Developmental Biology **248,** 369–383 (2002)
doi:10.1006/dbio.2002.0740

gcm2 Promotes Glial Cell Differentiation and Is Required with *glial cells missing* for Macrophage Development in *Drosophila*

Teresa B. Alfonso and Bradley W. Jones[1]

Molecular Neurobiology Program, Skirball Institute of Biomolecular Medicine, and Department of Pharmacology, New York University School of Medicine, New York NY 10016

glial cells missing **(*gcm*) is the primary regulator of glial cell fate in *Drosophila*. In addition, *gcm* has a role in the differentiation of the plasmatocyte/macrophage lineage of hemocytes. Since mutation of *gcm* causes only a decrease in plasmatocyte numbers without changing their ability to convert into macrophages, *gcm* cannot be the sole determinant of plasmatocyte/macrophage differentiation. We have characterized a *gcm* homolog, *gcm2*. *gcm2* is expressed at low levels in glial cells and hemocyte precursors. We show that *gcm2* has redundant functions with *gcm* and has a minor role promoting glial cell differentiation. More significant, like *gcm*, mutation of *gcm2* leads to reduced plasmatocyte numbers. A deletion removing both genes has allowed us to clarify the role of these redundant genes in plasmatocyte development. Animals deficient for both *gcm* and *gcm2* fail to express the macrophage receptor Croquemort. Plasmatocytes are reduced in number, but still express the early marker Peroxidasin. These Peroxidasin-expressing hemocytes fail to migrate to their normal locations and do not complete their conversion into macrophages. Our results suggest that both *gcm* and *gcm2* are required together for the proliferation of plasmatocyte precursors, the expression of Croquemort protein, and the ability of plasmatocytes to convert into macrophages.** **© 2002 Elsevier Science (USA)**

***Key Words: glial cells missing; gcm; gcm2*; glia; hemocyte; plasmatocyte; macrophage; blood cells; *Drosophila*.**

INTRODUCTION

The *glial cells missing* gene (*gcm*) is the primary regulator of glial cell determination in *Drosophila*. It encodes a transcription factor that is transiently expressed in all embryonic glia, except for the midline/mesectoderm-derived glia (Akiyama *et al.*, 1996; Hosoya *et al.*, 1995; Jones *et al.*, 1995; Schreiber *et al.*, 1997; Vincent *et al.*, 1996). *gcm* loss-of-function mutant embryos lack nearly all lateral glial cells, and presumptive glial cells are transformed into neurons (Hosoya *et al.*, 1995; Jones *et al.*, 1995; Vincent *et al.*, 1996). Conversely, when *gcm* is ectopically expressed, presumptive neurons are transformed into glia (Hosoya *et al.*, 1995; Jones *et al.*, 1995). Thus, within the nervous system, *gcm* acts as a genetic switch, with Gcm-positive cells becoming glia and Gcm-negative cells becoming neurons. Moreover, nonneural cells may adopt a glial cell fate when forced to express *gcm* (Akiyama-Oda *et al.*, 1998; Bernardoni *et al.*, 1998).

In addition to acting as a regulator of glial cell differentiation, *gcm* has been found to have an instructive role in the development of the plasmatocyte/macrophage lineage of blood cells (Bernardoni *et al.*, 1997; Lebestky *et al.*, 2000). Embryonic blood cells, or hemocytes, are derived from the procephalic mesoderm (Tepass *et al.*, 1994). Hemocyte precursors give rise to two populations of hemocytes in the embryo: plasmatocytes and crystal cells (Mathey-Prevot and Perrimon, 1998). Plasmatocytes constitute the majority of circulating hemocytes. They migrate throughout the embryo in the hemolymph space, where they convert into macrophages that are responsible for the phagocytosis of apoptotic cells (Abrams *et al.*, 1993; Tepass *et al.*, 1994). Early plasmatocytes, first seen in stage 10 embryos, express the extracellular matrix protein Peroxidasin. Peroxidasin expression is maintained throughout embryogenesis (Abrams *et al.*, 1993; Nelson *et al.*, 1994; Tepass *et al.*, 1994). In stage 11, plasmatocytes begin to express Croquemort, a CD36 related macrophage receptor (Franc *et al.*,

[1] To whom correspondence should be addressed, Fax: (212) 263-8214. E-mail: bjones@saturn.med.nyu.edu.

0012-1606/02 $35.00

1996), and they convert into macrophages. Croquemort expression is required for the efficient phagocytosis of apoptotic cells (Franc *et al.*, 1999). By the end of embryogenesis, nearly all plasmatocytes are macrophages that express the Croquemort receptor.

Before *gcm* is activated in glial progenitors, *gcm* is expressed in the primordium of hemocyte precursors (Bernardoni *et al.*, 1997; Jones *et al.*, 1995). *gcm* continues expression in hemocyte precursors as they develop into plasmatocytes through stage 11, after which it rapidly disappears (Bernardoni *et al.*, 1997). As plasmatocytes migrate out of the procephalon and convert into macrophages, *gcm* expression can no longer be detected.

gcm has been proposed to promote the differentiation of plasmatocytes (Bernardoni *et al.*, 1997; Lebestky *et al.*, 2000). Early misexpression of *gcm* induces an increase in circulating plasmatocyte numbers in the embryo (Bernardoni *et al.*, 1997). Ectopic expression of *gcm* in the crystal cell lineage of hemocytes induces Croquemort expression and the adoption of plasmatocyte characteristics (Lebestky *et al.*, 2000). *gcm* mutant embryos are deficient by 40% in plasmatocyte numbers (Bernardoni *et al.*, 1997). These results indicate an instructive role for *gcm* in plasmatocyte/macrophage development, and *gcm* has been proposed to be a master regulator of the plasmatocyte/macrophage lineage (Bernardoni *et al.*, 1997). However, since mutation of *gcm* causes only a decrease in plasmatocyte numbers (Bernardoni *et al.*, 1997). without changing their ability to differentiate into macrophages (Jones *et al.*, 1995), *gcm* cannot be the sole determinant of plasmatocyte/macrophage cell fate.

In this paper, we describe the characterization of a homolog of the *gcm* gene in *Drosophila*, *gcm2*. *gcm2* was previously identified as a PCR fragment containing a gcm-motif (Akiyama *et al.*, 1996), the highly conserved DNA-binding domain (Akiyama *et al.*, 1996; Schreiber *et al.*, 1997) that is shared with *gcm*'s two mammalian homologs (Akiyama *et al.*, 1996; Altshuller *et al.*, 1996; Kim *et al.*, 1998). We found *gcm2* located 27.9 kb proximal to *gcm*, and show that *gcm2* has partially redundant functions with *gcm*. *gcm2* is expressed in a pattern similar to *gcm*, but at lower levels, in glial cells and in hemocyte precursors. Ectopic expression of *gcm2* is sufficient to induce glial cell differentiation. Moreover, a deletion of both genes completely removes all glial cell development, apart from the midline glia. While this paper was in preparation, similar results were reported (Kammerer *et al.*, 2001); however, without a mutation in *gcm2*, its exact role in development was not clear. Here, we describe a null mutation of *gcm2* and its effects on both glial cell and hemocyte differentiation.

gcm2 loss-of-function has only a mild effect on glial cell differentiation. More significant is its role in plasmatocyte/macrophage differentiation. Like the *gcm* mutation, *gcm2* loss-of-function results in a deficit in plasmatocytes. Deletion of both *gcm* and *gcm2* results in the striking absence of Croquemort expression. However, Peroxidasin-expressing hemocytes are still present. These Peroxidasin-expressing hemocytes are reduced in numbers by over half, fail to undergo normal migration, and do not exhibit the morphology associated with their complete conversion into macrophages. We conclude that *gcm* and *gcm2* are not required for the initial specification of the plasmatocyte lineage but are necessary for the proliferation of plasmatocyte precursors, the expression of Croquemort protein, and the ability of plasmatocytes to convert into macrophages.

MATERIALS AND METHODS

Identification and Analysis of gcm2 Locus and cDNA

Database searches for *gcm* homologs using the BLAST program (Altschul *et al.*, 1997) identified an open reading frame (ORF) with homology to *gcm* located on a *Drosophila melanogaster* genomic library clone BACR24A22 (GenBank Accession No. AC007257), which had been generated and sequenced by the Berkeley Drosophila Genome Project (BDGP). A pair of oligonucleotide primers flanking the ORF (forward, AGTACGCATTCGGTGCATCC; and reverse, ACTGTGTTGTTGCTGTCGTC) was used to amplify a 0.7-kb PCR product from the BACR24A22 DNA template. This PCR product was used to screen a 9- to 12-h *D. melanogaster* embryonic cDNA library (Zinn *et al.*, 1988). A single *gcm2* cDNA was isolated; the 3-kb insert was subcloned into pBluescript SK(+) (Stratagene) and sequenced (GenBank Accession No. AF461416).

In Situ Transcript Localization

In situ hybridization in whole-mount embryos was performed by using digoxigenin-labeled riboprobes as described previously (Jones *et al.*, 1995). *gcm* antisense probes were generated from a full-length *gcm* cDNA (Jones *et al.*, 1995). *thioredoxin* probe was generated from a *thioredoxin* cDNA, GH24203, identified by the BDGP (Rubin *et al.*, 2000), and obtained from ResGen.

Immunohistochemistry

Horseradish peroxidase (HRP) immunohistochemistry and embryo dissection were done as previously described (Patel, 1994). Anti-Repo monoclonal antibody (MAb) (MAb 8D12; B.W.J., B. Blankemeier, and C. S. Goodman, unpublished observations) was used at a 1:5 dilution. Rabbit anti-Croquemort antibody (Ab) (Franc *et al.*, 1996) was used at a 1:1000 dilution, mouse anti-Peroxidasin Ab (Nelson *et al.*, 1994) at 1:500, mouse anti-β-galactosidase (β-gal) MAb (Roche) at 1:500, and rabbit anti-β-gal Ab (Cappel) at 1:10,000. Secondary antibodies conjugated to HRP or Biotin (Jackson ImmunoResearch) were used at 1:300 dilutions. Biotinylated secondary antibodies were detected by using Vectastain Elite ABC and HRP/diaminobenzidine (DAB) reaction. DAB reactions were enhanced to give a black color by addition of 0.067% $NiCl_2$.

