

***ming* is expressed in neuroblast sublineages and regulates gene expression in the *Drosophila* central nervous system**

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Summary

Cell diversity in the *Drosophila* central nervous system (CNS) is primarily generated by the invariant lineage of neural precursors called neuroblasts. We used an enhancer trap screen to identify the *ming* gene, which is transiently expressed in a subset of neuroblasts at reproducible points in their cell lineage (i.e. in neuroblast 'sublineages'), suggesting that neuroblast identity can be altered during its cell lineage. *ming* encodes a

predicted zinc finger protein and loss of *ming* function results in precise alterations in CNS gene expression, defects in axonogenesis and embryonic lethality. We propose that *ming* controls cell fate within neuroblast cell lineages.

Key words: *Drosophila*, neuroblast, cell lineage, enhancer trap, *ming*.

Introduction

The mechanisms controlling the generation of cellular diversity in the central nervous system (CNS) remain a fundamental question in neurobiology. In *Drosophila*, neuronal and glial diversity in the CNS is generated in two distinct steps. First, individual cells within the ventral ectoderm delaminate into the embryo and enlarge to form a subepidermal, two-dimensional array of neural precursors, most of which are neuroblasts (NBs; Wheeler, 1891; Poulson, 1950). Each NB can be uniquely identified by its time of formation, position of enlargement, pattern of gene expression and/or the neuronal progeny that it generates (reviewed in Doe, 1992a).

The second step of neurogenesis generates most of the cellular diversity in the CNS. NBs repeatedly undergo an oriented, asymmetric division, generating a smaller ganglion mother cell (GMC; Bauer, 1904), which is displaced into the embryo, and a new NB, which remains at the ventral cortex of the CNS. Each GMC divides to produce a characteristic pair of postmitotic neurons (Thomas et al., 1984; Goodman et al., 1984; Taghert and Goodman, 1984; Raper et al., 1983; Doe et al., 1988; Patel et al., 1989). Experimental evidence shows that NBs can produce a normal first GMC despite altered environmental cues in vivo (Doe and Goodman, 1985) or when isolated in vitro (Huff et al., 1989).

The fact that early NB lineages appear invariant in vivo and in vitro suggests that the information required to specify GMC and neuronal identity is contained within the NB lineage. It has been proposed that a class of genes, called

'NB identity' genes, are expressed in NBs and their progeny and control the cell lineage of each NB (Doe et al., 1991; Doe, 1992a). Expression of these genes could be modulated qualitatively or quantitatively during the NB lineage, such that each GMC inherits a different amount of gene product or unique combination of gene products. Loss-of-function mutations would switch NB or GMC identity to a different or novel fate. Currently only a few NB identity genes have been described. The *runt* gene is expressed in a subset of NBs, GMCs and neurons; it is required for the specification of several identified GMCs and neurons (Duffy et al., 1991). *polyhomeotic* is expressed in NBs and is required for correct gene expression in GMCs or neurons (Smouse et al., 1988; Decamillis et al., 1992). The *prospero* gene is transcribed in NBs and GMCs, but protein can only be detected in GMCs, where it is required to control GMC gene expression and their subsequent differentiation (Doe et al., 1991; Vaessin et al., 1991; Matsuzaki et al., 1992). Thus *prospero* is best considered a 'GMC identity' gene (Doe, 1992a).

We have used the enhancer trap technique (O'Kane and Gehring, 1987) to identify additional NB identity genes; in particular, we looked for genes expressed in qualitatively or quantitatively different patterns during identified NB cell lineages. Here we describe the *ming* gene ('fate' in Chinese), which is transcribed in a subset of NBs and GMCs, but not in neurons. Certain neuroblasts express *ming* from the time of their formation, whereas other identified neuroblasts express *ming* only after they have generated a stereotyped number of GMCs. *ming* encodes a predicted zinc finger protein and loss of *ming* function leads to pre-

cise alterations in CNS gene expression followed by defects in axonogenesis and embryonic lethality.

Materials and methods

Genetic analysis

The P element construct of Bier et al. (1989) was used to generate over 2,000 independent enhancer trap lines (C. Q. D., L. Rost, K. Schuske and M. P. Scott, unpublished results), including the embryonic lethal *ming*¹⁵³⁰ and *ming*²⁰⁹² alleles. We used the transposase-producing 2-3 chromosome (Robertson et al., 1988) to mobilize the 2092 P element insertion with the goal of creating deletions of the *ming* gene. We scored for the loss of the *w*⁺ marker on the P element. Among the 82 *w* flies examined, 60 were homozygous lethal and 22 were homozygous viable. 34 of the lethal lines were examined by Southern blots and three chromosomes (*ming*²⁴, *ming*³⁹ and *ming*⁷⁸) had deletions of genomic DNA at the insertion site. Embryos homozygous for any of these three alleles do not produce *ming* transcript (about 25% of the embryos from *ming*/TM3 parents had no detectable *ming* RNA expression by whole-mount hybridization) and do not complement the *ming*¹⁵³⁰ or *ming*²⁰⁹² insertion alleles. To identify accurately homozygous *ming* embryos for phenotypic analysis, we balanced the *ming*²⁴ allele over TM3 containing the *ftzlacZ* construct (Hiromi and Gehring, 1987); homozygous *ming*²⁴ embryos can be easily distinguished due to lack of the *ftzlacZ* expression.

Molecular analysis

The genomic DNA fragments flanking the 1530 and 2092 insertions were cloned by the plasmid rescue technique (Bier et al., 1989) and the flanking DNA was localized to cytological position 83C in polytene chromosomes by the method of Engels et al. (1986). The 4.9 kb fragment of genomic DNA between the 1530 insert and the *KspI* site (Fig. 1A) was used to isolate 20 cDNA clones from a *Drosophila* 0-24 hour embryonic ZAPII cDNA library (Stratagene). A deletion series was made across the longest cDNA, P1 (Henikoff, 1984; Promega Erase-a-Base Kit), and both strands were sequenced. Deduced amino acid sequences were compared to the Genbank Database (Pearson and Lipman, 1988). Southern blots were done using standard protocols (Sambrook et al., 1989) to assay the genomic DNA of P element revertant lines.

