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Neuronal Cell Fates in the *Drosophila* Central Nervous System

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In the *Drosophila* central nervous system, cellular diversity is generated through the asymmetric partitioning of cell fate determinants at cell division. Neural precursors (or neuroblasts) divide in a stem cell lineage to generate a series of ganglion mother cells, each of which divides once to produce a pair of postmitotic neurons or glial cells. An exception to this rule is the MP2 neuroblast, which divides only once to generate two neurons. We screened for genes expressed in the MP2 neuroblast and its progeny as a means of identifying the factors that specify cell fate in the MP2 lineage. We identified a P-element insertion line that expresses the reporter gene, tau-β-galactosidase, in the MP2 precursor and its progeny, the vMP2 and dMP2 neurons. The transposon disrupts the neurogenic gene, *mastermind*, but does not lead to neural hyperplasia. However, the vMP2 neuron is transformed into its sibling cell, dMP2. By contrast, expression of a dominant activated form of the Notch receptor in the MP2 lineage transforms dMP2 to vMP2. Notch signalling requires Mastermind, suggesting that Mastermind acts downstream of Notch to determine the vMP2 cell fate. We show that Mastermind plays a similar role in the neurons derived from ganglion mother cells 1-1a and 4-2a, where it specifies the pCC and RP2sib fates, respectively. This suggests that Notch signalling through Mastermind plays a wider role in specifying neuronal identity in the *Drosophila* central nervous system.

INTRODUCTION

As the nervous system develops, thousands of neurons are born, each of which assumes a distinct identity. Neuronal identity is specified both by characteristic patterns of gene expression ("intrinsic cues") (Chenn and McConnell, 1995; Doe *et al.*, 1991; Hirata *et al.*, 1995; Knoblich *et al.*, 1995; Rhyu *et al.*, 1994; Spana *et al.*, 1995; Spana and Doe, 1995; Vaessin *et al.*, 1991; Zhong *et al.*, 1996) and by cell–cell interactions ("extrinsic cues") (Frise *et al.*, 1996; Spana and Doe, 1996). One mechanism for generating cell diversity during neurogenesis is to ensure that when a neural precursor divides, each daughter cell assumes a distinct identity. This can be achieved by segregating a cell fate determinant to only one of the two daughter cells at division. The asymmetric partitioning of cell fate determinants is observed in the *Drosophila* and vertebrate nervous systems (Chenn and McConnell, 1995; Hirata *et al.*, 1995; Knoblich *et al.*, 1995; Rhyu *et al.*, 1994; Spana *et al.*, 1995; Spana and Doe, 1995; Zhong *et al.*, 1996). The mechanism for generating diversity is conserved between species, as are several of the cell fate determinants themselves (Zhong *et al.*, 1996).

In the *Drosophila* embryonic CNS, several hundred neurons are produced in each segment from a relatively small number of neuronal precursors (or neuroblasts). Neuroblasts divide in an asymmetric stem cell lineage, budding off a series of smaller ganglion mother cells (GMC), which in turn divide once to produce a pair of postmitotic neurons or glial cells.

The membrane-associated protein, Numb, and the homeodomain protein, Prospero, are localised within the neuroblast in a cell cycle-dependent manner (Hirata *et al.*, 1995; Knoblich *et al.*, 1995; Spana *et al.*, 1995; Spana and Doe, 1995). Numb and Prospero concentrate in a cortical crescent on the basal side of the neuroblast such that, upon division, they are segregated into only one daughter cell, the GMC. Prospero is then released from the cortex and enters the GMC nucleus while Numb remains localised at the membrane (Hirata *et al.*, 1995; Knoblich *et al.*, 1995; Spana and Doe, 1995). Prospero may act in the GMC to repress

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neuroblast genes and activate transcription of GMC-specific genes (Doe *et al.*, 1991; Vaessin *et al.*, 1991), thereby distinguishing the GMC from its parent.

One exception to this rule is the MP2 neuroblast, which divides only once to give two distinct neurons, vMP2 and dMP2 (Spana *et al.*, 1995). The MP2 division is similar to other neuroblast divisions in that Numb is asymmetrically localised and segregates only to the dMP2 cell. However, unlike other neuroblasts, Prospero is nuclear in the MP2 precursor and is distributed equally to both daughter cells at division (Spana *et al.*, 1995).

Notch is expressed by both MP2 progeny and acts to promote the vMP2 cell fate (Spana and Doe, 1996). Notch signalling is blocked by Numb, which segregates exclusively to dMP2 when the MP2 precursor divides. Numb interacts directly with the intracellular domain of Notch (Frise *et al.*, 1996). By antagonising Notch, Numb promotes the dMP2 cell fate (Spana and Doe, 1996). Mammalian homologues of Numb and Notch also interact directly, suggesting that this regulatory mechanism has been conserved through evolution (Zhong *et al.*, 1996).

Here we identify the neurogenic gene Mastermind as a member of the Notch signal transduction pathway that leads to the vMP2 cell fate. Mutations in *mastermind* transform the vMP2 neuron into dMP2. Our results show that Notch signals through Mastermind to direct neuronal fates not only in the MP2 lineage, but also during GMC divisions, where Mastermind specifies the identities of the pCC and RP2sib neurons.

MATERIALS AND METHODS

Drosophila Stocks

Flies were raised on standard *Drosophila* media at 25°C. Embryonic stages are as previously described (Campos-Ortega and Hartenstein, 1985). P[tau-lacZ]$^{3.177}$ (a kind gift from C. Callahan and J. Thomas) was one of a collection of tau-lacZ enhancer trap lines generated by Callahan and Thomas (1994). The transposon was balanced over *CyO, wg*^{en11} (Perrimon *et al.*, 1991). Wildtype embryos were distinguished by their expression of β-galactosidase in the *wingless* pattern.