D. melanogaster Stocks and Genetics

Wild-type embryos were w^{1118}. The *gcm* null allele $gcm^{\Delta PI}$ (FBa10045751) and *UAS-gcm* reporter lines *P{UAS-gcm.J}2* and *P{UAS-gcm.J}3* (FBal0045765) were described previously (Jones *et al.*, 1995) *Df(2L)200* (FBab0022176) was described by Lane and Kalderon (1993). *Df(2L)200* and $gcm^{\Delta PI}$ were balanced over *CyO*,

P{w^{+mC} Act5C-lacZ} (FBba0000031) for the identification of mutant embryos.

USA-gcm2 lines were generated by inserting the *gcm2* cDNA into pUAST, a UAS reporter P element vector (Brand and Perrimon, 1993), to generate P{UAS-gcm2}. Several lines carrying P {UAS-gcm2} insertions were generated by using P element-mediated transformation into *w^{1118}* flies using standard methods.

Ectopic expression of *gcm2* or *gcm* in neuroblasts was achieved by crossing *P{UAS-gcm2}* or *P{UAS-gcm.J}* with *sca-Gal4* (FBa10040466) (Klaes *et al.*, 1994). Ectopic expression of *gcm* in a *Df(2L)200* background was achieved by using *daughterless-Gal4* (*da-Gal4*) (FBti0013991) (Wodarz *et al.*, 1995), by crossing *Df(2L)200/CyO;P{UAS-gcm.J}3* with *Df(2L)200/CyO;da-Gal4*. Expression of a *lz* reporter gene (*lz-lacZ*) in a *Df(2L)200* background was achieved by crossing *lz-Gal4;Df(2L)200/+* males with *Df(2L)200/+;UAS-lacZ/+* females. *lz-Gal4* (FBti0003702) and *UAS-lacZ* (FBti0002140) have been described previously (Brand and Perrimon, 1993; Crew *et al.*, 1997; Lebestky *et al.*, 2000).

Deletions at the *gcm2* locus were generated by imprecise excision of a P element as previously described (Jones *et al.*, 1995), using the P element *P(EP)2018* (FBti0010775) (Liao *et al.*, 2000; Rorth *et al.*, 1998). Several hundred *w*$^{-}$ lines were generated and analyzed by Southern blot to detect deletions; one of these excision lines, *Df(2L)gcm2*, has a deletion that removes the entire ORF of *gcm2* as well as the *thioredoxin* promoter (see Fig. 5).

Molecular Characterization of Df(2L)200 and Df(2L)gcm2

The deletion breakpoints of *Df(2L)200* and *Df(2L)gcm2* were determined by Southern analysis using probes generated from genomic DNA walks of the region spanning 30B–30C (Jones *et al.*, 1995; Lane and Kalderon, 1993), as well as probes generated from the *gcm2* and *thioredoxin* cDNAs. The extent of the *Df(2L)gcm2* deletion was determined by amplifying a 0.9-kb PCR product using primers flanking the deletion (forward, CGAAGTGAGACGCTATCAGC; and reverse, CCTGGTACACCATGTTGGAT), with genomic DNA from homozygous *Df(2L)gcm2* flies as a template. The PCR product, cloned into pCR2.1 (Invitrogen), was sequenced.

RESULTS

Identification and Characterization of the gcm2 Locus

A second gcm-motif gene in *D. melanogaster*, named *gcm2*, was originally identified from a 137-bp PCR product amplified from *D. melanogaster* genomic DNA using degenerate primers (Akiyama *et al.*, 1996). Using BLAST searches (Altschul *et al.*, 1997), we found a sequence with significant homology to *gcm* on a *D. melanogaster* genomic library clone. This sequence has an ORF with a gcm-motif and identity to the predicted sequence of the *gcm2* PCR fragment. BLAST searches with the near complete sequence of the *D. melanogaster* genome (Adams *et al.*, 2000) failed to identify additional gcm-motif genes. We concluded that we had identified *gcm2*.

A 0.7-kb PCR product was amplified by using primers flanking the *gcm2* ORF. This PCR product was used as a probe to isolate a single 3-kb clone from an embryonic cDNA library. The cDNA clone was sequenced. It has an ORF that encodes a protein of 613 amino acids (Fig. 1B), a 928-bp 5′ UTR, and a 171-bp 3′ UTR with poly(A) tail. Comparison with the published genomic sequence (GenBank Accession No. AE003625; Adams *et al.*, 2000) revealed the *gcm2* transcription unit to have 3 exons and a location 27.9 kb 5′ to the *gcm* gene, in opposing orientation (Fig. 1A). Located between *gcm2* and *gcm* are two predicted genes encoding for carboxylesterases (CG3841 and CG4382). Immediately 3′ of *gcm2* is the *thioredoxin* gene, one of several Thioredoxin-like genes in *Drosophila*.

The predicted amino acid sequence and exon structure of *gcm2* are in agreement with the prediction of the Genome Project (GenBank Accession No. AAF52793), differing in amino acid sequence at only six residues (bold, underlined in Fig. 1B). However, our cDNA differs from that of Kammerer *et al.* (2001) at the 5′ end. Their cDNA (GenBank Accession No. AF184664) has a fourth, alternative 5′ exon, located upstream, that replaces the first 11 residues of our predicted protein with four different residues. These results suggest that *gcm2* has two separate promoters with alternate splicing at the 5′ end.

Gcm2 protein is similar in structure to other Gcm family members, sharing a highly conserved N-terminal gcm-motif of 156 amino acids (boxed in black in Fig. 1B). While all Gcm family members are very similar in the gcm-motif, Gcm2 and Gcm are more similar to each other (69% identity; 83% similarity) than to their two vertebrate counterparts Gcm1/GCMa and Gcm2/GCMb (55–64% identity; 70–78% similarity to human Gcm1 and Gcm2) (Fig. 1C).

Expression of gcm2 Transcripts

We followed *gcm2* expression in embryos using *in situ* hybridization to mRNA. Similar results were obtained whether we used a full-length probe for *gcm2* or a probe made from the first 930 bp of the *gcm2* cDNA that ensured no cross-hybridization with *gcm* mRNA. Cross-hybridization with *gcm* was of concern as the expression profiles of the two genes overlap, though *gcm2* is expressed at much lower levels. With *gcm2* and *gcm* probes of similar lengths under identical hybridization conditions, *gcm2* transcripts were first detected after 3 h of reaction, while *gcm* transcripts were detected after only 5 min of reaction.

The expression profiles of *gcm2* transcripts compared with *gcm* are shown in Fig. 2. Like *gcm*, *gcm2* is first detected in an anterior ventral region in stage 5 embryos (Fig. 2A). During gastrulation, these cells invaginate at the end of the ventral furrow just anterior to the cephalic furrow, in the primordium of presumptive hemocyte precursors (Fig. 2C). Expression in hemocyte precursors persists through stage 11, after which it rapidly fades. At late stage 9, *gcm2* is first detected in the neuroectoderm in each hemisegment in a single cell at the lateral edge of the CNS (Fig. 2E). At stage 11, *gcm2* continues to be expressed in a single cell per CNS hemisegment, which is now in the position of neuroblast NB1-3 or its progeny (Fig. 2G). NB1-3

gives rise to several CNS and peripheral glial (Schmidt *et al.*, 1997). By stage 12, *gcm2* is detected in longitudinal glia precursors, as well as other CNS glia, and in a stripe of ectodermal cells of the lateral body wall in each hemisegment (Fig. 2I). Through stage 15, *gcm2* continues to be detected in the longitudinal glia, in other CNS glia at very low levels, and in the lateral ectoderm (Figs. 2K and 2M). After stage 15, *gcm2* expression rapidly fades.

In summary, the expression pattern of *gcm2* in part mirrors that of *gcm*, but expression is at very low levels compared with that of *gcm*. *gcm2* expression is highest in the hemocyte primordia and in the longitudinal and nerve root glia.

Ectopic Expression of gcm2 Induces Glial Cell Differentiation

The similarity between Gcm2 and Gcm proteins, especially in the gcm-motif, suggested they have redundant functions. Ectopic expression of *gcm* is sufficient to promote glial cell differentiation, transforming presumptive neurons into glia (Hosoya *et al.*, 1995; Jones *et al.*, 1995). To test whether ectopic expression of *gcm2* is sufficient to promote glial cell differentiation, we used the Gal4/UAS binary system (Brand and Perrimon, 1993) to generate embryos that express *gcm2* in all presumptive neuroblasts. *UAS-gcm2* flies were crossed with *sca-Gal4* (Klaes *et al.*, 1994), which drives expression throughout the neurogenic ectoderm and developing neuroblasts. In *sca-Gal4;UAS-gcm2* embryos, there is a striking increase in the number of glial cells as revealed by anti-Repo antibody (Fig. 3C), a glial-specific marker (Campbell *et al.*, 1994; Halter *et al.*, 1995; Xiong *et al.*, 1994). Many of these Repo-positive cells exhibit the elongated or irregular cell shapes typical of glial cells. This phenotype is similar to that of ectopically expressed *gcm* (Fig. 3B). Similar results were also shown by Kammerer *et al.* (2001); in addition, they induced ectopic *gcm2* in neuroblasts in *gcm* null mutant embryos and demonstrated that *gcm2* is sufficient, in the absence of *gcm*, to induce gliogenesis.

gcm2 Is Necessary for Gliogenesis

gcm mutant embryos retain a low level of glial cell development (Hosoya *et al.*, 1995; Jones *et al.*, 1995; Vincent *et al.*, 1996). $gcm^{\Delta PI}$ is a null allele that deletes the transcription unit. In $gcm^{\Delta PI}$ mutant embryos, isolated single Repo-positive cells, as well as occasional small clusters of Repo-positive cells can be seen in many segments (Fig. 4C). These glial cells are typically, but not always, in the positions longitudinal glia and nerve root glia—cells in which *gcm2* expression is strongest. The number of Repo-positive cells per hemisegment may vary from 0 to as many as 8. We counted an average of 2 Repo-positive cells per hemisegment in the ventral nerve cord (n = 180 hemisegments) (Fig. 4C), compared with the wild-type number of 30 glial cells per hemisegment (Fig. 4B). To ask whether *gcm2* is required for the glial cell development in *gcm* mutants, we analyzed homozygous *Df(2L)200* embryos. Southern blot analysis established that *Df(2L)200* is a deletion removing approximately 120 kb that includes both *gcm* and *gcm2*, as well as 15 other predicted genes, none of which are known to affect glial cell differentiation (Fig. 4A). In *Df(2L)200* homozygous embryos, Repo staining is completely abolished (Fig. 4D). These results implicate *gcm2* in promoting glial cell differentiation.