Northern blots were done using total RNA from 0-12 hour embryos. Embryos were homogenized in lysis buffer containing 7 M Urea, 2% SDS, 0.35 M NaCl, 1 mM EDTA, 10 mM Tris pH 8.0. Following phenol-chloroform extraction, RNA was precipitated in the presence of 3 M sodium acetate at pH 5.0. 40 µg of total RNA was run on a 1% agarose-formaldehyde gel, transferred onto a nitrocellulose membrane and hybridized with ³²P-labeled P1 cDNA. Hybridization was at 68°C for 1.5 hour in QuickHyb solution (Stratagene). The filters were washed twice in 1× SSC/0.1% SDS at room temperature and once in 0.1× SSC/0.1% SDS at 62°C before autoradiography.

mRNA and protein localization

Localization of *ming* transcript was examined by in situ hybridization to whole embryos (Tautz and Pfeiffle, 1989). The P1 cDNA and the 4.5 kb genomic fragment from the 1530 insert to the *XbaI* site were used as nonradioactive probes. Embryos were processed identically to Doe et al. (1991). The following antibodies were used for protein localization: mouse anti-β-galactosidase monoclonal (1:1,000; Promega), mouse anti-engrailed monoclonal (1:1; Patel et al., 1989), mouse BP102 monoclonal (1:10; Klambt et al., 1991) and mouse anti-fasciclin III monoclonal (1:2; Patel et al., 1987). Immunocytochemistry was done by the procedure of Doe et al. (1991), with the exception of fasciclin III

staining which was done on nerve cord 'fillets' by the method of Thomas et al. (1984).

Results

Isolation and molecular analysis of the *ming* gene

Two enhancer trap lines, 1530 and 2092, express the marker gene *lacZ* in an identical subset of NBs. Genomic DNA flanking each insertion was cloned (Fig. 1A) and used to probe whole-mount embryos for RNA expression patterns. The 4.9 kb genomic DNA fragment extending from the 1530 insertion to the *KspI* site (Fig. 1A) detects a mRNA pattern indistinguishable from the 1530 *lacZ* pattern (see below) and was used to isolate a 1.3 kb cDNA, called P1 (Fig. 1B). The P1 cDNA hybridizes to mRNA matching the 1530 *lacZ* pattern in whole-mount embryos (data not shown) and detects a single band of 3.3 kb on northern blots (Fig. 1D). We call this transcription unit the *ming* gene.

The *ming* P1 cDNA contains a predicted open reading frame of 417 amino acids that extends to the end of the cDNA (Fig. 1E). Within this open reading frame are four repeated motifs (Fig. 1F), with part of each repeat showing homology to the TFIIIA-class zinc finger motif. The other portion of each repeat contains two cysteines and two histidines, which may form a metal-binding domain. The presence of four predicted zinc finger motifs in the *ming* P1 cDNA suggests that the *ming* protein could function to regulate gene expression in the CNS.

ming is specifically expressed in a subset of CNS neuroblasts

ming is expressed only in NBs, GMCs and midline of the CNS; it is not detectable in the peripheral nervous system or any other embryonic tissue. The *ming* transcript and the 1530 enhancer trap *lacZ* gene are expressed in virtually identical patterns (compare Fig. 2A, B, C, G [*lacZ*] to Fig. 2D, E, F, H [transcript]), although the 1530 line has a low level of *lacZ* expression in segmental stripes in the dorsal epidermis (which is a common artifact of this and possibly other enhancer trap constructs; C. Q. D. et al., unpublished results; Ghysen and O'Kane, 1989). *ming* transcripts are first detected in a subset of the midline CNS cells at stage 9 (data not shown; staging according to Campos-Ortega and Hartenstein, 1985). Slightly later, one NB in every hemisegment (NB 6-1) expresses *ming* (Fig. 2D). By late stage 11, *ming* transcripts are expressed in 17 identified NBs in every hemisegment of the CNS (Fig. 2E). By stage 14, *ming* transcript levels begin to decrease and, by stage 16, the transcript is undetectable except in a few cells in the thoracic and terminal abdominal CNS (Fig. 2F). β-galactosidase (β-gal) protein in 1530 embryos persists several hours later than the *ming* transcript can be detected, presumably due to the greater stability of the β-gal protein (Fig. 2C). *ming* expression is only detectable in NBs and GMCs, not in the neurogenic ectoderm or in neurons (Fig. 2H).

ming is expressed at different points during the lineage of identified neuroblasts

