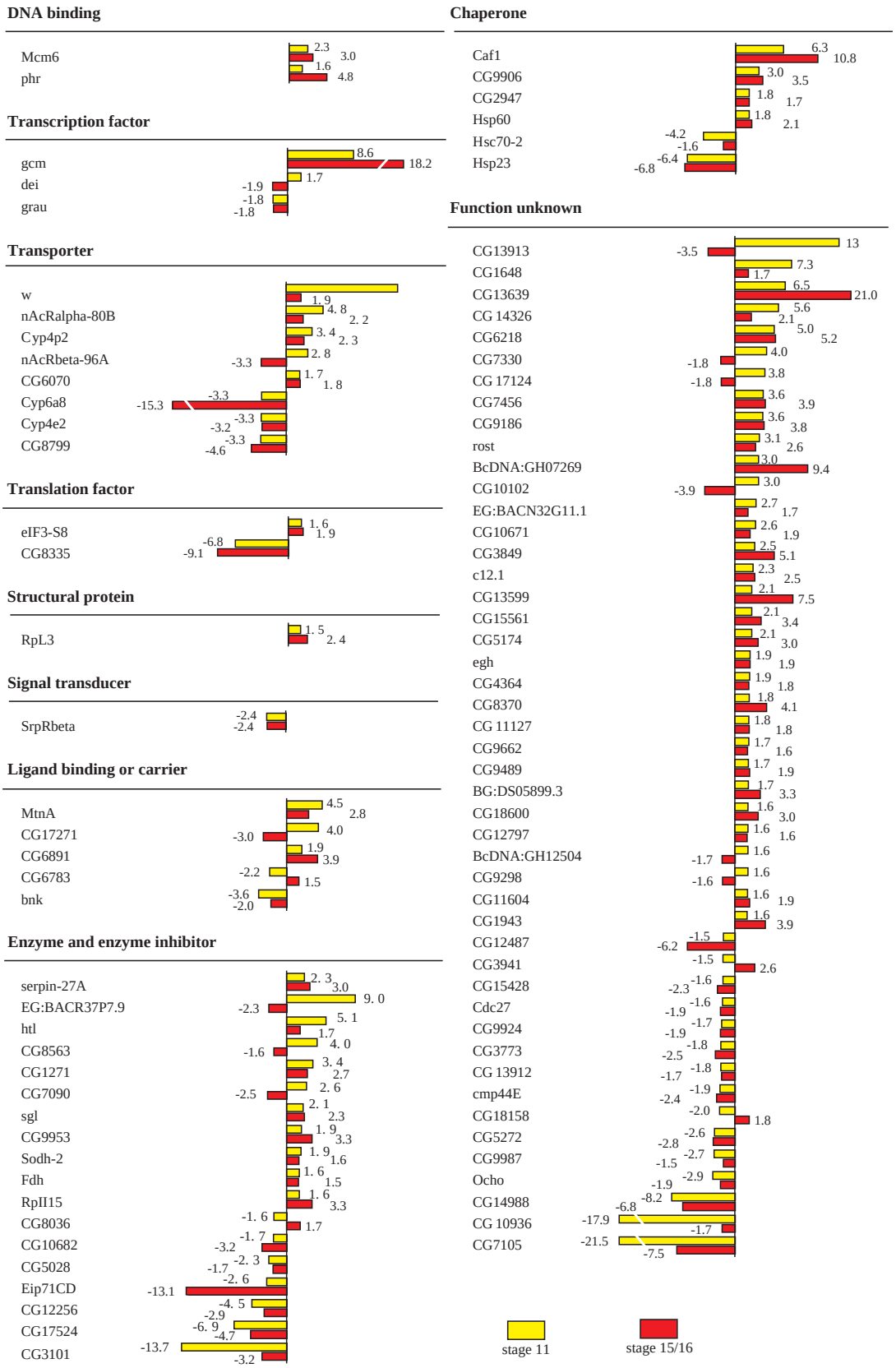


Fig. 6. Changes in transcript levels of the genes with differential expression in both early and late embryonic stages after *gcm* misexpression. Ninety-three genes show significant changes in expression levels in response to *gcm* misexpression at stage 11 (yellow), as well as at stage 15/16 (red). Bars represent the fold changes in gene expression levels between wild-type embryos and *sca-gcm* embryos. Positive values indicate that the relative expression level of a gene is increased (upregulation) and negative values indicate a decrease (downregulation).