To gain further evidence for a role for *gcm2* in gliogenesis, we isolated a deletion allele of *gcm2*. A P element inserted into the 5′ UTR of the *thioredoxin* gene, located 584 bp 3′ of the *gcm2* polyadenylation site, was mobilized to recover small deletions at the *gcm2* locus. We recovered one deletion, *Df(2L)gcm2*, that removes the entire *gcm2* ORF as well as the *thioredoxin* promoter (Fig. 5A). This mutation is homozygous viable. Homozygous *Df(2L)gcm2* embryos lack *gcm2* expression (data not shown). *Df(2L)gcm2* embryos also show altered *thioredoxin* expression. *thioredoxin* transcripts are normally expressed at high levels in the gut and at low levels throughout the embryo (data not shown). In *Df(2L)gcm2* embryos, the normal pattern of *thioredoxin* expression is abolished. But a low level of *thioredoxin* expression is detected in the pattern of *gcm2*, reflecting a fusion of *thioredoxin* with the *gcm2* promoter (data not shown). Smaller deletions removing just the *thioredoxin* promoter are homozygous viable but sterile, suggesting that the low level of *thioredoxin* expressed in *Df(2L)gcm2* rescues the sterility of *thioredoxin* mutation.

The pattern of Repo expression in *Df(2L)gcm2* homozygous embryos is normal (Fig. 5B), demonstrating that *gcm2* alone is not necessary for gliogenesis. However *gcm2*'s role in gliogenesis is revealed in a background where *gcm* expression is reduced by half. When *Df(2L)gcm2* is crossed with *Df(2L)200* embryos, removing one copy of *gcm* and two copies of *gcm2*, the resulting transheterozygotes are lethal, and glial cell deficiencies are consistently observed. Glial cell deficiencies are most notable in the longitudinal glia tracts, but are also observed with other CNS glial cell types (Figs. 5C–5E). To assay the penetrance of this glial cells missing phenotype, we counted longitudinal glia (LG) in *Df(2L)gcm2/Df(2L)200* embryos. In wild-type and in *Df(2L)gcm2* homozygous embryos, we counted an average of 8.5 LG per hemisegment (n = 656 767, respectively). In *Df(2L)gcm2/Df(2L)200* embryos, we counted an average of 6.4 LG per hemisegment (n = 1106). Thus, lowering the dosage of *gcm* and completely removing *gcm2* reveals the contribution of *gcm2* to glial cell differentiation, especially in the LG lineage.

Both gcm2 and gcm Are Required Together for the Terminal Differentiation of Plasmatocytes

Both *gcm* and *gcm2* are expressed in the primordia of embryonic hemocytes and in plasmatocytes through stage 11. In *gcm* mutant embryos, there is a decrease by 40% in the number of plasmatocytes (Bernardoni *et al.*, 1997).

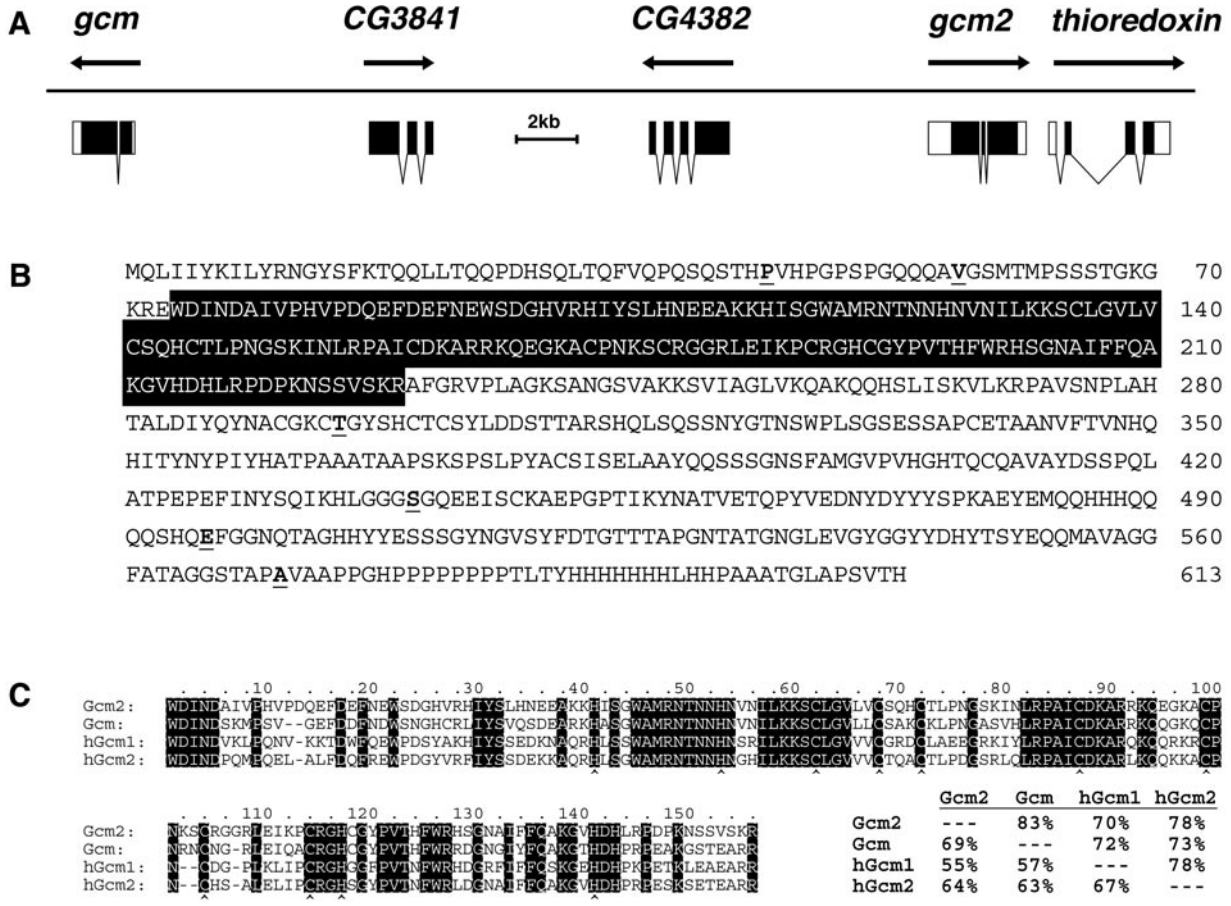
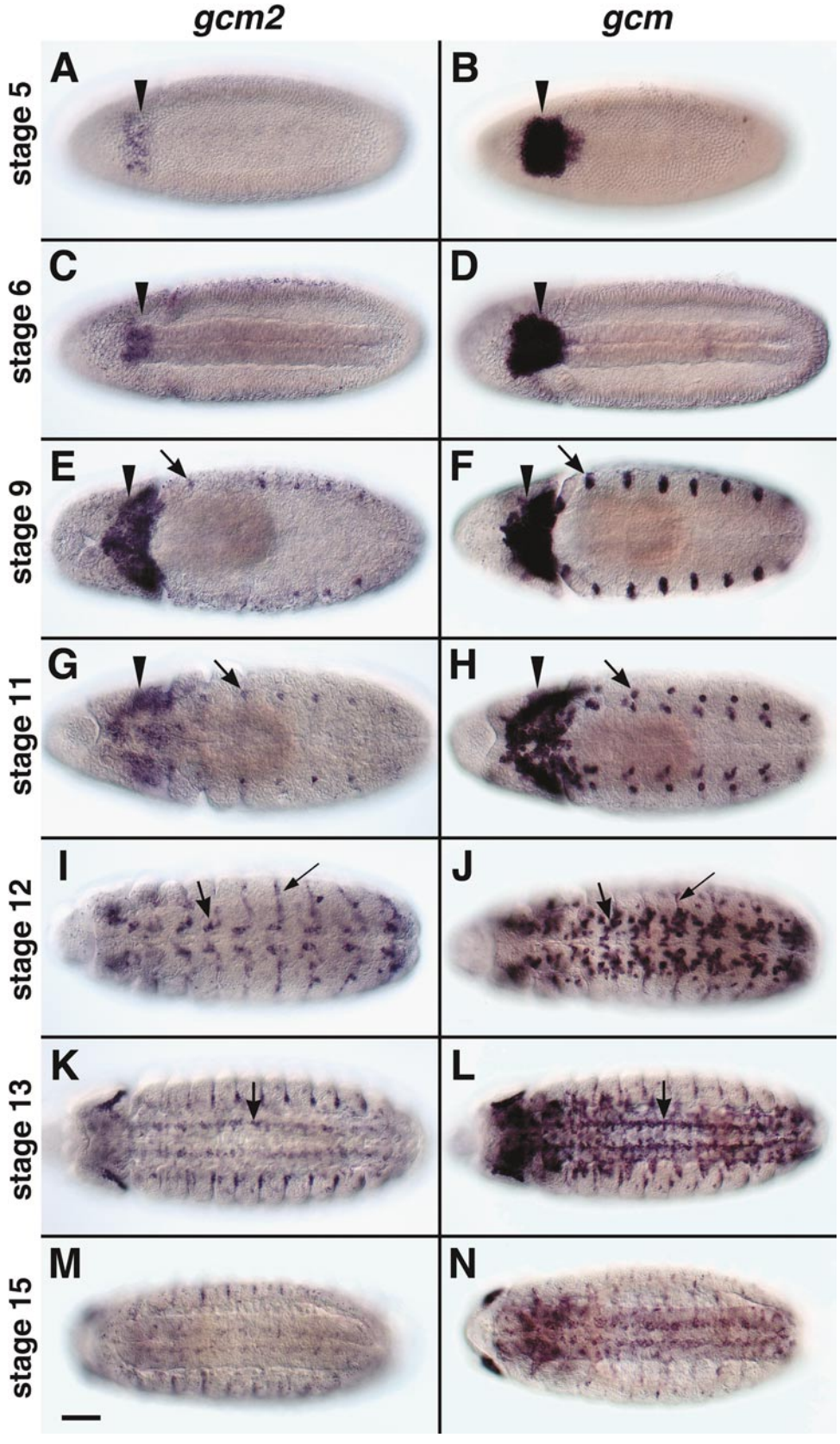