The initiation of *ming* expression in identified NBs was

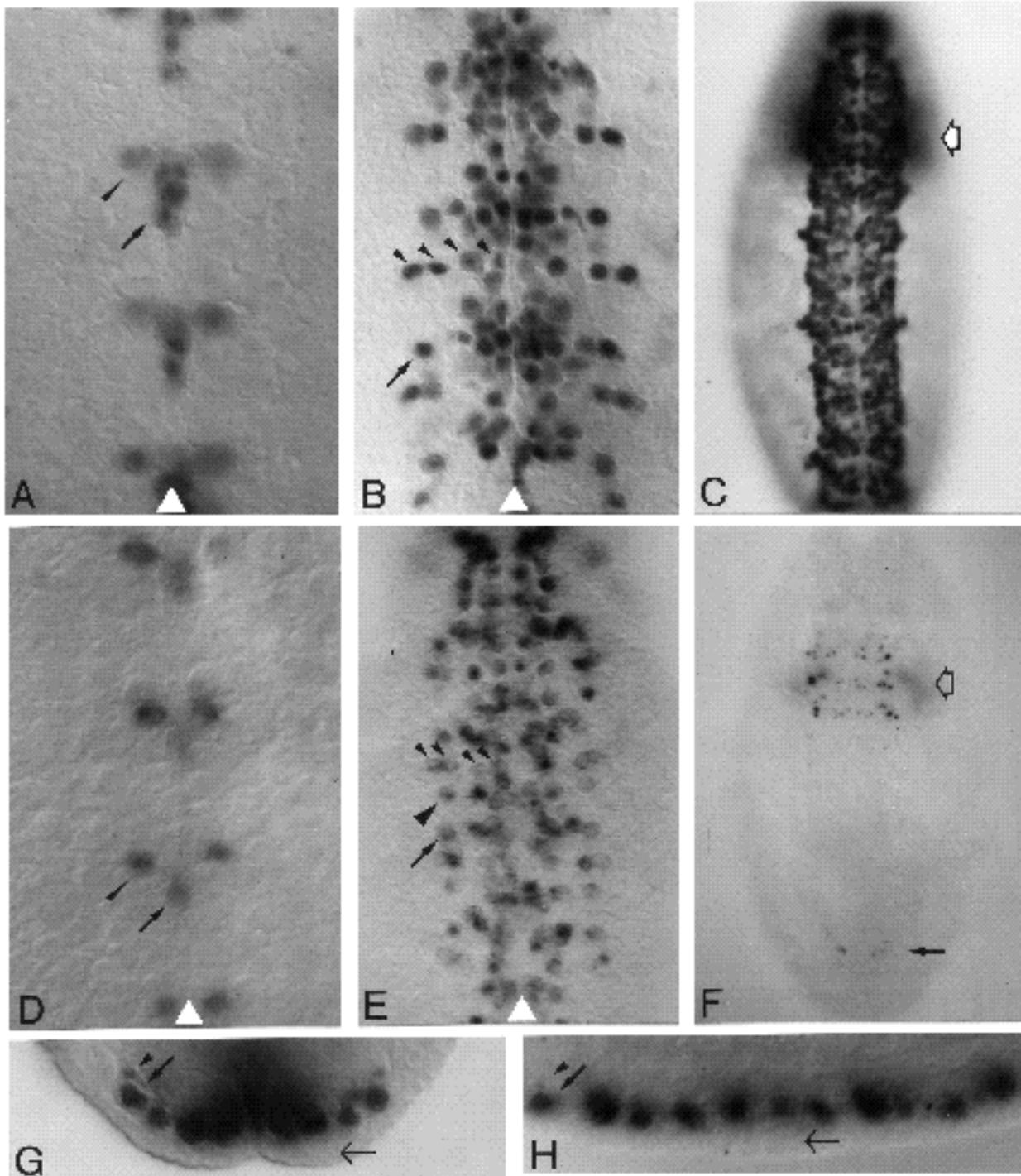


Fig. 2. *ming* is specifically expressed in a subset of embryonic NBs and GMCs. Detection of *gal* protein in the 1530 enhancer trap line (A-C, G) and *ming* mRNA (D-F, H). (A and D) Early stage 10 embryos (early S3 NB array). Only NB 6-1 (arrowhead) and a few midline cells (arrow) express *ming*. (B and E) Late stage 11 embryos (S5 NB array). The expression of *gal* in the 1530 line and *ming* mRNA is virtually identical; for example the indicated row 3 NBs (3-4, 3-3, 3-2 and 3-1, small arrowheads from left to right) and NB 7-4 (arrow). NB 5-6 expresses mRNA at this stage (E; large arrowhead), whereas the *gal* protein can be detected a short time later in the 1530 line (see Fig. 3). (C and F) Stage 15 embryos. (C) *gal* expression persists in most NBs in the 1530 line, probably due to the stability of the protein. (F) In contrast, *ming* transcript can only be detected in a few cells of the thoracic (wide arrowhead) and terminal CNS (arrow). (G and H) Stage 11 embryos. *ming* expression is only in NBs (small arrow) and some GMCs (arrowhead), not in ventral ectodermal cells (large arrow) or neurons (dorsal-most cells). (A-F) Ventral view, anterior is up. White triangle indicates the ventral midline. (G) Cross-section view, ventral side down. (H) Lateral view, anterior is left and ventral side down.

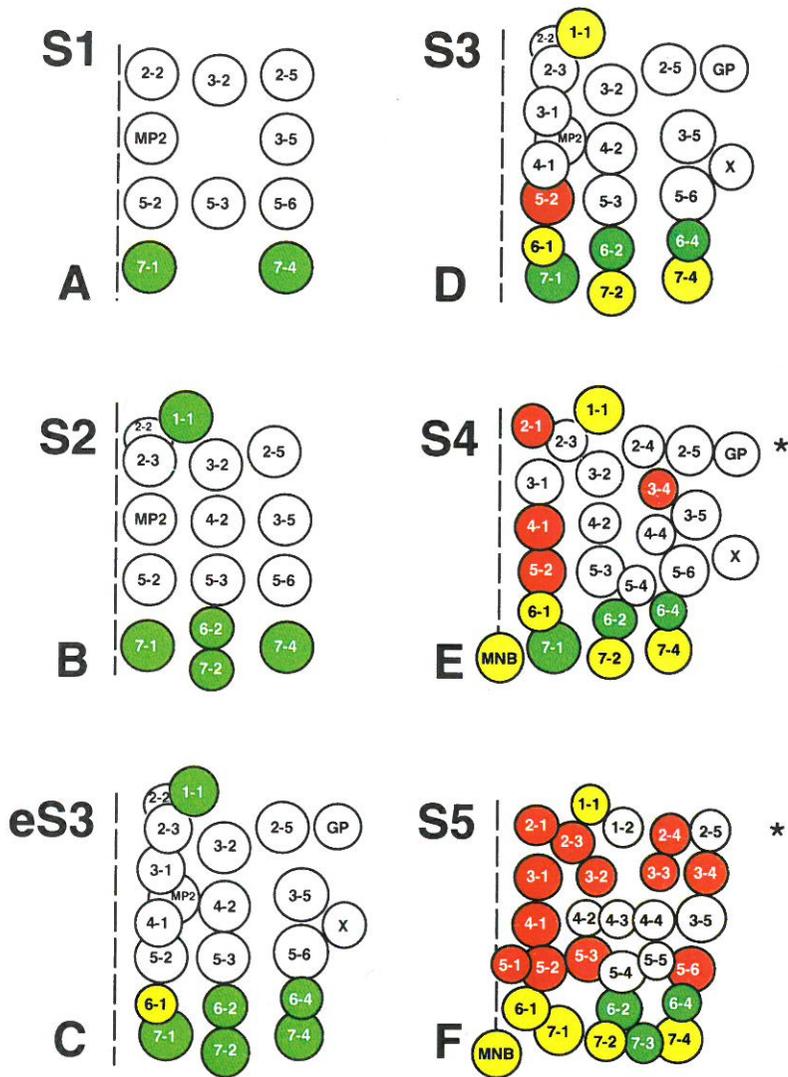


Fig. 3. Timing of *ming* expression in identified NBs. Expression of *en* protein (green) and β gal (*ming* pattern; red) in 1530 embryos. Co-expression is shown in yellow. (A, B) S1 and S2 neural precursors (late stage 8 and early stage 9), respectively. *ming* is not expressed, although several NBs have formed that will subsequently express *ming* (e.g. 1-1 and 7-4). (C) Early S3 neural precursors (early stage 10). *ming* is first expressed in 6-1, which also expresses *en* (see also Fig. 4A). (D) S3 neural precursors (late stage 10). Although no new NBs have formed, *ming* is now expressed in five NBs. Based on data from Hartenstein et al. (1987) and Doe (1992b), several of these NBs are likely to have generated progeny prior to expressing *ming* (e.g. 1-1, 5-2 and 7-4). (E) S4 neural precursors (early stage 11). *ming* is expressed in 9 NBs, including 5 of the 8 NBs that express *en*. (F) S5 neural precursors (late stage 11). *ming* is expressed in 9 more NBs (total of 18), including an S1 NB (3-2) and an S2 NB (2-3) which may have generated up to 5 GMCs prior to expressing *ming* (see text). Ventral view, anterior is up; Dotted line, ventral midline; asterisk, tracheal pit. Nomenclature from Doe (1992b).