Deficiencies Cx1 and Mk1 were a kind gift from N. Brown. *mam*^{1l113} and UAS-Notch^{intra} were kind gifts from S. Bray. Expression of UAS-Notch^{intra} was driven by ftzN GAL4 (Lin *et al.*, 1995); (a gift from D. Van Vactor). ftzN GAL4 expresses GAL4, from the *ftz* neurogenic enhancer, in the MP2 precursor from stage 11 and, after cell division, in both MP2 neurons. The epistatic relationship between Notch and Mastermind was determined using stocks: *P[tau-lacZ*$^{3.177}$*], UAS-N*^{intra}*/CyO, wg*^{en11}, and *P[tau-lacZ*$^{3.177}$*]/CyO, ftz-lacZ; ftzN GAL4/ftzN GAL4.*

Immunohistochemistry

Antibody staining was performed as previously described (Patel, 1994). Primary antibodies were diluted as follows: rabbit anti-β-galactosidase 1:1000 (Cappel), mouse mAb1D4 anti- Fasciclin II at 1:5 (Van Vactor *et al.*, 1993) (kindly provided by C. Goodman), rabbit anti-Oddskipped at 1:1000 (a kind gift from Ellen Ward), rabbit anti-Evenskipped at 1:500 (a kind gift from M. Frasch), and mouse mAb22C10 (Fujita *et al.*, 1982). Secondary antibodies, directly conjugated to horseradish peroxidase (Jackson Labs), were used at a dilution of 1:300. HRP was detected using diaminobenzidine as a substrate (0.3 mg/ml in PBS, 0.1% Triton X-100). Where necessary, the precipitate was intensified with NiCl (final concentration 0.06%). Embryos were cleared in 50% glycerol, mounted in 70% glycerol, and dissected with tungsten needles. Transmitted light images were generated on a Zeiss Axiophot microscope with DIC optics. Z-series projections were carried out in Adobe Photoshop 4.0.

For fluorescence microscopy, secondary antibodies conjugated to Texas red, FITC, or Cy5 (Jackson Labs) were used at a 1:200 dilution. Embryos were mounted in Vectashield (Vector Labs) and visualised on a Bio-Rad MRC1024 confocal microscope. Z-series projections of 1 to 20 1-μm sections are presented. Images were imported into Adobe Photoshop 4.0, assembled in Adobe Illustrator 6.0, and printed on a Tektronix Phaser 440 printer.

RESULTS

P[tau-lacZ]$^{3.177}$ Is Expressed in the MP2 Neurons

To identify genes that play a role in distinguishing the fates of the MP2 neurons, we screened a collection of enhancer trap lines that express the reporter protein, tau-β-galactosidase (Callahan and Thomas, 1994). Tau-β-galactosidase labels neuronal cell bodies and is transported along microtubules into axons, such that the MP2 neurons can easily be identified by their shape, cell body position, and characteristic axon projections. The tau-lacZ insertion line, P[tau-lacZ]$^{3.177}$, drives expression in vMP2 and dMP2 from stage 11 (Fig. 1a). By stage 13, tau-β-galactosidase expression is seen also in MP1 (data not shown). To confirm the identity of the tau-β-galactosidase-expressing cells, embryos were double labelled with antibodies against Fasciclin II, which labels the cell bodies and axons of the MP1 and MP2 neurons (Van Vactor *et al.*, 1993). Consistent with the axon projections of the MP2 and MP1 neurons, which project into the most medial and the intermediate longitudinal axon fascicles, respectively (Hidalgo and Brand, 1997), tau-β-galactosidase expression is seen in the longitudinal axon tracts at stage 16 (Fig. 1b).

vMP2 Is Transformed to dMP2 in Homozygous P[tau-lacZ]$^{3.177}$ Embryos

In embryos that are homozygous for the P[tau-lacZ]$^{3.177}$ insertion, the vMP2 neuron is present but no longer projects an axon anteriorly (Fig. 2b). Instead, the vMP2 axon projects posteriorly along the pathway taken by dMP2. To determine whether this phenotype results from axon misrouting, or is due to a cell fate transformation, we assayed expression of the gene *oddskipped*. *oddskipped* is expressed by the MP2 precursor prior to cell division, and by the MP1 neurons. After the MP2 precursor divides, *oddskipped* expression is maintained in dMP2, but is quickly repressed in vMP2. In a wildtype embryo at stage 14, four cells per segment, two MP1 and two dMP2 neurons, express *odd-*

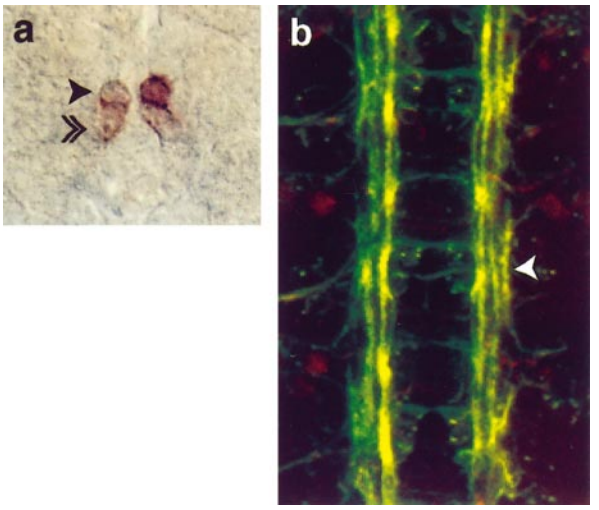

FIG. 1. P[tau-lacZ$^{3.177}$] is expressed in the MP2 cell lineage. P[tau-lacZ$^{3.177}$] drives expression of tau-β-galactosidase in two cells adjacent to the midline in each hemisegment (a; stage 11). The position of the cell bodies and their shape and size are characteristic of the vMP2 (arrowhead) and dMP2 (double arrowhead) neurons. Later in development (b; stage 16), consistent with the axonal projections of the MP2 neurons in the medial and intermediate longitudinal fascicles, tau-β-galactosidase labels the longitudinal fascicles. At this stage, other neurons also express tau-β-galactosidase, as the outermost fascicle is also labelled (arrowhead). Colocalisation of Fasciclin II (green) and tau-β-galactosidase (red) in the longitudinal tracts labels axons in yellow.