FIG. 1. Map of the *gcm/gcm2* locus, Gcm2 sequence, and comparison to human homologs. (A) Map of genomic region 30B–30C, showing locations of *gcm2*, *gcm*, and three other predicted genes, derived from sequences and gene predictions from the *Drosophila* Genome Project (GenBank Accession No. AE003625; Adams *et al.*, 2000) and from experimental data. Arrows indicate direction of transcription. Predicted transcript structures are shown below the line, with rectangles representing exons; black regions within the rectangles represent predicted ORFs. Distal to proximal orientation is from left to right. (B) Predicted amino acid sequence of Gcm2 protein. Gcm-motif is boxed in black. The six amino acid residues that differ from the predicted sequence of the Genome Project are underlined in bold. (C) Sequence alignments of gcm-motifs of *D. melanogaster* Gcm2, *D. melanogaster* Gcm (GenBank Accession No. AAC46912), *H. sapiens* hGcm1 (GenBank Accession No. BAA13651), and *H. sapiens* hGcm2 (GenBank Accession No. AAC33792). Sequence identities are boxed in black. Conserved cysteine and histidine residues are marked underneath. The percentage sequence identity is shown in the lower half, and the percentage sequence similarity is shown in the upper half of the inserted table. Sequence similarities are based on BLAST analysis. Note that Gcm2 and Gcm are more similar to each other than to their human homologs.

FIG. 2. *In situ* localization of *gcm2* and *gcm* transcripts in whole-mount embryos at different stages, viewed ventrally, with anterior to the left. *gcm2* expression (A, C, E, G, I, K, M) is shown after 6 h of staining reaction. *gcm* expression (B, D, F, H, J, L, N) is shown after 1 h of staining reaction. (A, B) Stage 5: *gcm2* (A) and *gcm* (B) are expressed in a ventral anterior patch of cells in presumptive procephalic mesoderm (arrowheads). (C, D) Stage 6: *gcm2* (C)-and *gcm* (D)-expressing cells invaginate into the ventral furrow anterior to the cephalic furrow (arrowheads). (E, F) Stage 9: *gcm2* (E) is expressed in procephalic mesoderm (arrowhead) and in a single cell per hemisegment at the lateral edge of the neuroectoderm (arrow); *gcm* (F) is expressed in a patch of cells at the same position as *gcm2* at the lateral edge of the neuroectoderm (arrow), as well as in procephalic mesoderm (arrowhead). (G, H) Stage 11: *gcm2* (G) continues to be expressed in a single cell per CNS hemisegment (arrow) and in hemocyte precursors (arrowhead); *gcm* (H) is expressed in the same cell as *gcm2* in the CNS (arrow) as well as other glial precursors and in hemocyte precursors (arrowhead). (I, J) Stage 12: *gcm2* (I) is expressed in migrating longitudinal glia (arrow) at low levels in other CNS glia; *gcm* (J) is expressed at high levels in all developing glia, including the longitudinal glia (arrow). Both *gcm2* and *gcm* begin expression in a stripe of lateral ectodermal cells (thin arrows). Note that *gcm2* and *gcm* are no longer expressed in hemocytes at stage 12. (K, L) Stage 13: *gcm2* (K) is detected in longitudinal glia (arrow), as well as in other glia; *gcm* (L) is expressed in all glia. (M, N) Stage 15: *gcm2* and *gcm* expression fades in the ventral nerve cord. Scale bar, 50 μm.



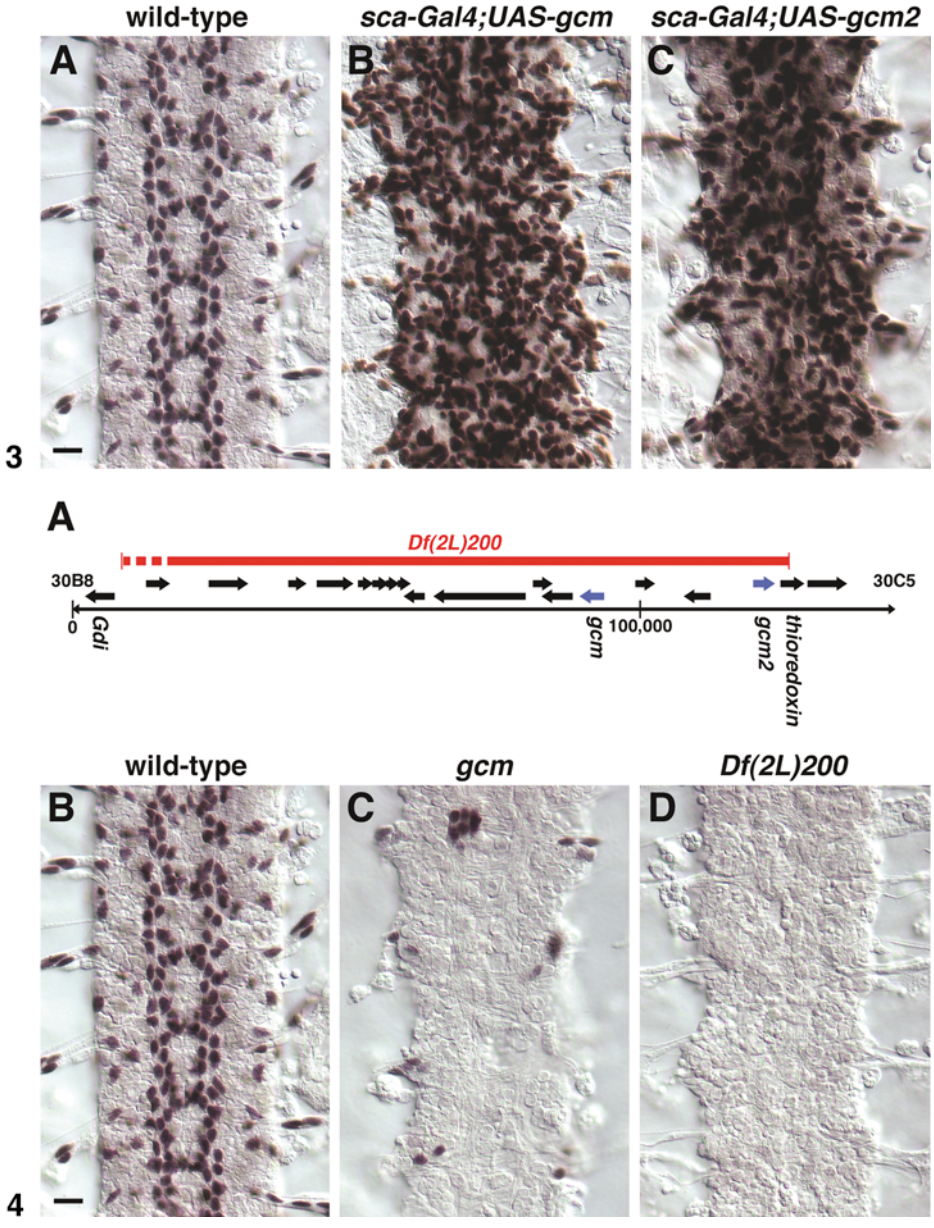


FIG. 3. Ectopic expression of *gcm2* promotes glial cell differentiation. (A–C) Dissected stage 16 embryos showing five adjacent segments of the CNS labeled with Repo monoclonal antibody. (A) Wild-type. (B) *sca-Gal4;UAS-gcm*. (C) *sca-Gal4;UAS-gcm2*. Panneural expression of *gcm* (B) or *gcm2* (C) induces a significant increase in CNS cells expressing Repo protein that adopt glial cell morphologies. Anterior is up. Scale bar, 10 μm.
FIG. 4. A deletion removing both *gcm* and *gcm2* results in loss of all lateral glial cells. (A) Map of 30B–30C region of chromosome 2 showing the 120 kb of DNA removed by the *Df(2L)200* deletion. Red line shows extent of deletion, with the dashed part showing uncertainty. Predicted genes are represented with arrows, with names shown underneath. Solid black line represents scale in DNA base pairs. (B–D) Dissected stage 16 embryos showing five adjacent segments of the CNS labeled with Repo monoclonal antibody with the following genotypes: (B) wild-type, (C) $gcm^{\Delta PI}$, and (D) *Df(2L)200*. In the *gcm* null mutant embryo (C), there remains a low level of glial cell development in the CNS as shown by Repo expression. *Df(2L)200* mutant embryo (D), deleting both *gcm* and *gcm2*, completely lacks Repo expression. Anterior is up. Scale bar, 10 μm.