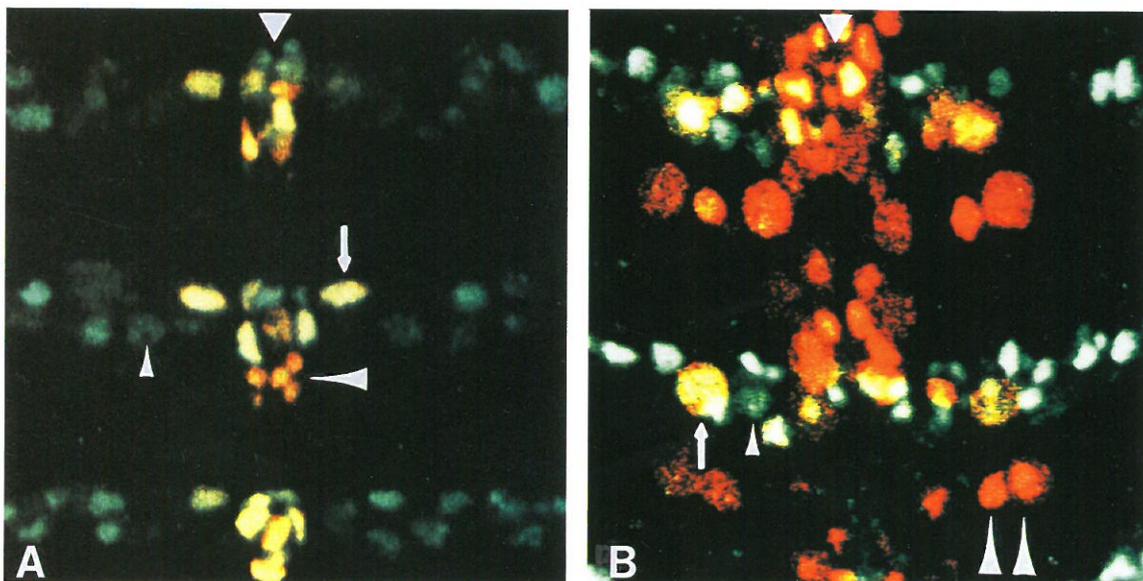


Fig. 4 *ming* and *en* are co-expressed in neural precursors. CNS expression of *ming* (as detected by β gal expression in 1530 embryos; red) and *en* (as detected with an antibody to *en* protein; green) using confocal microscopy. (A) Early stage 10 embryo showing co-expression in NB 6-1 and a subset of midline CNS cells (yellow; arrow). NBs expressing only *en* (green; small arrowhead) and midline cells expressing only *ming* (red; large arrowhead) can also be observed. (B) Late stage 11 embryo showing a subset of the NBs expressing *ming* and *en* (other NBs are out of the focal plane). *ming* and *en* co-expression can be observed in NB 7-4 (yellow; arrow). *en* expression in NB 7-3 (green; small arrowhead) and *ming* expression in NBs 3-3 and 3-4 (red; large arrowheads) can be observed. Ventral view, anterior is up. White triangle indicates the ventral midline.

stein et al., 1987). Based on these data, we predict that many of the S1 NBs (e.g. NBs 3-2, 5-2, 5-3, 7-1 and 7-4) only express ming transcript after generating one or more GMCs (Figs 2B,E, 3).

Generation of mutations at the ming locus

The enhancer trap insertions 1530 and 2092 are located within 200 bp of each other at cytological position 83C on the third chromosome (Fig. 1A). Both insertions are homozygous embryonic lethal and do not complement each other; neither insertion mutation noticeably affects the distribution or abundance of the embryonic ming transcript.

To obtain mutations that abolish expression of the ming transcript, the P elements in the 1530 and 2092 lines were mobilized using the transposase-producing 2-3 chromosome (Robertson et al., 1988). We generated both homozygous viable and homozygous lethal chromosomes lacking the w^+ P element marker; the viable chromosomes show that lethality of the original enhancer trap chromosome is due to the enhancer trap insertion. Southern blots show that three 2092 revertant chromosomes lack some of the genomic DNA flanking the P element insertion (data not shown). These three excision mutations are embryonic lethal, do not complement either insertion mutation and do not express detectable ming transcript by whole-mount hybridization. We refer to these P element excision mutations as *ming*²⁴, *ming*³⁹ and *ming*⁷⁸, and the insertion mutations as *ming*¹⁵³⁰ and *ming*²⁰⁹². The smallest of the deficiencies, *ming*²⁴, removes 850 bp of DNA at the site of the enhancer trap insertion (Fig. 1C). Because *ming*²⁴ abolishes expression of the ming transcript without removing coding sequences for other embryonically expressed genes (Fig. 1), we used embryos homozygous for *ming*²⁴ (called 'ming embryos' below) to characterize the loss-of-function *ming* CNS phenotype.

ming is required for the correct CNS expression of the engrailed gene

The NB expression of *ming* (as shown by gal localization in 1530 embryos) has been compared to seven other genes expressed in subsets of NBs (Doe, 1992b), with the hope of identifying potential 'downstream' targets of the *ming* gene. Substantial overlap is observed between expression of *ming* and *engrailed* (*en*) (Figs 3, 4). At early stage 10, *ming* is expressed in several midline cells and the single NB 6-1; the NB and most of the midline cells also express *en* (Figs 3C, 4A). By stage 11, *ming* and *en* are co-expressed in six NBs and a subset of midline CNS cells (Figs 3F, 4B). There is also overlap between *ming* and *en* in a number of GMCs (data not shown), but we have not yet uniquely identified these cells. Due to extensive co-expression of *ming* and *en* in NBs and GMCs, we looked in *ming* embryos for defects in *en* CNS expression.