skipped. (Fig. 2c) (Spana *et al.*, 1995). In contrast, P[tau-lacZ]$^{3.177}$ mutant embryos express *oddskipped* in five to six cells per segment (Fig. 2d). The extra *oddskipped*-expressing cells are found at the position of the vMP2 cell bodies, suggesting that *oddskipped* expression is maintained in the vMP2 cell. The vMP2 neuron exhibits the same *oddskipped* expression profile and axon trajectory as its sibling, dMP2, suggesting that vMP2 has been transformed to dMP2. P[tau-lacZ]$^{3.177}$ appears, therefore, to have inserted in a gene that is required to specify the vMP2 cell fate.

P[tau-lacZ]$^{3.177}$ Is Inserted at the Mastermind Locus

P[tau-lacZ]$^{3.177}$ was mapped to position 50 C-D on chromosome II. We carried out complementation tests with two deficiencies in the region, Cx1 and Mk1. P[tau-lacZ]$^{3.177}$ does not complement Cx1, and only partially complements Mk1, suggesting that the insert maps within the Cx1 deficiency, as does the neurogenic gene, *mastermind* (Lehmann *et al.*, 1983). We tested whether P[tau-lacZ]$^{3.177}$ is inserted at the *mastermind* locus. A strong allele of *mastermind* (*mam^{II13}*) is unable to complement lethality of P[tau-lacZ]$^{3.177}$, suggesting that the transposon has inserted within the *mastermind* gene.

mam$^{3.177}$ Specifies MP2 Cell Fate

The neurogenic genes, which include *Notch* and *mastermind*, act early in neural development to regulate the number of neural precursors (Campos-Ortega and Harten-

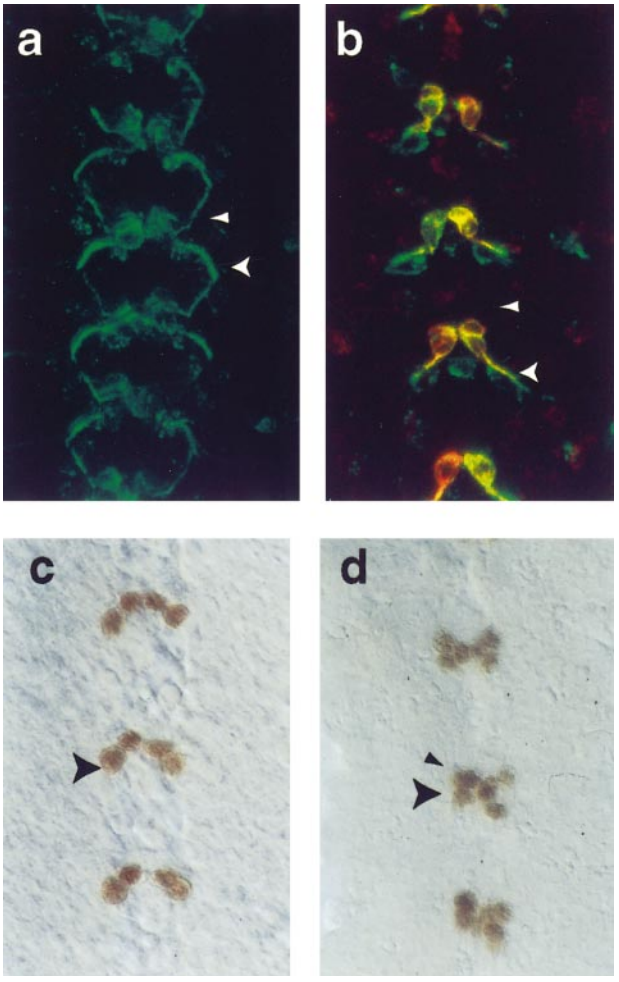

FIG. 2. vMP2 is transformed into dMP2 in embryos homozygous for P[tau-lacZ$^{3.177}$]. In wildtype embryos at stage 12 (a), vMP2 projects an axon anteriorly (small triangular arrowhead) and dMP2 projects an axon posteriorly (larger arrowhead), as seen by labelling with mAb22C10 (green). In embryos homozygous for the P[tau-lacZ$^{3.177}$] insertion (b), vMP2 projects its axon posteriorly along the pathway followed by dMP2 (arrowhead). No anterior axon projection is seen (small triangular arrowhead). tau-β-galactosidase (red) colocalises with 22C10 (green) both in the MP2 cell bodies and in the posteriorly projecting axons (yellow). At stage 14, in wildtype embryos (c), four cells per segment express Oddskipped; two MP1 neurons and two dMP2 neurons (arrowhead). In embryos homozygous for P[tau-lacZ$^{3.177}$], 5–6 cells per segment express Oddskipped (d). The position of the extra Oddskipped-expressing cells corresponds with vMP2 (small arowhead), suggesting that vMP2 is transformed to dMP2 (large arrowhead) in the P[tau-lacZ$^{3.177}$] mutant background.