Given that *gcm2* may have redundant functions with *gcm* in the plasmatocyte lineage, we counted Croquemort-positive cells in *Df(2L)gcm2* embryos. At stage 15, we found an average of 219 per half embryo in *Df(2L)gcm2* mutants (n = 6; 226, 202, 223, 196, 210, 259), as compared with 294 per half embryo in wild type (n = 6; 303, 314, 292,

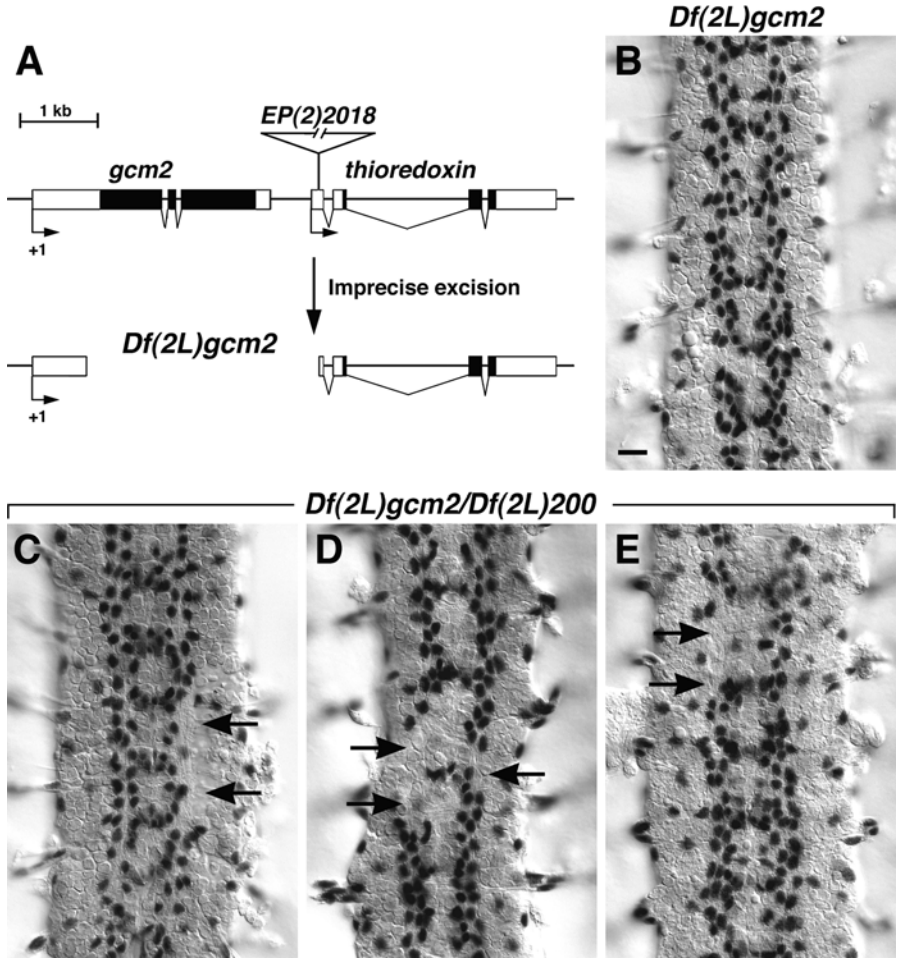


FIG. 5. Mutation of *gcm2* effects glial cell differentiation. (A) Map of *gcm2* deletion. Upper part, Wild-type exon structure of *gcm2* and *thioredoxin* are shown as rectangles, with black regions denoting predicted ORFs. Arrows below, at start of genes, denote the beginning of the *gcm2* cDNA (+1), and the start site of *thioredoxin* based on the longest ESTs from BDGP (Rubin *et al.*, 2000). P element *P(EP)2018* is inserted in the 5′UTR of *thioredoxin* and is located 584 bp downstream of the *gcm2* polyadenylation site. Lower part, Map of deletion *Df(2L)gcm2* induced by imprecise excision of *P(EP)2018*. The deletion starts at position +736 and ends at position +3629 relative to the 5′ end of the *gcm2* cDNA. Sequence analysis revealed a deletion of 2894 bp, replaced with nine unrelated nucleotides. This deletion removes the entire *gcm2* ORF as well as the 5′ end of the *thioredoxin* gene. (B–E) Dissected stage 16 embryos showing five adjacent segments of the CNS labeled with Repo monoclonal antibody. (B) *Df(2L)gcm2* homoygous embryo. Deletion of *gcm2* is viable and has normal pattern of Repo expression. (C–E) *Df(2L)gcm2/Df(2L)200* embryos. A twofold reduction of *gcm* and the complete removal of *gcm2* result in glial cell deficiencies. Missing longitudinal glia are denoted by arrows. Anterior is up. Scale bar, 10 μm.

283, 310). This constitutes a 25% reduction in the number of Croquemort-positive hemocytes in *Df(2L)gcm2* embryos compared with wild-type. We also looked at Peroxidasin protein expression, which is an early marker of the plasmatocyte lineage (Abrams *et al.*, 1993; Nelson *et al.*, 1994; Tepass *et al.*, 1994). At stage 15, we counted an average of 234 Peroxidasin-labeled hemocytes per half embryo in *Df(2L)gcm2* mutants (n = 6; 205, 240, 248, 204, 257, 251), as compared with 314 per half embryo in wild type (n = 6; 306, 341, 297, 314, 322, 301). This constitutes a 26% reduction in Peroxidasin-labeled hemocytes in *Df(2L)gcm2* embryos compared with wild type.

In *Df(2L)200* mutant embryos, deleting both *gcm* and *gcm2*, we found a complete absence of Croquemort expression (Fig. 6B). To ascertain the fate of hemocytes in *Df(2L)200* embryos, we also looked at Peroxidasin expression. In *Df(2L)200* embryos, Peroxidasin-labeled hemocytes are still present (Fig. 7); however, they are reduced in number and have aberrant morphologies and migration behavior. In wild-type stage 13 embryos, plasmatocytes migrate from the head along different routes: midventrally between the ventral epidermis and ventral nerve cord (Fig. 7C), between the dorsal surface of the nerve cord and the mesoderm, along the dorsal boundary of the epidermal

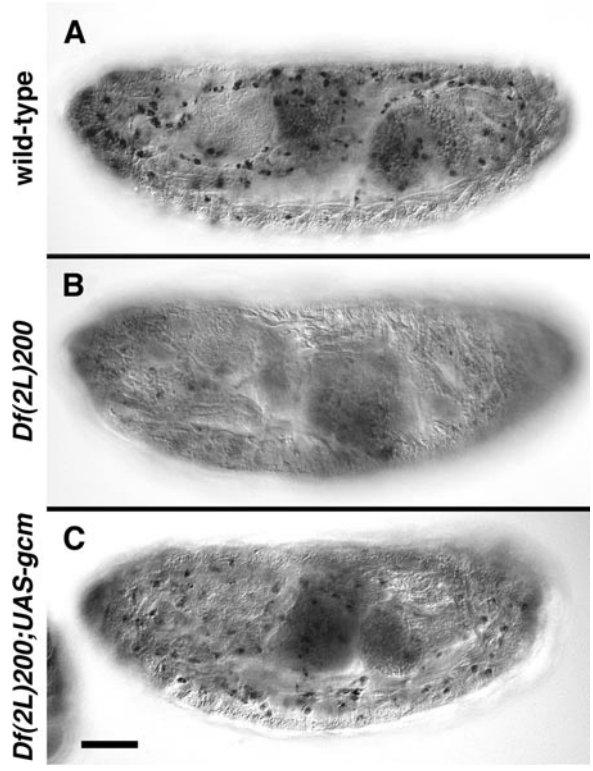


FIG. 6. *gcm/gcm2*-deficient embryos lack Croquemort expression. Ubiquitous expression of *gcm* in *gcm/gcm2*-deficient embryos rescues Croquemort expression in hemocytes. (A–C) Whole-mount stage 16 embryos (lateral view, anterior to left) stained with anti-Croquemort antibody. (A) Wild-type. (B) *Df(2L)200*. (C) *Df(2L)200; UAS-gcm/da-Gal4*. *Df(2L)200* mutant embryos (B), deficient for both *gcm2* and *gcm*, completely lack Croquemort expression in hemocytes. Ubiquitous expression of *gcm* in *Df(2L)200* embryos (C) restores Croquemort expression in hemocytes. Scale bar, 50 μm.

primordium (Fig. 7A), and along the gut primordium. In *Df(2L)200* stage 13 embryos, most Peroxidasin-labeled hemocytes remain in the cephalic region. They fail to migrate ventrally past the 2nd thoracic segment (Fig. 7D), and those that migrate dorsally stay clumped together along the dorsal boundary of the epidermis (Fig. 7B). By stage 15, wild-type plasmatocytes are distributed throughout the embryo, with many found on the ventral surface of the CNS (Figs. 7E and 7G). In *Df(2L)200* stage 15 embryos, a reduced number of Peroxidasin-labeled hemocytes is evident; most of them are in cephalic and dorsal positions, as well as around the gut (Fig. 7F). Very few hemocytes are found along the ventral surface of the CNS (Fig. 7H). At stage 15, we counted an average of 127 Peroxidasin-labeled hemocytes per half embryo in *Df(2L)200* mutants (n = 6; 132, 119, 151, 133, 121, 110), as compared with 314 per half embryo in wild type. This constitutes a 60% reduction in the number of Peroxidasin-labeled hemocytes in *Df(2L)200* embryos compared with wild type.

By stage 16, 90% of Peroxidasin-labeled hemocytes in wild-type embryos have converted into macrophages that are enlarged due to phagocytic activity (Tepass *et al.*, 1994). Macrophages are typically 8–15 microns in diameter and contain large vacuoles filled with dark inclusions (Figs. 8A and 8C). In *Df(2L)200* embryos, the majority of Peroxidasin-labeled hemocytes are small, rounded, but irregular in shape, and less than 5 microns in diameter; some contain small vacuoles and occasionally enlarge up to 8 microns (Figs. 8B and 8D).

Given these phenotypes, we were curious to test whether expression of *gcm* in *gcm/gcm2*-deficient embryos was sufficient to rescue Croquemort expression in hemocytes. We used *daughterless-Gal4* (*da-Gal4*) (Wodarz *et al.*, 1995) to drive ubiquitous expression of *UAS-gcm* throughout embryogenesis in *Df(2L)200* embryos. In *Df(2L)200;UAS-gcm/da-Gal4* embryos, there is a partial rescue of Croquemort expression in hemocytes (Fig. 6C). We counted an average of 68 Croquemort-positive hemocytes per half stage 15 embryo (n = 6; 54, 63, 57, 76, 85, 72), which is 23% of the normal complement of Croquemort-positive hemocytes. These Croquemort-positive hemocytes are distributed throughout the embryo and have the enlarged characteristics of wild-type macrophages. These results demonstrate that expression of *gcm* alone is sufficient to rescue both Croquemort expression and the migration and morphological defects associated with hemocytes that lack endogenous *gcm* and *gcm2* genes.