Between stage 11 and stage 13, *en* is expressed in many GMCs and neurons derived from the two rows of *en*-expressing (en^+) NBs (Fig. 3). During stage 14 and 15, the *en* protein pattern 'sharpen up' into several discrete clusters of cells, which are shown in Fig. 5 and schematized in Fig. 6. Within each segment of the CNS, *en* protein is detectable in three large ventral midline cells (VUMs; Patel et al., 1989), a cluster of dorsal medial (DM) cells and two

pair of small non-neuronal median support (MS) cells just posterior to the median NB (Fig. 5D,E). In addition, there are four bilateral clusters of en^+ cells in each segment: a posterior intermediate (PI) group of 4-6 cells (Fig. 5C), a posterior lateral (PL) group of 8-10 cells (Fig. 5C) and two pair of neurons located near the ventral surface of the anterior region of the segment, the NH cells (Fig. 5A,B).

In *ming* embryos, epidermal and NB *en* protein patterns are normal (data not shown). Expression of *en* in GMCs is roughly normal but, due to the difficulty of identifying individual GMCs, we may be missing subtle defects in *en* expression in these cells. By stage 16, however, there are clear alterations in *en* CNS expression. *en* protein cannot be detected in the NH or MS cells (Figs 5F,G,I, 6). Because these cells can only be unambiguously detected at stage 16, we do not know when the defects first appear or whether these cells derive from *ming*-expressing precursors. In contrast, the number of en^+ cells in the PL cluster increases from the 8-10 in wild-type embryos to 20-25 in *ming* embryos (Fig. 5C,H). Moreover, in *ming* embryos the size of the PL cells is abnormal, with each cell about half its normal size (Fig. 5H). Three independent *ming* transcript-negative mutants all show the same altered *en* expression pattern.

Interestingly, embryos heterozygous for a *ming* insertion mutation (*ming*²⁰⁹²) and a *ming* transcript-negative mutation (*ming*²⁴ or *ming*³⁹) have a pattern of *en* expression intermediate in severity between homozygous insertion chromosome (identical to wild type) and homozygous null (described above). The intermediate phenotype of the *ming*²⁰⁹²/*ming*²⁴ embryos is a complete loss of en^+ MS cells, a reduction in the number of en^+ NH cells and a slight increase in the number of en^+ PL neurons (data not shown). This shows that the 2092 insertion is a weak *ming* allele, still homozygous lethal despite having no detectable alterations in *ming* transcript pattern or *en* CNS expression.

ming is required for the formation of an identified commissural fascicle

Altered GMC or neuronal cell fate is expected to affect later events during CNS development, including elaboration of the axonal scaffold. In fact, *ming* embryos show subtle, but clear, defects in the pattern of CNS axons. The wild-type CNS has two longitudinal connectives and a segmentally repeated pair of commissures (anterior and posterior), which can be detected with the BP102 monoclonal antibody (Fig. 7A; Klambt et al., 1991). In *ming* embryos, the posterior commissure is thinner than normal, with the average diameter decreased almost to half (Fig. 7B). To define further the posterior commissure defects in *ming* embryos, anti-fasciclin III monoclonal antibody was used to detect a subset of fascicles in the posterior commissure (Patel et al., 1987). The fasciclin III pattern is diagrammed in Fig. 6. In stage-16 wild-type embryos, fasciclin III is expressed in several clusters of cells as well as in three axonal fascicles in the anterior commissure and two fascicles in the posterior commissure (Fig. 7C). In *ming* embryos, fasciclin III expression in the cell bodies and anterior commissure is normal; however, one fasciclin III-positive fascicle is missing from the posterior commissure (Fig. 7D). It is not known whether fasciclin III gene expression is lost in some