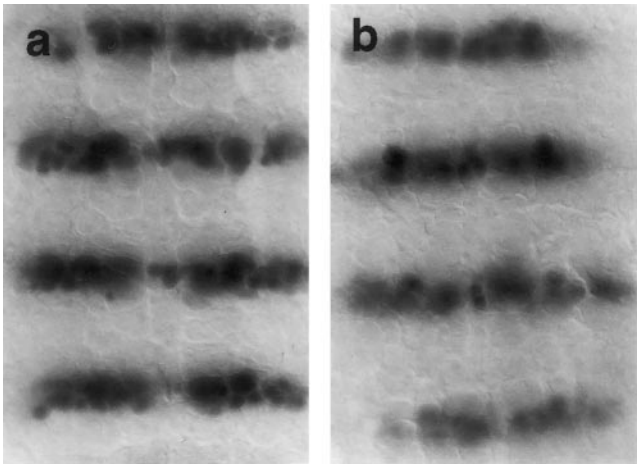

FIG. 3. *mam*$^{3.177}$ does not exhibit neural hyperplasia. At stage 9, gsb-d is expressed in a two rows of neuroblasts in each segment (a). Expression of gsb-d is normal in *mam*$^{3.177}$ embryos (b), suggesting that the number of neuroblasts is similar to that found in wildtype embryos.

stein, 1993). Neural precursors originate from small clusters of ectodermal cells, defined by expression of the proneural genes, within which only one cell will become a neuroblast. Once selected, the neuroblast prevents neighbouring cells from adopting a neural fate through a process called "lateral inhibition," which is mediated by the ligand, Delta, and the receptor, Notch. The remaining ectodermal cells within each proneural cluster then assume an epidermal cell fate.

Mastermind is a nuclear protein that acts in the Notch signal transduction pathway during lateral inhibition (de la Concha *et al.*, 1988; Lehmann *et al.*, 1983; Schmid *et al.*, 1996; Smoller *et al.*, 1990; Yedvobnick *et al.*, 1988). *Notch* and *mastermind* mutants are both characterised by an excess number of neuroblasts, which form at the expense of epidermal cells. Notch is also required later in development to specify the vMP2 cell fate (Spana and Doe, 1996). A role for *mastermind* in nervous system development after lateral inhibition has not been demonstrated, although *mastermind* is expressed in the MP2 neurons (Bettler, 1996). *mam*$^{3.177}$ recapitulates a subset of *mastermind* expression. It is expressed at high levels in the MP2s (Fig. 1) and other neural cells (data not shown). Other low levels of *mastermind* may be due to maternal expression.

If *mastermind* only acts to limit the number of neuroblasts, then in *mam*$^{3.177}$ mutant embryos, the extra *oddskipped*-expressing cells might be the result of neural hyperplasia, rather than cell fate transformation. To assess whether the number of neural precursors is normal in *mam*$^{3.177}$, or whether *mam*$^{3.177}$ shows a neurogenic phenotype, we stained embryos for a number of neuroblast markers, including *gsb-d* (Fig. 3) and *oddskipped* (data not shown). The expression of *gsb-d* is unaltered, suggesting that the number of neuroblasts is similar to wildtype (Fig. 3b). This is supported by the fact that we see only one or two extra *oddskipped*-expressing cells in *mam*$^{3.177}$, as one would expect if vMP2 adopted a dMP2 cell fate. In neurogenic mutants, extra neuroblasts are formed at the expense of epidermal precursor cells, and there is a concomitant loss of the ventral cuticle. Whereas strong *mastermind* alleles show a loss of ventral cuticle (Yedvobnick *et al.*, 1988), *mam*$^{3.177}$ does not, suggesting that the normal complement of epidermal cells is present (data not shown). Therefore, the extra cells that express *oddskipped* in *mam*$^{3.177}$ result from a cell fate transformation, rather than neural hyperplasia.

Mastermind Acts Downstream of Notch during Cell Fate Determination

Our results suggest that *mastermind* acts in the Notch signalling pathway not only during lateral inhibition but also during MP2 cell fate determination. Mastermind has been proposed to act upstream of Notch (Lieber *et al.*, 1993). It has also been shown to enhance the Su(H) phenotype, which is downstream of Notch (Fortini, 1994). The epistatic relationship between Notch and *mastermind* may not be linear, or signalling may occur through different tissue- or stage-specific effector molecules. For example, during the asymmetric divisions of sensory organ precursor cells (SOPs), only a subset of the cell fate decisions mediated by Notch require Su(H) (Wang, 1997), and Notch signals independently of Su(H) in the mesectoderm (Lecourtois, 1995).

We tested the epistatic relationship between Notch and Mastermind during MP2 cell fate determination. Using the GAL4 system to target transcription (Brand *et al.*, 1994; Brand and Perrimon, 1993), we expressed a dominant activated form of the Notch receptor, N^{intra}, in the MP2 precursor prior to division, and subsequently in both MP2 daughter cells. N^{intra} is a truncated form of the receptor, consisting of only the intracellular domain (Lieber *et al.*, 1993; Rebay *et al.*, 1993; Struhl *et al.*, 1993); S. Bray and J. de Celis, unpublished). Expression of N^{intra} in the MP2 lineage represses *oddskipped* in dMP2 (Fig. 4d). Furthermore, dMP2 now projects its axon along the pathway of vMP2, in the anterior direction (Fig. 4b). In wildtype embryos, Numb is segregated to dMP2 and antagonises the Notch pathway, thereby promoting the dMP2 cell fate (Spana *et al.*, 1995). Expression of N^{intra} at high levels is able to override inhibition by Numb, and two vMP2s are formed at the expense of dMP2.