Hemocyte precursors generate two populations of embryonic hemocytes: plasmatocytes (approximately 600–700) that migrate throughout the embryo, and crystal cells (approximately 40–60) that are clustered around the proventriculus (Lebestky *et al.*, 2000; Mathey-Prevot and Perrimon, 1998; Tepass *et al.*, 1994). Crystal cell differentiation is promoted by the *lozenge* (*lz*) gene, and *gcm* expression is excluded from the crystal cell population (Lebestky *et al.*, 2000). *lz* expression was found not to be altered in *gcm* mutants (Lebestky *et al.*, 2000). To test whether the number of crystal cells is altered in *gcm/gcm2*-deficient embryos, we looked at *lz* expression as monitored by a *UAS lacZ* reporter driven by a *lz-Gal4* driver (*lz-lacZ*) in *Df(2L)200* embryos. We detected no change in the number or position of *lz-lacZ*-expressing cells (data not shown). These results exclude the possibility that some presumptive plasmatocytes had been transformed into crystal cells in embryos that lack both *gcm* and *gcm2*.

DISCUSSION

gcm2 Functions Overlap with gcm in Both Glial Cell and Hemocyte Development

gcm has previously been shown to be the primary regulator of lateral glial cell fate in *Drosophila*. In addition, *gcm* has been implicated in promoting the differentiation of the plasmatocyte/macrophage lineage of blood cells. In this paper, we describe a second *gcm*-like gene, *gcm2*, which has

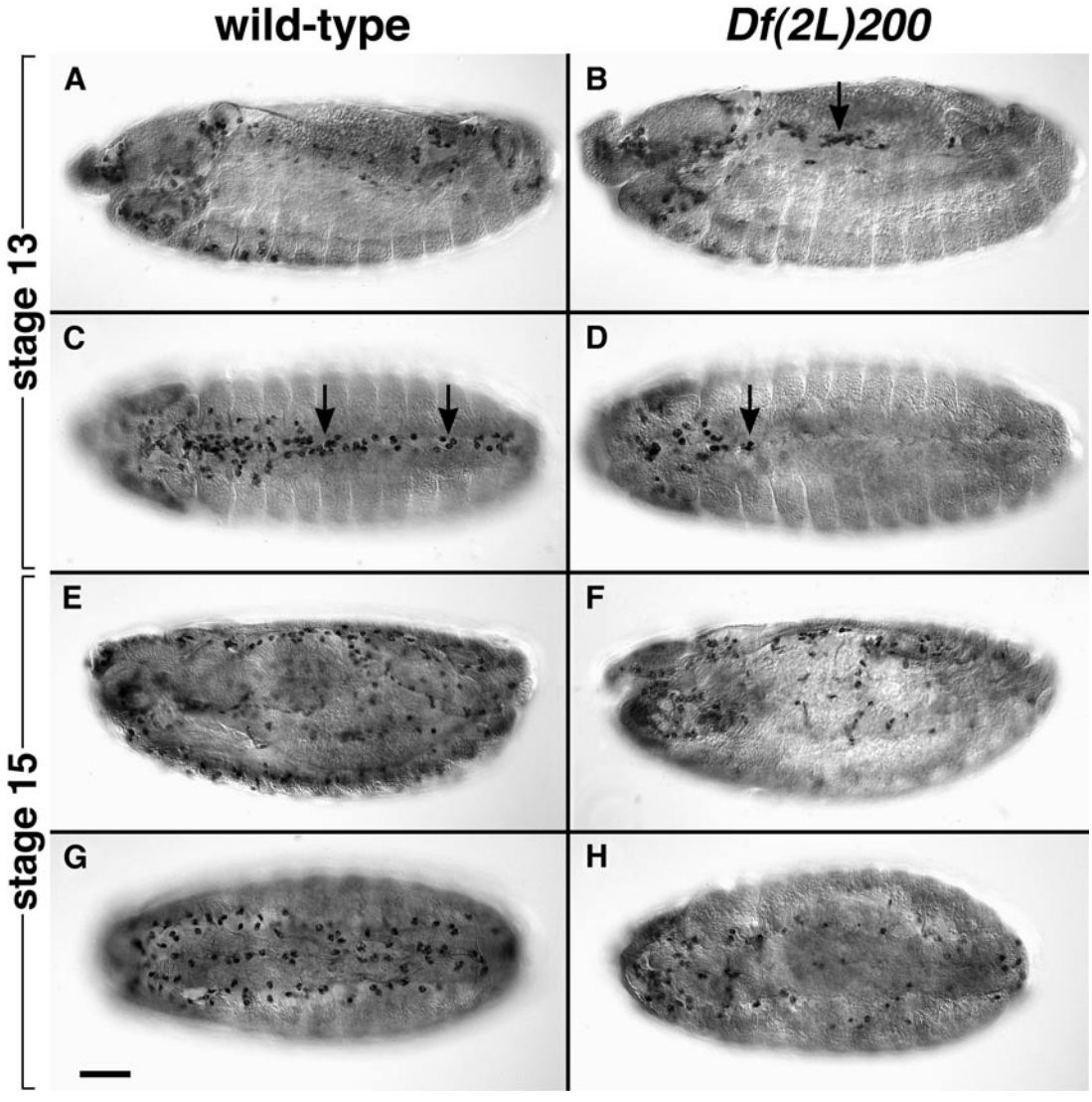


FIG. 7. *gcm/gcm2*-deficient embryos show aberrant plasmatocyte differentiation. (A–H) Whole-mount embryos at different stages stained with anti-Peroxidasin antibody (anterior to left). (A, C) Wild-type stage 13. Lateral view (A) shows plasmatocytes located in the head and migrating dorsally along the edge of the epidermis and ventrally along the developing CNS. Ventral view (C) shows plasmatocytes migrating along the ventral surface of the CNS (arrows). (B, D) *Df(2L)200, gcm/gcm2*-deficient embryos, stage 13. Lateral view (B) shows fewer plasmatocytes, most located in the head; some have migrated out of the head, but are clustered at the dorsal edge of the epidermis (arrow). Ventral view (D) shows that plasmatocytes do not migrate along the ventral surface of the CNS past the second thoracic segment (arrow). (E, G) Wild-type stage 15. Lateral view (E) shows plasmatocytes distributed throughout the hemolymph. Ventral view (G) shows plasmatocytes distributed around the ventral surface of the CNS. (F, H) *Df(2L)200, gcm/gcm2*-deficient embryos, stage 15. Lateral view (F) shows fewer plasmatocytes; most are still located in the head, though some plasmatocytes are scattered in the hemolymph of the dorsal half of the embryo. Ventral view (H) shows very few plasmatocytes on the ventral surface of the CNS. Scale bar, 50 μm.

functions that overlap those of *gcm*. We have shown that *gcm2* has a minor role in gliogenesis, and a more significant role in hemocyte differentiation. Analysis of a deletion of both genes has allowed us to clarify the role of these two transcription factors in plasmatocyte development. We present evidence that *gcm* and *gcm2* are required for the proliferation and maturation of plasmatocytes, but not their initial specification.

The sequence of Gcm2 protein is closely related to Gcm, especially in the highly conserved gcm-motif DNA-binding domain, where it shares 69% identity. While the degree of similarity between the gcm-motifs of all Gcm family members is close. *D. melanogaster* Gcm2 and Gcm are more similar to each other than to their two mammalian counterparts Gcm1/GCMa and Gcm2/GCMb, suggesting that independent duplications produced these two gene

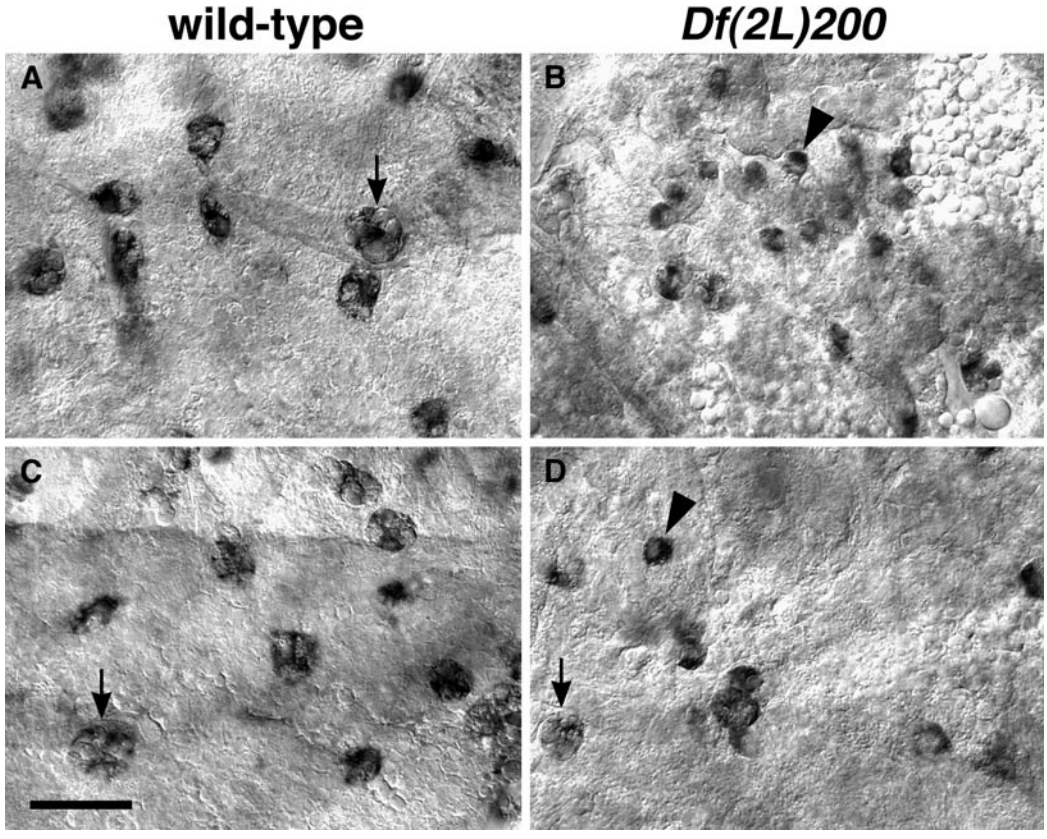


FIG. 8. In *gcm/gcm2*-deficient embryos, plasmatocytes do not convert into normal macrophages. (A–D) Magnified views of anti-Peroxidasin-labeled plasmatocytes at stage 16. (A, C) Wild-type viewed from the dorsal surface (A) and on the ventral anterior surface of the CNS (C). Nearly all wild-type plasmatocytes have become enlarged macrophages that are typically 8–15 microns in diameter and contain large vacuoles filled with dark inclusions (arrows). (B, D) *Df(2L)200, gcm/gcm2*-deficient embryos viewed from the dorsal surface (B) and on the ventral anterior surface of the CNS (C). In *Df(2L)200, gcm/gcm2*-deficient embryos, most Peroxidasin-labeled hemocytes remain small and irregular in shape (arrow heads). An occasional Peroxidasin-labeled hemocyte may contain vacuoles and enlarge up to 8 microns in diameter. (D, arrow). Scale bar, 20 μm.