cells, and differential gene expression in these embryos when compared with wild-type embryos reflects this fact. Although the transcript image obtained at this stage will, therefore, identify genes that are involved in the maintenance of differentiated glial versus neuronal cells, nonspecific side effects of *gcm* misexpression on differential gene expression cannot be ruled out. This is because the marked loss of neurons in stage 15/16 *sca-gcm* embryos results in morphological changes such as defective condensation of the CNS or reduction of peripheral innervation, and these morphological alterations might be accompanied by changes in gene expression.

Nevertheless, given that the strongest phenotype of stage 15/16 *sca-gcm* embryos is the gain of glial cells at the expense of neurons, we postulate that most of the observed differential gene expression at this stage is directly related to the replacement of differentiated neurons by differentiated glial cells. This is supported by the fact that several genes that are known to be expressed in differentiated neurons, such as *elav*, *lark*, *Ten-m* and *CadN*, are downregulated whereas genes that are expressed in differentiated glial cells such as *htl*, *wrapper* and *bnb* are upregulated in stage 15/16 *sca-gcm* embryos. In several cases, however, genes encoding markers for lateral or peripheral glia were not judged to be upregulated by our data analysis. For example, for the genes *repo*, *locomotion defects* (*loco*) and *gliotactin* (*gli*), the normalized expression levels, the fold change levels or the statistical significance levels were below our threshold filter values, so that these genes were not considered to be upregulated in our microarray experiments.

Cell fate determination is often controlled at the transcriptional level by key regulatory factors that are expressed transiently, whereas the gene expression patterns that they establish persist. Maintenance of the transcriptional state in differentiated cells is then achieved by control elements involved in chromatin remodeling and modification (Francis and Kingston, 2001). Accordingly, in our analysis of stage 15/16 *sca-gcm* versus wild-type embryos, we identified several differentially expressed genes that are involved in chromatin remodeling, such as *Bap60*, *dalao*, *Snr1* and *ash2*. In specific glial lineages, the onset of differentiation is thought to require cell cycle progression (Akiyama-Oda et al., 2000). In our analysis, differential expression of genes encoding cell cycle regulators or proteins involved in chromatin condensation and segregation during mitosis was also observed. Examples of this are cyclin-encoding genes such as *CycB*, *CycA*, *CycD* and *CycE*, which are differentially expressed in stage 15/16 embryos. The differential expression of these genes after *gcm* misexpression provides further support for the general notion that cell cycle regulators are key elements in cellular differentiation processes (Ohnuma et al., 2001).

In summary, this study combines in vivo transgenic analysis with genome-wide expression analysis based on oligonucleotide arrays to identify genes that are downstream of *gcm*, a key transcriptional control element in gliogenesis. The results of this study should be helpful in obtaining a comprehensive view of the molecular mechanisms of cell fate specification and cell type maintenance in the developing nervous system.

We thank A. Roth, M. Tessier, M. Wilhelm-Seiler, C. Broger, M. Neeb, R. Brem, A. Giangrande, K. Takizawa, B. W. Jones, K. J. Schmid, M. Affolter and his group, A. Ebner, L. Michaut, A. Tomlinson, S. Baumgartner, U. Walldorf, R. A. H. White, J. N. Noordermeer and H. Montalta-He; the Bloomington Stock Center for technical support, reagents and flies; and M. Müller, F. Hirth and M. Götz for helpful comments on the manuscript.

REFERENCES

Adams, M. D., Celniker, S. E., Holt, R. A., Evans, C. A., Gocayne, J. D., Amanatides, P. G., Scherer, S. E., Li, P. W., Hoskins, R. A., Galle, R. F. et al. (2000). The genome sequence 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 cells 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. (2000). Distinct mechanisms triggering glial differentiation in *Drosophila* thoracic and abdominal neuroblasts 6-4. *Dev. Biol.* **222**, 429-439.

Anderson, M. G., Perkins, G. L., Chittick, P., Shrigley, R. J. and Johnson, W. A. (1995). *drifter*, a *Drosophila* POU-domain transcription factor, is required for correct differentiation and migration of tracheal cells and midline glia. *Genes Dev.* **9**, 123-137.