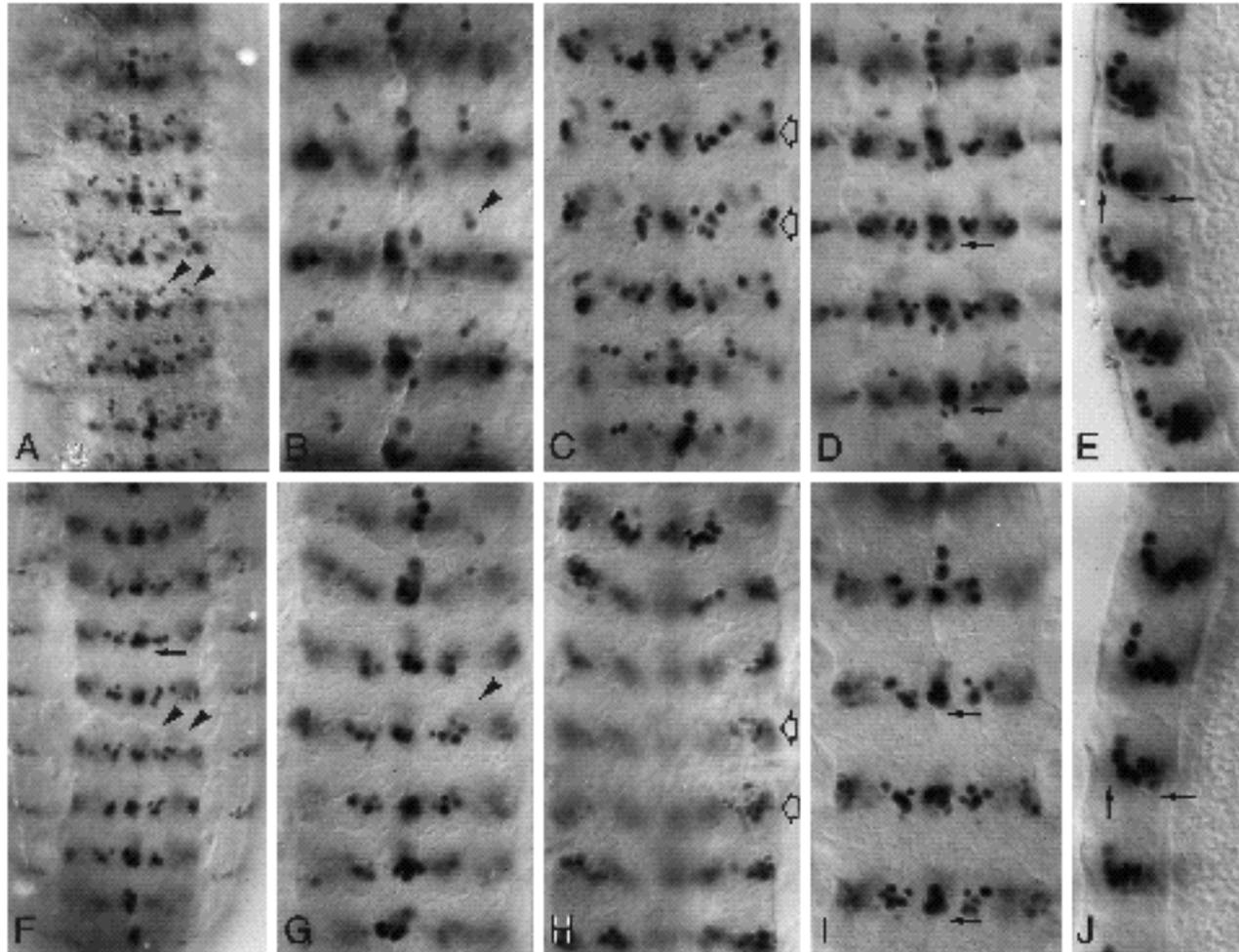


Fig. 5. *ming* is required for correct *engrailed* CNS expression. CNS expression of engrailed protein in stage 15 or 16 wild-type embryos (A-E) and stage 16 homozygous *ming*²⁴ embryos (F-J). (A and F) Overall pattern of the *en* expression. Two pairs of NH neurons (arrowheads) and one pair of MS cells (arrow) can be seen in wild-type embryos, but not in *ming* embryos. (B and G) The en⁺ NH neurons (arrowhead) are detectable in wild-type embryos, but not in *ming* embryos. (C and H) The en⁺ PL group (wide arrowhead) has about twice the number of cells in the *ming* mutants; the size of each PL cell is reduced in *ming* embryos. Focal plane in H is slightly more dorsal than in C. (D and E) Wild-type embryos have two pair of en⁺ MS cells (arrows); (I and J) these cells are not detectable in *ming* embryos. (A-D and F-I) ventral view, anterior up; (E and J) lateral view, anterior is up and ventral surface is to the left.

neurons, whether these neurons express fasciclin III but grow in another fasciclin III-positive axon bundle, or whether the neurons die.

Discussion

Do neuroblasts change fate with every cell cycle?

NBs of the *Drosophila* CNS appear positionally and morphologically unaltered as they divide to produce GMCs, yet the fact that most early-born GMCs in a lineage exhibit a unique fate suggests that NB identity may change with each cell cycle. This hypothesis is supported by our finding that *ming* expression is altered at precise points during the lineage of identified NBs. We have determined the time of birth for the first GMC from NB 1-1 and NB 7-4 (Doe, 1992b). Combining these data with the timing of *ming* expression (Fig. 3), we observe that *ming* transcription is

initiated in NBs 1-1 and 7-4 after GMC-1 is born, but prior to the generation of GMC-2. We predict that *ming* is similarly expressed at specific, reproducible points during the lineage of many different NBs. For example, the early-forming NB 3-2 always expresses *ming* about 2.5 hours after its formation, suggesting that it has divided many times prior to triggering *ming* transcription. Thus *ming* expression can be reproducibly initiated at different points during the lineage of identified NBs. Two conclusions can be drawn from these results. First, NB identity may change during the course of its cell lineage (as reflected by altered *ming* expression). Second, GMCs born at different times during a NB lineage may differentially inherit *ming* gene product.

The change in *ming* expression within NB lineages is extremely reproducible, but the function of differential *ming* expression within a NB lineage is not clear. *ming* is expressed in the latter portion of the NB 7-4 lineage and,

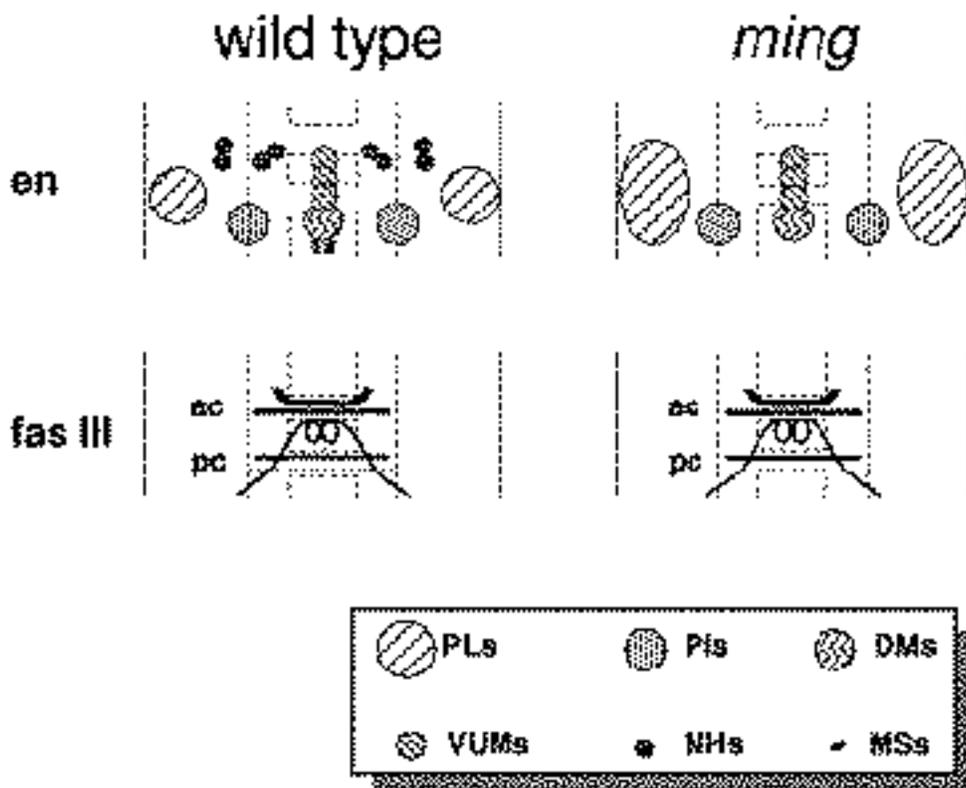


Fig. 6. Summary of engrailed and fasciclin III expression pattern in wild-type and *ming* embryos. Schematic drawing based on camera-lucida tracings of engrailed (*en*) and fasciclin III (*fasIII*)-stained embryos. *ming* embryos lack NH and MS cells, and have twice the normal number of PL cells; there is a loss of one fasciclin III-positive fascicle in the posterior commissure. See text for details. Dorsal view, anterior is up; vertical lines, nerve cord boundaries; dotted lines, outline of longitudinal and commissural axons.