pairs. Gcm and its mammalian homologs have all been shown to bind the same octamer-binding site sequence with similar affinities (Akiyama *et al.,* 1996; Schreiber *et al.,* 1997, 1998; Tuerk *et al.,* 2000). Expression of rat or mouse Gcm1/GCMa in *Drosophila* is sufficient to activate glial cell development (Kim *et al.,* 1998; Reifegerste *et al.,* 1999). Given these results, it is likely that *D. melanogaster* Gcm2 has similar DNA-binding and functional specificities. *gcm2*'s location, only 27.9 kb from *gcm*, and their similar expression patterns suggest the possibility that *gcm* and *gcm2* share regulatory regions (Kammerer *et al.,* 2001).

gcm2 Promotes Gliogenesis

The presence of a second factor promoting gliogenesis explains why a small number of glial cells still develop in *gcm* null mutants. *gcm2* is expressed at very low levels in lateral glial cells. The complete deletion of both *gcm* and *gcm2* results in the elimination of all lateral glial cell development. Ectopic expression of *gcm2* induces gliogenesis, and its effectiveness is indistinguishable from *gcm* (Fig. 3). These results suggest that Gcm and Gcm2 proteins have redundant biochemical capabilities, which are likely to be mediated through the similar gcm-motif DNA-binding domains.

Mutation of *gcm2* alone is viable and has little effect on glial cell differentiation, confirming that the presence of normal *gcm* expression is sufficient to carry out gliogenesis in the absence of *gcm2*. However, when *gcm2* mutation is crossed with a deletion removing both *gcm* and *gcm2*, the resulting transheterozygote is lethal, and glial cell deficiencies are visible. Thus, a twofold reduction of *gcm* and the complete removal of *gcm2* reveal a small contribution of *gcm2* to glial cell differentiation, especially in the LG lineage, where *gcm2* expression is highest. This phenotype shows that glial cell differentiation is sensitive to the dosage of *gcm* and *gcm2* gene products. Kammerer *et al.* (2001) demonstrated that in *gcm* mutant embryos there is a

reduction in the glial expression of *gcm2* transcripts; in addition, they showed that ectopic *gcm* expression induces *gcm2* expression, and vice versa. These results suggest that cross-regulation between the two genes may contribute to the phenotypes observed.

Dosage sensitivity and the regulation of *gcm2* by *gcm* may explain why *gcm2* has a weak effect on glial cell differentiation in *gcm* mutants. *gcm2* is expressed at such low levels that Gcm2 protein may be at concentrations below a threshold that triggers glial cell differentiation. Sporadically it surpasses this threshold in some neural progenitors, triggering occasional longitudinal glia and nerve root glia differentiation in the absence of *gcm*. It has been proposed that a fairly high threshold for Gcm protein is required to trigger glial cell differentiation in neuroglioblasts that give rise to both neurons and glia. In these neuroglioblasts, low levels of Gcm expression are not sufficient to trigger glial cell fate, but may be necessary to confer glial potential when upregulated in daughter cells (Freeman and Doe, 2001).

gcm and gcm2 Are Required for the Proliferation of Plasmatocyte Precursors but Not Their Initial Specification

It is clear that both *gcm* and *gcm2* are required for the proper differentiation of the plasmatocyte lineage. Mutation of either *gcm* or *gcm2* results in deficits in plasmatocyte numbers (Bernardoni *et al.,* 1997; this study), and the ectopic expression of *gcm* leads to extra plasmatocytes (Bernardoni *et al.,* 1997). Previous reports had suggested that *gcm* specifies the plasmatocyte lineage, and that perhaps the existence of a second gcm-motif gene in *Drosophila* accounted for the presence of only reduced number of plasmatocytes in *gcm* mutants rather than their elimination (Bernardoni *et al.,* 1997).

In light of this, we were surprised to find, in embryos deficient for both *gcm* and *gcm2*, plasmatocyte-like cells still developing and expressing the early plasmatocyte marker Peroxidasin. However the number of Peroxidasin-labeled hemocytes in *gcm/gcm2*-deficient embryos is reduced by 60% compared with wild type. This reduction roughly corresponds to the combined reduction in plasmatocytes in *gcm* and *gcm2* mutant embryos together (40% for *gcm* mutants plus 25% for *gcm2* mutants). It has previously been observed that the procephalic mesoderm, from which plasmatocytes develop, forms a mitotic domain that undergoes four divisions during embryonic stages 8–11 (Tepass *et al.,* 1994). After the final division, most procephalic mesoderm cells are recognizable as plasmatocytes and undergo no further divisions (Tepass *et al.,* 1994). These cell divisions are coincident with the highest levels of *gcm* and *gcm2* expression. These results suggest that *gcm* and *gcm2* promote the proliferation of plasmatocyte precursors, rather than their initial specification.

We believe that the increased number of plasmatocytes observed when embryos receive an overexpression of *gcm* under control of the heat shock promoter (Bernardoni *et al.,* 1997) is due to an over-proliferation of plasmatocytes in the procephalic mesoderm, which subsequently migrate throughout the embryo. Bernardoni *et al.* (1997) observed that ectopic expression of *gcm* in the nervous system leads to an increase in Peroxidasin-labeled hemocytes clustered around the CNS. They ascribed this phenotype to a transformation of CNS to hemocyte cell fate. We counted the number of Peroxidasin-labeled hemocytes in *sca-Gal4; UAS-gcm* embryos, where *gcm* is ectopically expressed in all neuroblasts, and found that the number of plasmatocytes in stage 15 embryos is not significantly increased (average of 320 Peroxidasin-labeled hemocytes per half embryo; $n = 6$; 292, 339, 287, 309, 358, 337), even though their distribution has changed, with more macrophages observed around the CNS (data not shown). We believe this phenotype arises from the recruitment of macrophages to the CNS due to increased apoptosis, rather than transformation of neurons into macrophages. Our results, and those of others, show that ectopic expression of *gcm* transforms presumptive neurons into glial cells (Hosoya *et al.,* 1995; Jones *et al.,* 1995) but not into plasmatocytes, consistent with combinatorial models of transcription factor action.

Hemocyte precursors in the embryo give rise to two populations of blood cells, crystal cells and plasmatocytes (Mathey-Prevot and Perrimon, 1998), promoted by the GATA transcription factor Serpent (Rehorn *et al.,* 1996). Lack of Serpent results in the complete absence of hemocytes (Rehorn *et al.,* 1996). Crystal cells development is promoted by the AML-1 transcription factor homolog Lozenge (Lebestky *et al.,* 2000). In the absence of both *gcm* and *gcm2*, the number and location of crystal cells remains the same. We conclude that *gcm* and *gcm2* do not act as genetic switches between plasmatocyte and crystal cell fate. However, ectopic expression of Gcm in the crystal cell lineage causes them to express Croquemort and assume plasmatocyte-like morphology (Lebestky *et al.,* 2000) showing that there is some plasticity between hemocyte lineages.

The persistent expression of Serpent suggests that it may continue to have a role in plasmatocyte development after the initial specification of hemocyte precursors (Rehorn *et al.,* 1996). In addition, a second factor, U-shaped, is expressed in plasmatocytes (Fossett *et al.,* 2001). U-shaped acts to limit the proliferation of crystal cells. In the absence of U-shaped, there is an increase in the crystal cell population, and forced expression of U-shaped reduces the crystal cell population (Fossett *et al.,* 2001). We suggest that a combination of Serpent, U-shaped, lack of Lozenge expression, and perhaps unidentified factors promotes the initial specification of plasmatocytes and Peroxidasin expression.

gcm and gcm2 Promote Macrophage Development

Our results are consistent with a requirement of *gcm* and *gcm2* for the conversion of plasmatocytes into macro-