Aoyagi, N. and Wassarman, D. A. (2000). Genes encoding *Drosophila melanogaster* RNA polymerase II general transcription factors: diversity in TFIIA and TFIID components contributes to gene-specific transcriptional regulation. *J. Cell Biol.* **150**, F45-F50.

Baumgartner, S., Martin, D., Hagios, C. and Chiquet-Ehrismann, R. (1994). *ten^m*, a *Drosophila* gene related to tenascin, is a new pair-rule gene. *EMBO J.* **13**, 3728-3740.

Bernardoni, R., Vivancos, B. 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., Vonesch, J. L. and Giangrande, A. (1999). Gliogenesis depends on *glide*/*gcm* through asymmetric division of neuroglioblasts. *Dev. Biol.* **216**, 265-275.

Bhat, K. M., van Beers, E. H. and Bhat, P. (2000). Sloppy paired acts as the downstream target of Wingless in the *Drosophila* CNS and interaction between *sloppy paired* and *gooseberry* inhibits *sloppy paired* during neurogenesis. *Development* **127**, 655-665.

Bonkowsky, J. L., Yoshikawa, S., O'Keefe, D. D., Scully, A. L. and Thomas, J. B. (1999). Axon routing across the midline controlled by the *Drosophila* Derailed receptor. *Nature* **402**, 540-544.

Bourouis, M., Heitzler, P., el Messal, M. and Simpson, P. (1989). Mutant *Drosophila* embryos in which all cells adopt a neural fate. *Nature* **341**, 442-444.

Campbell, G., Goring, H., Lin, T., Spana, E., Andersson, S., Doe, C. Q. and Tomlinson, A. (1994). RK2, a glial-specific homeodomain protein required for embryonic nerve cord condensation and viability in *Drosophila*. *Development* **120**, 2957-2966.

Campos-Ortega, J. and Hartenstein, V. (1997). *The Embryonic Development of* Drosophila melanogaster. Heidelberg: Springer.

Condron, B. G., Patel, N. H. and Zinn, K. (1994). Engrailed controls glial/neuronal cell fate decisions at the midline of the central nervous system. *Neuron* **13**, 541-554.

Connell-Crowley, L., le Gall, M., Vo, D. J. and Giniger, E. (2000). The cyclin-dependent kinase Cdk5 controls multiple aspects of axon patterning in vivo. *Curr. Biol.* **10**, 599-602.

Cubadda, Y., Heitzler, P., Ray, R. P., Bourouis, M., Ramain, P., Gelbart, W., Simpson, P. and Haenlin, M. (1997). *u-shaped* encodes a zinc finger protein that regulates the proneural genes *achaete* and *scute* during the formation of bristles in *Drosophila*. *Genes Dev.* **11**, 3083-3095.

Daffre, S., Kylsten, P., Samakovlis, C. and Hultmark, D. (1994). The

lysozyme locus in *Drosophila melanogaster*: an expanded gene family adapted for expression in the digestive tract. *Mol. Gen. Genet.* **242**, 152-162.

Delidakis, C. and Artavanis-Tsakonas, S. (1992). The Enhancer of split [E(spl)] locus of *Drosophila* encodes seven independent helix-loop-helix proteins. *Proc. Natl. Acad. Sci. USA* **89**, 8731-8735.

Doe, C. Q. and Skeath, J. B. (1996). Neurogenesis in the insect central nervous system. *Curr. Opin. Neurobiol.* **6**, 18-24.

Follette, P. J. and O'Farrell, P. H. (1997). Cdks and the *Drosophila* cell cycle. *Curr. Opin. Genet. Dev.* **7**, 17-22.

Francis, N. J. and Kingston, R. E. (2001). Mechanisms of transcriptional memory. *Nat. Rev. Mol. Cell. Biol.* **2**, 409-421.

Frank, D. J., Tyree, C. M., George, C. P. and Kadonaga, J. T. (1995). Structure and function of the small subunit of TFIIF (RAP30) from *Drosophila* melanogaster. *J. Biol. Chem.* **270**, 6292-6297.

Furukawa, T., Mukherjee, S., Bao, Z. Z., Morrow, E. M. and Cepko, C. L. (2000). rax, Hes1, and notch1 promote the formation of Muller glia by postnatal retinal progenitor cells. *Neuron* **26**, 383-394.