If Mastermind acts downstream of Notch during MP2 cell fate determination, then N^{intra} should be unable to rescue the vMP2 to dMP2 transformation in *mam*$^{3.177}$. When N^{intra} is expressed in *mam*$^{3.177}$, the vMP2 neurons are still transformed into dMP2s (Figs. 5 and 6). Therefore, Mastermind acts downstream of Notch to determine neuronal cell fate in the MP2 cell lineage.

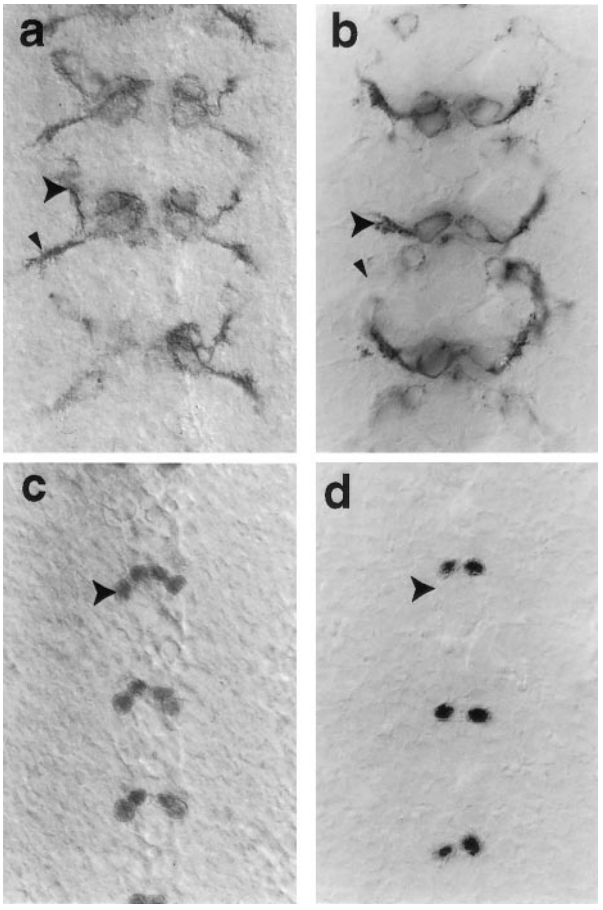

FIG. 4. Activated Notch transforms dMP2 into vMP2. A dominant activated form of the Notch receptor (N^{intra}) was expressed in the MP2 cell lineage. Expression of *UAS-N^{intra}* was driven by *ftzN GAL4*, which activates transcription in the MP2 precursor prior to cell division, and subsequently in both MP2 daughter cells. In wildtype embryos at stage 12 (a; labelled with 22C10), vMP2 projects its axon anteriorly (large arrowhead) and dMP2 projects posteriorly (small arrowhead). In embryos expressing N^{intra} in the MP2 lineage (b), the posteriorly projecting dMP2 axon is lost (small arrowhead), and only anteriorly projecting axons are seen (large arrowhead). In wildtype embryos at stage 14 (c), Oddskipped is expressed in four cells per segment: two medial MP1 neurons and two lateral dMP2 neurons (arrowhead). Oddskipped is repressed in the vMP2 neurons at this stage. In embryos expressing N^{intra} in the MP2 lineage (d), Oddskipped is repressed in the dMP2 neurons, leaving only two Oddskipped-expressing cells, the MP1 neurons, per segment.

Mastermind Specifies the RP2sib and pCC Neuronal Cell Fates

Notch and Mastermind may act solely in the MP2 lineage, or they may direct other cell fate decisions in the CNS. We tested whether the loss of Mastermind affects the identities of other neurons in the CNS. The RP2 and RP2sib neurons are the progeny of the first ganglion mother cell generated by NB 4-2 (Doe *et al.*, 1988). At first both RP2 and RP2sib express *evenskipped* (*eve*), but *eve* is rapidly repressed in RP2sib (Fig. 7a). In *mam$^{3.177}$* embryos, both RP2 and RP2sib continue to express *eve* (Fig. 7b), suggesting that two RP2 neurons are formed at the expense of RP2sib.

Next we examined the NB1-1 lineage, where the the first GMC gives rise to the aCC neuron and its sibling, pCC (Doe *et al.*, 1988). aCC is the motorneuron that pioneers the intersegmental nerve; pCC is an interneuron that pioneers the most medial longitudinal axon fascicle (Jacobs and Goodman, 1989) (Fig. 7c). Both neurons express *eve*. In *mam$^{3.177}$* embryos, two *eve*-expressing cells are found at the position of aCC and pCC, but both neurons project their axons into the intersegmental nerve (Fig. 7d). Therefore, in the absence of Mastermind, pCC adopts the fate of its sibling, aCC. Furthermore, the segmental nerve is missing in *mam$^{3.177}$* embryos, suggesting that the motorneurons that extend along this pathway may also adopt alternative fates (Fig. 7d).