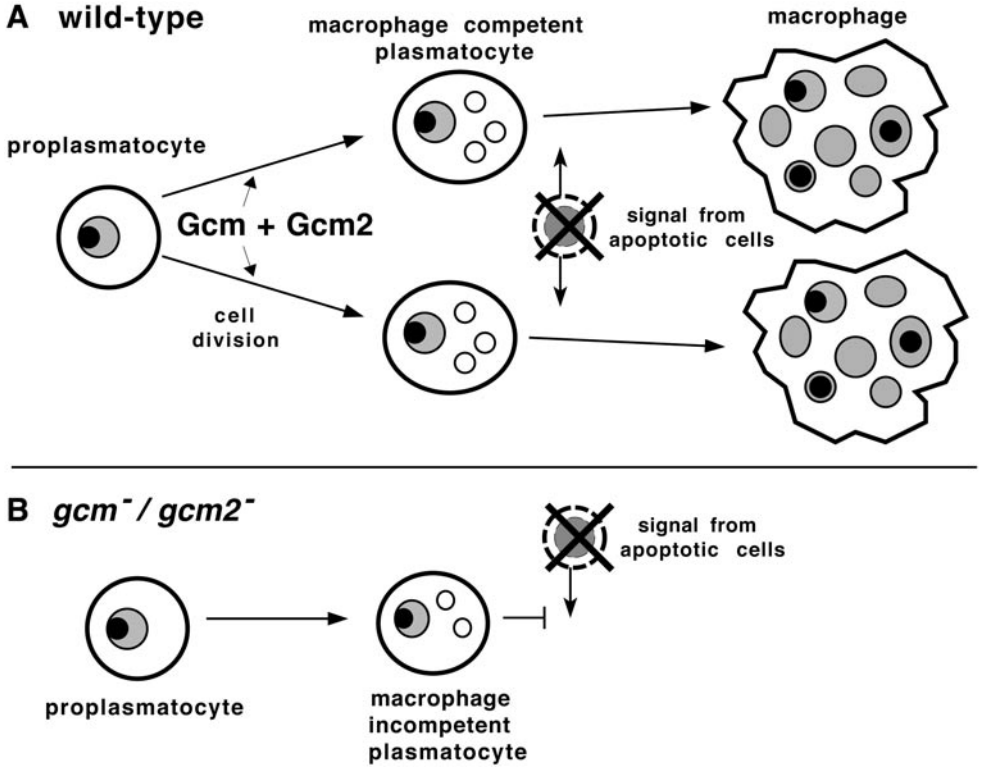


FIG. 9. Model for *gcm* and *gcm2* functions in plasmatocyte differentiation. (A) Wild-type. Hemocyte precursors differentiate into a population of proplasmatocytes that express Gcm, Gcm2, and Peroxidasin. Gcm and Gcm2 stimulate the proliferation and differentiation of proplasmatocytes into plasmatocytes that are competent to become macrophages by activating Croquemort and other macrophage genes. After stage 11, Gcm and Gcm2 expression disappears. Macrophage competent plasmatocytes convert into macrophages in response to signals from cells undergoing apoptosis. (B) *gcm*$^{-}$/*gcm2*$^{-}$. In the absence of Gcm and Gcm2 proteins, proplasmatocytes fail to proliferate. They express Peroxidasin and partially differentiate into cells with characteristics of plasmatocytes. However, these plasmatocytes are not competent to convert into macrophages in response to signals from apoptotic cells.

phages. Deletion of both *gcm* and *gcm2* results in the complete absence of Croquemort expression in hemocytes. These mutant hemocytes retain Peroxidasin expression and have some migratory properties characteristic of plasmatocytes; some move out of the procephalon and are ultimately distributed in the hemolymph. However, many of these mutant hemocytes remain in the head; those that do migrate, migrate dorsally, and do not follow stereotypic ventral paths along the surface of the CNS. Normal plasmatocytes migrate to prominent sites of programmed cell death (Abrams *et al.,* 1993; Tepass *et al.,* 1994). The distribution of plasmatocytes in embryos lacking *gcm* and *gcm2* functions suggests that they are not attracted to cells undergoing programmed cell death or that their ability to migrate is impaired.

In addition, the majority of mutant plasmatocytes fail to enlarge, nor do they contain large vacuoles filled with dark inclusions. This phenotype suggests that most mutant plasmatocytes lack phagocytic activity. We cannot rule out that they are incabable of phagocytosis; the fact that a small number of mutant plasmatocytes enlarge slightly may indicate that they are competent for phagocytosis. Plasmatocytes mutant for Croquemort protein are impaired in their ability to engulf apoptotic cells, but will still recognize and engulf bacteria (Franc *et al.,* 1999). Whether *gcm/gcm2*-deficient plasmatocytes will convert into macrophages in response to bacterial invasion or other insults remains to be explored. The morphology of these cells and high level of Peroxidasin expression suggest characteristics of plasmatocytes just before their conversion to macrophages (Tepass *et al.,* 1994). We suggest a model (Fig. 9) whereby *gcm* and *gcm2* promote the expansion and differentiation of a Peroxidasin-expressing population of hemocytes, or "proplasmatocytes." Expression of *gcm* and *gcm2* is required for the differentiation of these proplasmatocytes into plasmatocytes that are competent for macrophage conversion, and they do so by initiating the expression of the macrophage receptor Croquemort and other genes that promote macrophage morphogenesis and function.

ACKNOWLEDGMENTS

We thank JoMichelle Corrales and Rithwick Rajagopal for the cloning and initial characterization of the *gcm2* cDNA; Melissa Yee for technical help; Nathalie Franc for Croquemort antisera; Liselotte Fessler for Peroxidasin antisera; the Bloomington Stock Center for fly stocks; Ruth Lehmann, Michelle Starz-Gaiano, and Jones Lab members for comments on the manuscript. This research was funded in part by the City of New York Council Speaker's Fund for Biomedical Research: Toward the Science of Patient Care, and NIH Grant RO1 NS39373.

REFERENCES

Abrams, J. M., White, K., Fessler, L. I., and Steller, H. (1993). Programmed cell death during Drosophila embryogenesis. *Development* **117,** 29–43.

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.

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.

Altshuller, Y., Copeland, N. G., Gilbert, D. J., Jenkins, N. A., and Frohman, M. A. (1996). Gcm1, a mammalian homolog of Drosophila glial cells missing. *FEBS Lett.* **393,** 201–204.

Bernardoni, R., Miller, A. A., and Giangrande, A. (1998). Glial differentiation does not require a neural ground state. *Development* **125,** 3189–3200.

Bernardoni, R., Vivancos, B., and Giangrande, A. (1997). glide/gcm is expressed and required in the scavenger cell lineage. *Dev. Biol.* **191,** 118–130.

Brand, A. H., and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118,** 401–415.

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.

Crew, J. R., Batterham, P., and Pollock, J. A. (1997). Developing compound eye in lozenge mutants of Drosophila: Lozenge expression in the R7 equivalence group. *Dev. Genes Evol.* **206,** 481–493.

Fossett, N., Tevosian, S. G., Gajewski, K., Zhang, Q., Orkin, S. H., and Schulz, R. A. (2001). The Friend of GATA proteins U-shaped, FOG-1, and FOG-2 function as negative regulators of blood, heart, and eye development in Drosophila. *Proc. Natl. Acad. Sci. USA* **98,** 7342–7347.

Franc, N. C., Dimarcq, J. L., Lagueux, M., Hoffmann, J., and Ezekowitz, R. A. (1996). Croquemort, a novel Drosophila hemocyte/macrophage receptor that recognizes apoptotic cells. *Immunity* **4,** 431–443.

Franc, N. C., Heitzler, P., Ezekowitz, R. A., and White, K. (1999). Requirement for croquemort in phagocytosis of apoptotic cells in Drosophila. *Science* **284,** 1991–1994.

Freeman, M. R., and Doe, C. Q. (2001). Asymmetric Prospero localization is required to generate mixed neuronal/glial lineages in the Drosophila CNS. *Development* **128,** 4103–4112.

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.

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.

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.

Kammerer, M., and Giangrande, A. (2001). Glide2, a second glial promoting factor in Drosophila melanogaster. *EMBO J.* **20,** 4664–4673.

Kim, J., Jones, B. W., Zock, C., Chen, Z., Wang, H., Goodman, C. S., and Anderson, D. J. (1998). Isolation and characterization of mammalian homologs of the Drosophila gene glial cells missing. *Proc. Natl. Acad. Sci. USA* **95,** 12364–12369.

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

Lane, M. E., and Kalderon, D. (1993). Genetic investigation of cAMP-dependent protein kinase function in Drosophila development. *Genes Dev.* **7,** 1229–1243.

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

Liao, G. C., Rehm, E. J., and Rubin, G. M. (2000). Insertion site preferences of the P transposable element in *Drosophila melanogaster. Proc. Natl. Acad. Sci. USA* **97,** 3347–3351.

Mathey-Prevot, B., and Perrimon, N. (1998). Mammalian and Drosophila blood: JAK of all trades? *Cell* **92,** 697–700.

Nelson, R. E., Fessler, L. I., Takagi, Y., Blumberg, B., Keene, D. R., Olson, P. F., Parker, C. G., and Fessler, J. H. (1994). Peroxidasin: A novel enzyme-matrix protein of Drosophila development. *EMBO J.* **13,** 3438–3447.

Patel, N. H. (1994). Imaging neuronal subsets and other cell types in whole-mount Drosophila embryos and larvae using antibody probes. *Methods Cell Biol.* **44,** 445–487.

Rehorn, K. P., Thelen, H., Michelson, A. M., and Reuter, R. (1996). A molecular aspect of hematopoiesis and endoderm development common to vertebrates and Drosophila. *Development* **122,** 4023–4031.

Reifegerste, R., Schreiber, J., Gulland, S., Ludemann, A., and Wegner, M. (1999). mGCMa is a murine transcription factor that overrides cell fate decisions in Drosophila. *Mech. Dev.* **82,** 141–150.

Rorth, P., Szabo, K., Bailey, A., Laverty, T., Rehm, J., Rubin, G. M., Weigmann, K., Milan, M., Benes, V., Ansorge, W., and Cohen, S. M. (1998). Systematic gain-of-function genetics in Drosophila. *Development* **125,** 1049–1057.

Rubin, G. M., Hong, L., Brokstein, P., Evans-Holm, M., Frise, E., Stapleton, M., and Harvey, D. A. (2000). A Drosophila complementary DNA resource. *Science* **287,** 2222–2224.

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.

Schreiber, J., Enderich, J., and Wegner, M. (1998). Structural requirements for DNA binding of GCM proteins. *Nucleic Acids Res.* **26,** 2337–2343.

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.

Tepass, U., Fessler, L. I., Aziz, A., and Hartenstein, V. (1994). Embryonic origin of hemocytes and their relationship to cell death in Drosophila. *Development* **120,** 1829–1837.

Tuerk, E. E., Schreiber, J., and Wegner, M. (2000). Protein stability and domain topology determine the transcriptional activity of the mammalian glial cells missing homolog, GCMb. *J. Biol. Chem*. **275,** 4774–4782.

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.

Wodarz, A., Hinz, U., Engelbert, M., and Knust, E. (1995). Expression of crumbs confers apical character on plasma membrane domains of ectodermal epithelia of Drosophila. *Cell* **82,** 67–76.

Xiong, W. C., Okano, H., Patel, N. H., Blendy, J. A., and Montell, C. (1994). repo encodes a glial-specific homeo domain protein required in the Drosophila nervous system. *Genes Dev*. **8,** 981–994.

Zinn, K., McAllister, L., and Goodman, C. S. (1988). Sequence analysis and neuronal expression of fasciclin I in grasshopper and Drosophila. *Cell* **53,** 577–587.

Received for publication March 22, 2002
Revised May 23, 2002
Accepted May 23, 2002
Published online July 22, 2002