Gage, F. H. (2000). Mammalian neural stem cells. *Science* **287**, 1433-1438.

Giesen, K., Hummel, T., Stollewerk, A., Harrison, S., Travers, A. and Klämbt, C. (1997). Glial development in the *Drosophila* CNS requires concomitant activation of glial and repression of neuronal differentiation genes. *Development* **124**, 2307-2316.

Granderath, S. and Klämbt, C. (1999). Glia development in the embryonic CNS of *Drosophila*. *Curr. Opin. Neurobiol.* **9**, 531-536.

Hacker, U., Grossniklaus, U., Gehring, W. J. and Jäckle, H. (1992). Developmentally regulated *Drosophila* gene family encoding the fork head domain. *Proc. Natl. Acad. Sci. USA* **89**, 8754-8758.

Hahn, M. and Jäckle, H. (1996). *Drosophila goosecoid* participates in neural development but not in body axis formation. *EMBO J.* **15**, 3077-3084.

Hirth, F., Hartmann, B. and Reichert, H. (1998). Homeotic gene action in embryonic brain development of *Drosophila*. *Development* **125**, 1579-1589.

Hojo, M., Ohtsuka, T., Hashimoto, N., Gradwohl, G., Guillemot, F. and Kageyama, R. (2000). Glial cell fate specification modulated by the bHLH gene Hes5 in mouse retina. *Development* **127**, 2515-2522.

Hosoya, T., Takizawa, K., Nitta, K. and Hotta, Y. (1995). *glial cells missing*: a binary switch between neuronal and glial determination in *Drosophila*. *Cell* **82**, 1025-1036.

Ito, K., Urban, J. and Technau, G. M. (1995). Distribution, classification, and development of *Drosophila* glial cells in the late embryonic and early larval ventral nerve cord. *Roux's Arch. Dev. Biol.* **204**, 284-307.

Iwai, Y., Usui, T., Hirano, S., Steward, R., Takeichi, M. and Uemura, T. (1997). Axon patterning requires DN-cadherin, a novel neuronal adhesion receptor, in the *Drosophila* embryonic CNS. *Neuron* **19**, 77-89.

Jennings, B., Preiss, A., Delidakis, C. and Bray, S. (1994). The Notch signalling pathway is required for Enhancer of split bHLH protein expression during neurogenesis in the *Drosophila* embryo. *Development* **120**, 3537-3548.

Jones, B. W., Fetter, R. D., Tear, G. and Goodman, C. S. (1995). *glial cells missing*: a genetic switch that controls glial versus neuronal fate. *Cell* **82**, 1013-1023.

Jones, B. W. (2001). Glial cell development in the *Drosophila* embryo. *BioEssays* **23**, 877-887.

Kammermeier, L., Leemans, R., Hirth, F., Flister, S., Wenger, U., Walldorf, U., Gehring, W. J. and Reichert, H. (2001). Differential expression and function of the *Drosophila* Pax6 genes *eyeless* and *twin of eyeless* in embryonic central nervous system development. *Mech. Dev.* **103**, 71-78.

Kania, A., Han, P. L., Kim, Y. T. and Bellen, H. (1993). *neuromusculin*, a *Drosophila* gene expressed in peripheral neuronal precursors and muscles, encodes a cell adhesion molecule. *Neuron* **11**, 673-687.

Keene, J. D. (2001). Ribonucleoprotein infrastructure regulating the flow of genetic information between the genome and the proteome. *Proc. Natl. Acad. Sci. USA* **98**, 7018-7024.

Klaes, A., Menne, T., Stollewerk, A., Scholz, H. and Klämbt, C. (1994). The Ets transcription factors encoded by the *Drosophila* gene *pointed* direct glial cell differentiation in the embryonic CNS. *Cell* **78**, 149-160.

Klämbt, C. and Goodman, C. S. (1991). The diversity and pattern of glia during axon pathway formation in the *Drosophila* embryo. *Glia* **4**, 205-213.

Knoblich, J. A., Sauer, K., Jones, L., Richardson, H., Saint, R. and Lehner, C. F. (1994). Cyclin E controls S phase progression and its down-regulation during *Drosophila* embryogenesis is required for the arrest of cell proliferation. *Cell* **77**, 107-120.