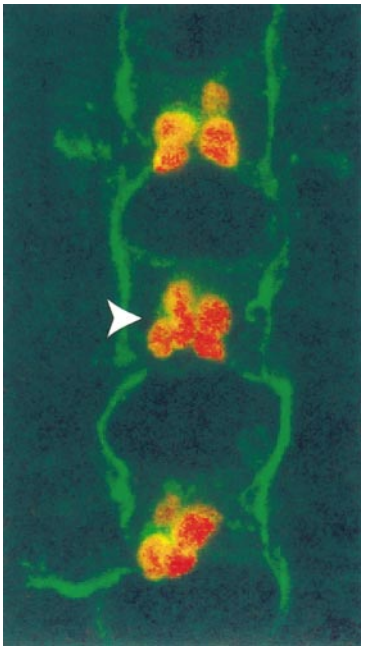

FIG. 5. Mastermind acts downstream of Notch during MP2 cell fate determination. In *mam$^{3.177}$* embryos, vMP2 is transformed to dMP2 and both express Oddskipped. As a result, 5–6 cells (four dMP2 neurons, two MP1 neurons) per segment express Oddskipped (Fig. 2d). Conversely, in embryos expressing a dominant activated form of the Notch receptor, dMP2 is transformed to vMP2., and only two cells (two MP1 neurons) per segment express Oddskipped (Fig. 4d). If Mastermind acts downstream of Notch, then activated Notch will be unable to rescue the mutant *mam$^{3.177}$* phenotype: four dMP2 neurons will form at the expense of vMP2. Expression of N^{intra} in the MP2 lineage of *mam$^{3.177}$* embryos does not suppress the *mam$^{3.177}$* phenotype (a): 5–6 cells (four dMP2 neurons, two MP1 neurons) continue to express Oddskipped (in red; arrowhead).

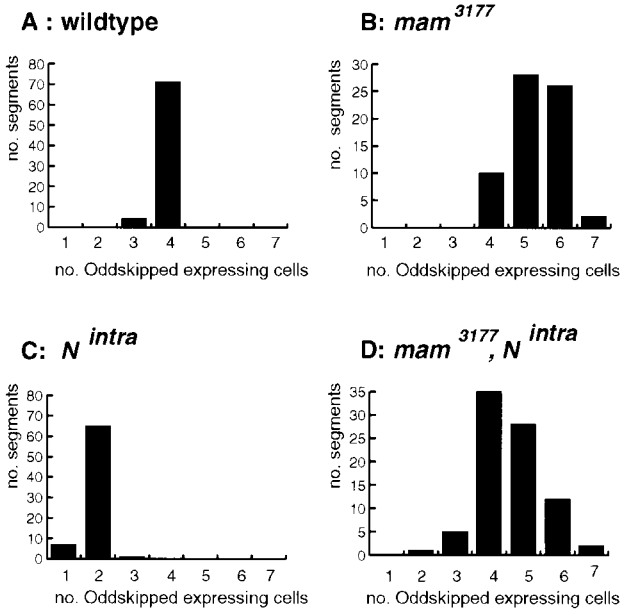

FIG. 6. *mam*$^{3.177}$ is epistatic to Notch^{intra}. In wildtype embryos (A), four cells per segment express Oddskipped, two dMP2 neurons and two MP1 neurons. In *mam*$^{3.177}$ embryos (B), vMP2 assumes the dMP2 fate and, as a result, 5–6 cells per segment express Oddskipped. Constitutive activation of the Notch signalling pathway (C) causes the opposite transformation, dMP2 to vMP2, and only two cells per segment express Oddskipped. When N^{intra} is expressed in *mam*$^{3.177}$ embryos (D), 4–6 cells continue to express Oddskipped.

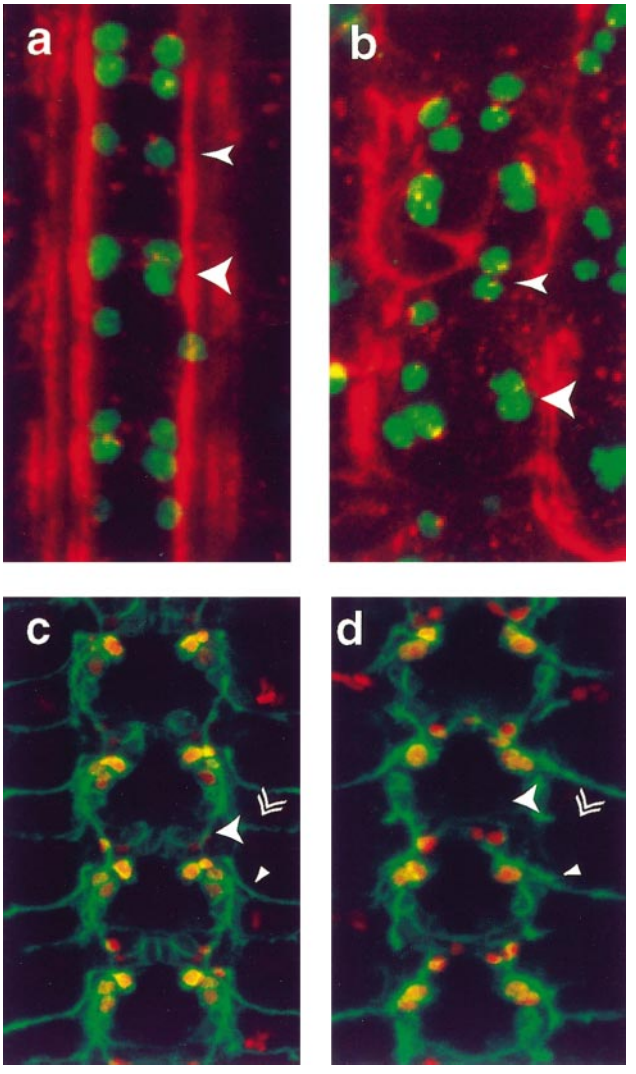

FIG. 7. Mastermind directs cell fates in other neuroblast lineages. By stage 16 in wildtype embryos (a), Evenskipped (green) is expressed in aCC, pCC (large arrowhead), and RP2 (small arrowhead). In *mam*$^{3.177}$ embryos (b) two cells in the RP2 cluster express Evenskipped (small arrowhead), suggesting that RP2sib has taken on the identity of its sister, RP2. Both aCC and pCC still express Evenskipped (large arrowhead).Longitudinal axon tracts are labelled with fasII (red)(a, b). In a wildtype embryo at late stage 12 (c), the pCC interneuron, labelled with FasII (green), projects its axon anteriorly in the longitudinal axon tract (large arrowhead). The axon of the aCC motorneuron, labelled with FasII (green) projects ipsilaterally along the intersegmental nerve (small arrowhead). aCC and pCC are marked by Evenskipped expression (red)(c, d). In *mam*$^{3.177}$ embryos (d), the anteriorly projecting axon of pCC is missing (large arrowhead) and both aCC and pCC project their axons in the intersegmental nerve (small arrowhead). The segmental nerve is missing in *mam*$^{3.177}$ embryos (double arrowhead), indicating that Mastermind specifies the neuronal identity of one or more of the motorneurons that normally extend along this axon pathway.