when gene expression is abolished throughout the lineage, there are defects in the *en*-expressing cells that are likely progeny of this NB (they are located dorsal to NB 7-4). Whether 'ectopic' expression of *ming* in the initial portion of the 7-4 lineage (normally *ming*-negative) can generate a phenotype remains to be seen. Nevertheless, it is attractive to think that differential gene expression in a NB lineage might play a role in specifying GMC identity. The mechanism controlling differential gene activity in NB lineages is unknown. Timing of gene expression has been proposed to be regulated by the number of rounds of DNA synthesis (e.g. Mita-Miyazawa et al., 1985), the nuclear:cytoplasmic ratio (e.g. Newport and Kirschner, 1982a,b; Edgar et al., 1986), the stability of regulatory factors (discussed in Nasmyth et al., 1990), or a combination of these mechanisms (e.g. Edgar and McGhee, 1988). The *ming* transcript and 1530 line provide excellent markers to address the question of both initial NB specification as well as how NB identity is modified during its cell lineage.

ming encodes a predicted zinc finger protein

Conceptual translation of the *ming* cDNA reveals a repetitive zinc finger motif which shares homology with the TFIIIA class proteins. The TFIIIA superfamily proteins regulate gene activity primarily by binding target DNA through the zinc finger domains (Berg, 1990). It also has been shown that some of the TFIIIA class proteins can bind both DNA and RNA (Pelham and Brown, 1980), and some bind exclusively to RNA (Joho et al., 1990). In addition to the TFIIIA consensus sequences, there is another domain in each repeat with two cysteines and two histidines, which may represent a novel metal-binding motif. Although the predicted *ming* protein has strong homology to TFIIIA class

zinc finger domains, it is not clear whether *ming* protein would be more likely to bind DNA or RNA. In either case, the predicted protein structure is consistent with a role for the *ming* protein in regulating gene activity.

ming is required for cell specification in the CNS

Loss of *ming* function results in abnormal *en* CNS expression: some neurons and glia lose *en* expression (e.g. the NH and MS cells), whereas elsewhere additional *en*⁺ cells are detected (e.g. PL cells). The exact cell lineage of the *en*⁺ NH, MS and PL neurons is not known. The closest *en*⁺ NB to the PL neurons is NB 7-4, which suggests that it is their likely precursor. NB 7-4 expresses *ming* during the latter portion of its embryonic cell lineage. Loss of *ming* expression could alter the NB 7-4 lineage in a number of ways. It may be that the GMCs or neurons are transformed to a novel cell fate, with a subsequent change in their pattern of division resulting in more PL cells of a smaller size. Alternatively, the later-born GMCs in the 7-4 lineage normally may not express *en*, but are transformed towards an earlier (*en*⁺) GMC fate in *ming* embryos. Another possibility is that the NB 7-4 lineage may be extended in *ming* embryos. Asymmetric NB divisions tend to decrease the volume of the NB (Hartenstein et al., 1987), which may lead to the observed decrease in size of the PL neurons in *ming* embryos. Finally, a combination of these effects may occur, such as the transformation of all GMCs towards the fate of the first (*ming*-negative) GMC, which may lead to the extension of the NB lineage (if the NB continues to divide because it never produces the 'last' GMC in the lineage). Cell lineage studies of NBs in wild-type and *ming* embryos will be required to distinguish among these alternatives.