Lebestky, T., Chang, T., Hartenstein, V. and Banerjee, U. (2000). Specification of *Drosophila* hematopoietic lineage by conserved transcription factors. *Science* **288**, 146-149.

Lee, K. S., Oh, Y., Baek, G., Yoon, J., Han, K., Cho, N. and Baek, K. (1997). Analysis of the structure and expression of the *TFIIB* gene in *Drosophila melanogaster*. *Mol. Cell* **7**, 374-379.

Leemans, R., Loop, T., Egger, B., He, H., Kammermeier, L., Hartmann, B., Certa, U., Reichert, H. and Hirth, F. (2001). Identification of candidate downstream genes for the homeodomain transcription factor Labial in *Drosophila* through oligonucleotide-array transcript imaging. *Genome Biol.* **2**, 0015.1-0015.9.

Levine, A., Bashan-Ahrend, A., Budai-Hadrian, O., Gartenberg, D., Menasherow, S. and Wides, R. (1994). *Odd Oz*: a novel *Drosophila* pair rule gene. *Cell* **77**, 587-598.

Lipshutz, R. J., Fodor, S. P., Gingeras, T. R. and Lockhart, D. J. (1999). High density synthetic oligonucleotide arrays. *Nat. Genet.* **21**, 20-24.

Llamazares, S., Moreira, A., Tavares, A., Girdham, C., Spruce, B. A., Gonzalez, C., Karess, R. E., Glover, D. M. and Sunkel, C. E. (1991). *polo* encodes a protein kinase homolog required for mitosis in *Drosophila*. *Genes Dev.* **5**, 2153-2165.

Long, A. R., Wilkins, J. C. and Shepherd, D. (1998). Dynamic developmental expression of *smallminded*, a *Drosophila* gene required for cell division. *Mech. Dev.* **76**, 33-43.

Lundell, M. J. and Hirsh, J. (1992). The *zfh-2* gene product is a potential regulator of neuron-specific dopa decarboxylase gene expression in *Drosophila*. *Dev. Biol.* **154**, 84-94.

Malatesta, P., Hartfuss, E. and Götz, M. (2000). Isolation of radial glial cells by fluorescent-activated cell sorting reveals a neuronal lineage. *Development* **127**, 5253-5263.

Montalta-He, H., Leemans, R., Loop, T., Strahm, M., Certa, U., Primig, M., Acampora, D., Simeone, A. and Reichert, H. (2002). Evolutionary conservation of *otd/OTX2* transcription factor action: a genome-wide microarray analysis in *Drosophila*. *Genome Biol.* **3**, 0015.1-0015.15.

Ng, S. C., Perkins, L. A., Conboy, G., Perrimon, N. and Fishman, M. C. (1989). A *Drosophila* gene expressed in the embryonic CNS shares one conserved domain with the mammalian GAP-43. *Development* **105**, 629-638.

Noordermeer, J. N., Kopczynski, C. C., Fetter, R. D., Bland, K. S., Chen, W. Y. and Goodman, C. S. (1998). Wrapper, a novel member of the Ig superfamily, is expressed by midline glia and is required for them to ensheath commissural axons in *Drosophila*. *Neuron* **21**, 991-1001.

Ohno, K., Hirose, F., Inoue, Y. H., Takisawa, H., Mimura, S., Hashimoto, Y., Kiyono, T., Nishida, Y. and Matsukage, A. (1998). cDNA cloning and expression during development of *Drosophila melanogaster* MCM3, MCM6 and MCM7. *Gene* **217**, 177-185.

Ohnuma, S., Philpott, A. and Harris, W. A. (2001). Cell cycle and cell fate in the nervous system. *Curr. Opin. Neurobiol.* **11**, 66-73.

Oishi, I., Sugiyama, S., Liu, Z. J., Yamamura, H., Nishida, Y. and Minami, Y. (1997). A novel *Drosophila* receptor tyrosine kinase expressed specifically in the nervous system. Unique structural features and implication in developmental signaling. *J. Biol. Chem.* **272**, 11916-11923.

Pearson, A., Lux, A. and Krieger, M. (1995). Expression cloning of *dSR-CI*, a class C macrophage-specific scavenger receptor from *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **92**, 4056-4060.