DISCUSSION

Distinct Roles for the Neurogenic Genes at Different Stages of Nervous System Development

Notch is well known for its role in lateral inhibition where, in conjunction with Delta, it mediates the cell–cell interactions that ensure that only one cell in each proneural cluster becomes a neuroblast. *Notch* mutants are characterised by neural hyperplasia, as cells normally destined to become epidermal assume a neural fate. More recently, Notch has been shown to play a role later in nervous system development, in specifying cell fates (Frise *et al.*, 1996; Guo *et al.*, 1996; Spana and Doe, 1996). In the MP2 cell lineage, Notch signalling promotes the vMP2 neuronal fate. Numb, which segregates to only one of the two daughter cells when the MP2 neuroblast divides, blocks Notch signal transduction and promotes the dMP2 fate (Spana and Doe, 1996). In the absence of both Numb and Notch, two dMP2 neurons are formed, suggesting that Numb is not required to specify the dMP2 cell fate, but merely to inhibit Notch function.

Several other neurogenic genes act in the Notch signalling pathway during lateral inhibition (Artavanis-Tsakonas, 1995). Some of these may also play a role in specifying neuronal identity. Alternatively, Notch signalling may

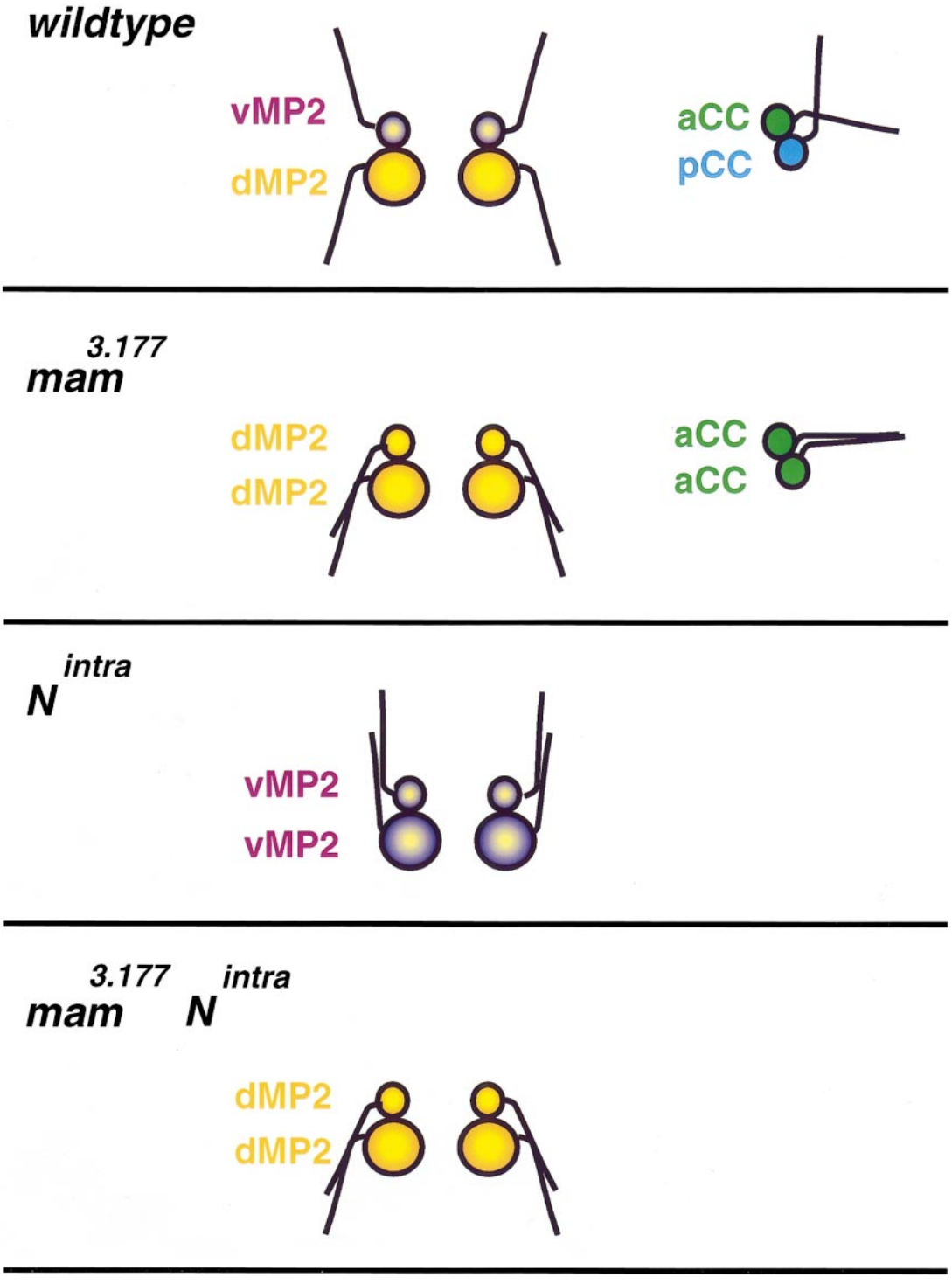

FIG. 8. Mastermind specifies neuronal cell fates in the CNS. Mastermind acts downstream of Notch to promote the vMP2 neuronal cell fate. In wildtype embryos, vMP2 (purple) projects an axon anteriorly, while its sister, dMP2 (yellow), projects an axon posteriorly. In the absence of Notch signalling, in $mam^{3.177}$ embryos, vMP2 is transformed into dMP2 (yellow) and both neurons project axons posteriorly. In contrast, constitutive activation of the Notch signalling pathway (N^{intra}) converts dMP2 into vMP2 (purple) and both neurons project axons anteriorly. Notch signalling requires Mastermind. Constitutive activation of Notch signalling has little effect in $mam^{3.177}$ embryos ($mam^{3.177}$, N^{intra}); vMP2 is still transformed to dMP2 (yellow). Mastermind is required in the lineage of NB 1-1 to promote the pCC neuronal cell fate (blue). In $mam^{3.177}$ embryos, pCC takes on the identity of its sister, aCC (green), and projects its axon along the intersegmental nerve.

utilise downstream effectors that are specific to each tissue or developmental stage. Here we show that at least one of the neurogenic genes, *mastermind,* functions in neuronal cell fate specification.

It is often difficult to assess the later roles of a gene that performs several different functions in development. If the requirement for gene activity early in development can be rescued, for example, by the use of conditional mutant alleles, it then becomes possible to study later functions. We identified $mam^{3.177}$ in a screen for genes expressed in the MP2 neurons. This mutation does not lead to neural hyperplasia, but uncovers a role for Mastermind later in nervous system development, in specifying the vMP2 cell fate. *mastermind* is expressed maternally, and this early expression may rescue the neurogenic phenotype. $mam^{3.177}$ appears to be a hypomorphic allele and the function of Mastermind during neurogenesis may be less sensitive to reduced Mastermind activity. Consistent with this, we still see expression of Mastermind in $mam^{3.177}$ (data not shown).

Mastermind Acts Downstream of Notch during MP2 Cell Fate Determination