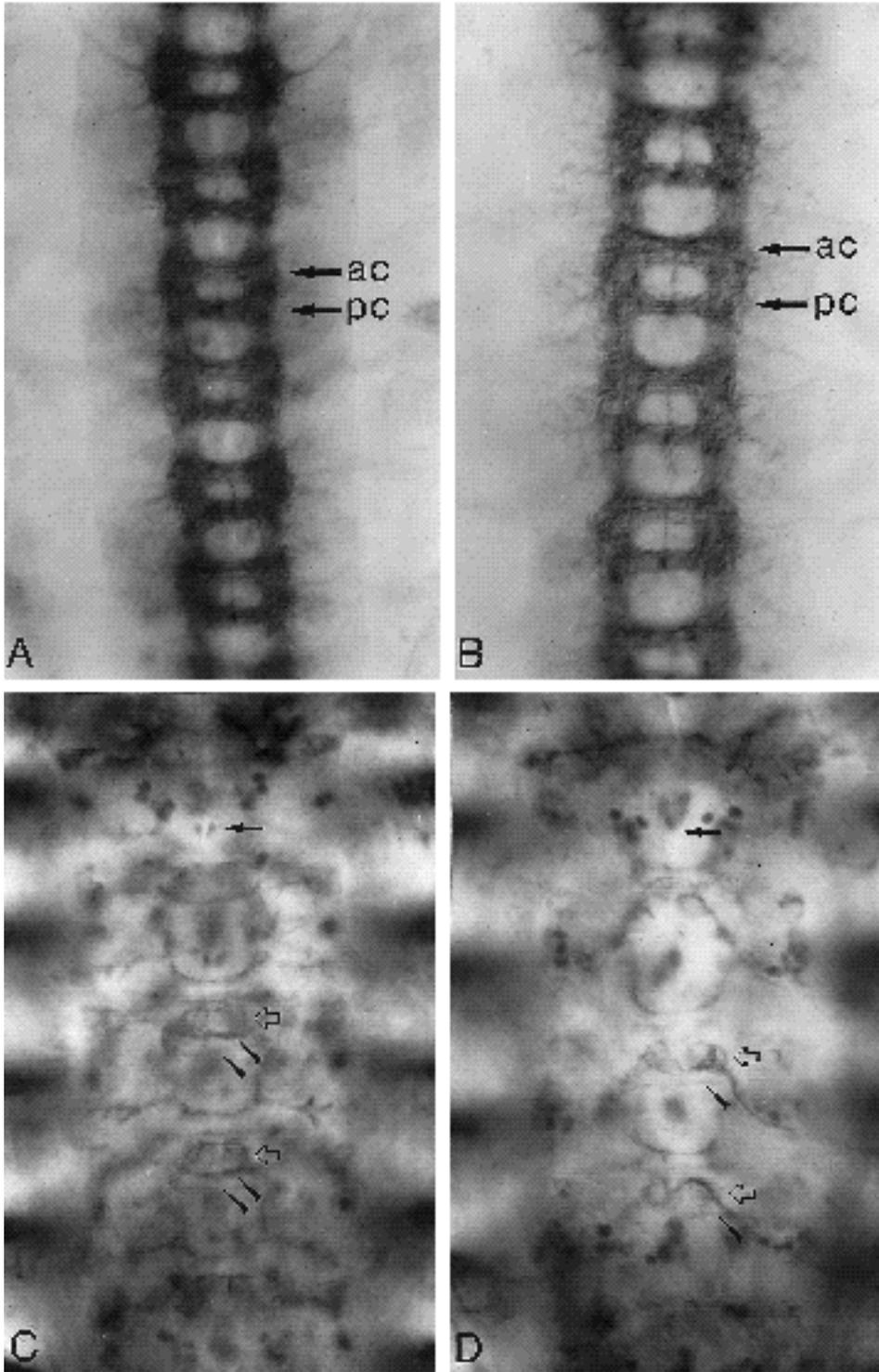


Fig. 7. *ming* is required to establish the normal CNS axonal pattern. (A,B) CNS axons detected with the BP102 antibody in stage 16 embryos. (A) In wild-type embryos, the anterior and posterior commissures (ac, pc) have the same diameter; (B) in *ming* embryos the posterior commissure has a reduced number of axons. (C and D) There are fewer fasciclin III-positive fascicles in *ming* embryos. Embryos were double labeled with anti-engrailed antibody to identify the *ming* embryos. (C) Wild-type embryos (note the *en*⁺ MS cells; thin arrow) have two fasciclin III-positive fascicles in the posterior commissure (arrowheads); the RP1 neurons (wide arrow) are indicated. (D) *ming* embryos (note lack of *en*⁺ MS cells; thin arrow) have only one fasciclin III-positive fascicle in the posterior commissure (arrowhead); the RP1 neurons (wide arrow) are indicated. Ventral view, anterior is up.

Many cells along the posterior midline of the CNS co-express *ming* and *en* (Fig. 3). If the MS cells develop in situ, they almost certainly co-express *ming* and *en*; however, we cannot rule out the possibility that the MS cells migrate to their final position, as has been observed for midline glia and longitudinal glia (Klambt and Goodman, 1991; Jacobs et al., 1989). The NH cells are located near the ven-

tral side of the CNS, anterior to the *en*⁺ NBs and neurons of the same segment. These neurons could develop in situ from *en*-negative NBs or they may be generated by *en*⁺ NBs and migrate anteriorly. Again, cell lineage studies will be required to determine the parental NBs of the NH cells, whether they express *ming*, and the fate of the NH and MS cells in *ming* embryos. Regardless of the mechanism, it is

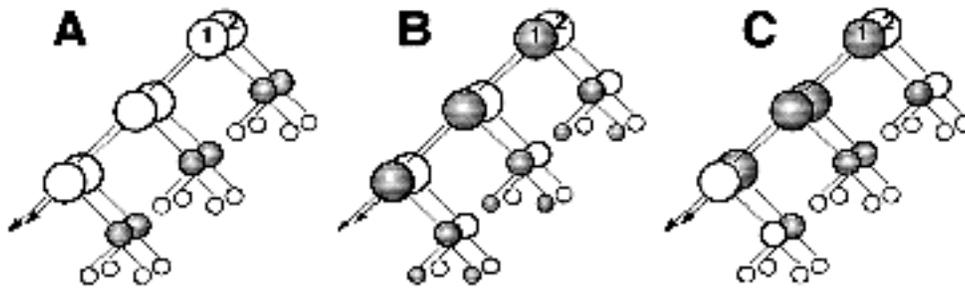


Fig. 8. Differential gene expression during NB cell lineages. Two NB lineages (1, 2) are shown in each panel. (A) Cell type-specific expression could be specifically in NBs, GMCs, or neurons; here prospero protein expression is shown in GMCs but not NBs or neurons. (B) Lineage-specific expression in the entire lineage of a subset of NBs; this type of

expression may control NB-specific aspects of neural development. (C) Sublineage-specific expression in NBs during stereotyped portions of their lineages. *ming* may be reproducibly expressed at different points in the lineages of NBs 1-1, 2-3, 2-4, 3-1, 3-2, 4-1, 5-2, 5-3, 7-1, 7-2 and 7-4. Large circle, NB; medium circle, GMC; small circle, neuron. Shading indicates mRNA or protein expression.

clear that *ming* is required for the normal specification of cell fate in the CNS, as detected by altered *en* neuronal and glial expression.

The *ming* gene is not transcribed in neurons, yet we observe defects in the axonal architecture of the CNS. There are fewer axons in the posterior commissure and an identified fasciclin III-positive fascicle is missing. Due to the early expression of *ming* transcript (in NBs and GMCs, but not neurons) and the nature of the product (predicted zinc finger protein), we feel this phenotype is a secondary effect due to altered NB or GMC fates. A similar phenotype has been observed in *prospero* mutant embryos (Doe et al., 1991). The nuclear prospero protein can only be detected in GMCs and a subset of glia within the CNS (Vaessin et al., 1991; Matsuzaki et al., 1992), where it regulates gene expression in GMCs (Doe et al., 1991; Vaessin et al., 1991). However, *prospero* mutants show dramatic alterations in axon morphology, either due to prior defects in GMC or glial specification (Doe et al., 1991) or general defects in neuronal differentiation (Vaessin et al., 1991).

We have proposed that GMC identity is specified, in part, by the unique combination of gene products inherited from the parental NB (Doe, 1992a). Some genes might be expressed in specific cell types (e.g. the prospero protein is expressed in GMCs only; Fig. 8A). Other genes might be expressed in the entire cell lineage of a subset of NBs (Fig. 8B). Finally, many genes required for generating cell diversity in the CNS are likely to be expressed in 'sublineages' within a NB cell lineage (Fig. 8C). These genes are likely to contribute to the specification of both NB and GMCs during an individual NB cell lineage. The *ming* gene belongs in this class; it is reproducibly expressed in specific sublineages of identified NBs. Based on the *ming* expression pattern (NB sublineages), predicted protein (zinc finger motif) and mutant phenotype (altered CNS gene expression), we suggest that *ming* is a NB identity gene that controls cell fate during NB cell lineages in the embryonic CNS.

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