Platt, N., da Silva, R. P. and Gordon, S. (1998). Recognizing death: the phagocytosis of apoptotic cells. *Trends Cell Biol.* **8**, 365-372.

Prokopenko, S. N., He, Y., Lu, Y. and Bellen, H. J. (2000). Mutations affecting the development of the peripheral nervous system in *Drosophila*: a molecular screen for novel proteins. *Genetics* **156**, 1691-1715.

Qian, X., Shen, Q., Goderie, S. K., He, W., Capela, A., Davis, A. A. and Temple, S. (2000). Timing of CNS cell generation: a programmed sequence of neuron and glial cell production from isolated murine cortical stem cells. *Neuron* **28**, 69-80.

Roark, M., Sturtevant, M. A., Emery, J., Vaessin, H., Grell, E. and Bier, E. (1995). *scratch*, a pan-neural gene encoding a zinc finger protein related to snail, promotes neuronal development. *Genes Dev.* **9**, 2384-2398.

Sanchez-Soriano, N. and Russell, S. (2000). Regulatory mutations of the *Drosophila* Sox gene *Dichaete* reveal new functions in embryonic brain and hindgut development. *Dev. Biol.* **220**, 307-321.

Schmidt, H., Rickert, C., Bossing, T., Vef, O., Urban, J. and Technau, G. M. (1997). The embryonic central nervous system lineages of Drosophila

melanogaster. II. Neuroblast lineages derived from the dorsal part of the neuroectoderm. *Dev. Biol.* **189**, 186-204.

Scholz, H., Sadlowski, E., Klaes, A. and Klämbt, C. (1997). Control of midline glia development in the embryonic *Drosophila* CNS. *Mech. Dev.* **64**, 137-151.

Schultz, S. J., Fry, A. M., Sutterlin, C., Ried, T. and Nigg, E. A. (1994). Cell cycle-dependent expression of *Nek2*, a novel human protein kinase related to the NIMA mitotic regulator of *Aspergillus nidulans*. *Cell Growth Differ.* **5**, 625-635.

Shishido, E., Ono, N., Kojima, T. and Saigo, K. (1997). Requirements of DFR1/Heartless, a mesoderm-specific *Drosophila* FGF- receptor, for the formation of heart, visceral and somatic muscles, and ensheathing of longitudinal axon tracts in CNS. *Development* **124**, 2119-2128.

Soriano, N. S. and Russell, S. (1998). The *Drosophila* SOX-domain protein Dichaete is required for the development of the central nervous system midline. *Development* **125**, 3989-3996.

Steffensen, S., Coelho, P. A., Cobbe, N., Vass, S., Costa, M., Hassan, B., Prokopenko, S. N., Bellen, H., Heck, M. M. and Sunkel, C. E. (2001). A role for *Drosophila* SMC4 in the resolution of sister chromatids in mitosis. *Curr. Biol.* **11**, 295-307.

Tautz, D. and Pfeifle, C. (1989). A non-radioactive in situ hybridization method for the localization of specific RNAs in *Drosophila* embryos reveals translational control of the segmentation gene *hunchback*. *Chromosoma* **98**, 81-85.

Therianos, S., Leuzinger, S., Hirth, F., Goodman, C. S. and Reichert, H. (1995). Embryonic development of the *Drosophila* brain: formation of commissural and descending pathways. *Development* **121**, 3849-3860.

Vincent, S., Vonesch, J. L. and Giangrande, A. (1996). *Glide* directs glial fate commitment and cell fate switch between neurones and glia. *Development* **122**, 131-139.

Wech, I., Bray, S., Delidakis, C. and Preiss, A. (1999). Distinct expression patterns of different enhancer of split bHLH genes during embryogenesis of *Drosophila melanogaster*. *Dev. Genes Evol.* **209**, 370-375.

Wegner, M. and Riethmacher, D. (2001). Chronicles of a switch hunt: gcm genes in development. *Trends Genet.* **17**, 286-290.

Zilian, O., Frei, E., Burke, R., Brentrup, D., Gutjahr, T., Bryant, P. J. and Noll, M. (1999). *double-time* is identical to *discs overgrown*, which is required for cell survival, proliferation and growth arrest in *Drosophila* imaginal discs. *Development* **126**, 5409-5420.