Expression of a constitutively active Notch receptor transforms dMP2 into vMP2. In these experiments, endogenous Numb is unable to inhibit activated Notch, which is expressed at high levels by GAL4-mediated transcription. This is consistent with the work of Frise *et al.* (Frise *et al.,* 1996), who suggest that the relative levels of Numb and Notch are important in regulating signal transduction. When activated Notch is expressed in $mam^{3.177}$embryos, however, two dMP2 neurons are produced, demonstrating that Notch signalling requires Mastermind. High levels of activated Notch can partially suppress the $mam^{3.177}$phenotype (Fig. 6), confirming that $mam^{3.177}$ retains some Mastermind activity.

Notch signalling may regulate transcription of *mastermind*. Previously it has been shown that mastermind shows phenotypic interactions with Su(H) (Fortini, 1994). It is unclear whether this also holds true during neuronal cell fate specification. For example, it has recently been shown that Notch and Numb act in a Su(H)-independent manner during some asymmetric cell divisions (Wang, 1997). We see expression of P[tau-lacZ]$^{3.177}$ in both vMP2 and dMP2 (Fig. 1a), suggesting that regulation of Mastermind activity occurs posttranscriptionally. If Notch were required for *mastermind* transcription, we would expect to see Mastermind expression only where Notch is active, i.e., in vMP2 but not dMP2.

Mastermind is a nuclear factor (Smoller *et al.,* 1990) and might regulate the genes that specify vMP2 identity. It has been shown to associate with approximately 100 different sites on polytene chromosomes (Bettler, 1996), some of which may correspond to neuronal identity genes. Several genomic sites overlap those recognised by Groucho, a transcriptional repressor, and it has been suggested that Mastermind may act as a corepressor when bound to promoters in conjunction with Groucho (Bettler, 1996).

Mastermind Directs Cell Fate in Other Neuroblast Lineages

Mastermind specifies neuronal identity in neuroblast cell lineages other than MP2. We have shown that at least two other sibling cell fate decisions are altered in $mam^{3.177}$. In the NB4-2 lineage, RP2sib is transformed into its sister, RP2, while in the NB1-1 lineage, pCC is transformed into aCC. Notch signalling appears, therefore, to be required during GMC divisions. Does Numb also play a role during GMC divisions, by segregating to one of the two neuronal progeny and inhibiting Notch signal transduction? Previous reports suggest that *numb* mutants show few defects in the CNS (Rhyu *et al.,* 1994; Spana *et al.,* 1995). For example, RP2 and RP2sib appear to assume their correct identities, as there is no change in *evenskipped* expression in the RP2 cluster. The number of *eve*-expressing cells in the EL neuron cluster is reduced, however, indicating a role for Numb in the CNS. It is possible that maternally expressed Numb rescues the CNS phenotype, or that the *numb* alleles used in these experiments retain some activity. Alternatively, other factors may selectively repress Notch signal transduction during GMC divisions.

In summary, we show that Mastermind acts in the MP2 cell lineage to promote the vMP2 cell fate (Fig. 8). A constitutively activated form of the Notch receptor is unable to rescue the $mam^{3.177}$ phenotype, placing Mastermind downstream of Notch in the signalling pathway that specifies the vMP2 fate. Mastermind also directs the fates of the RP2sib and pCC neurons, suggesting that Notch signalling through Mastermind plays a wider role in specifying neuronal identity in the *Drosophila* central nervous system.

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