INTRODUCTION

How cell diversity is generated is a central question in developmental biology. While a great deal has been learned recently about how extrinsic cues between cells are used to control cell determination, it is not clear to what extent intrinsic cues regulate cell fates between two cells (Greenwald and Rubin, 1992; Posakony, 1994; Jan and Jan, 1995). Perhaps the best examples of intrinsic cues are the regionally localized gene products in the early Drosophila embryo: pole plasm triggers germ cell fate (St Johnston, 1993), bicoid activates anterior embryonic cell fates (Driever, 1993), and nanos specifies posterior cell identity (St Johnston, 1993). However, few examples are known where a determinant (RNA or protein) is responsible for differential specification of cell fate due to asymmetric localization into one daughter cell. Mutations exist which perturb binary cell fate decisions of sibling cells in several organisms (Horvitz and Herskowitz, 1992; Posakony, 1994; Jan and Jan, 1995). Perhaps the best examples of intrinsic cues are the regionally localized gene products in the early Drosophila embryo: pole plasm triggers germ cell fate (St Johnston, 1993), bicoid activates anterior embryonic cell fates (Driever, 1993), and nanos specifies posterior cell identity (St Johnston, 1993). However, few examples are known where a determinant (RNA or protein) is responsible for differential specification of cell fate due to asymmetric localization into one daughter cell. Mutations exist which perturb binary cell fate decisions of sibling cells in several organisms (Horvitz and Herskowitz, 1992), but the relevant gene products are not asymmetrically localized or their distribution has not been determined.

Only one gene, numb, is known to encode an asymmetrically localized protein that is required for a binary cell fate decision. numb is a novel cytoplasmic or membrane-associated protein that specifies cell identity in the external sensory bristle organ (ES organ) lineage in the Drosophila peripheral nervous system (PNS) (Uemura et al., 1989; Rhyu et al., 1994). The 4-cell ES organ develops from a sense organ precursor (SOP) that divides to produce two daughter cells, A and B. The A cell divides to produce a bristle cell and a socket cell, while the B cell makes a neuron and a glial cell. Both cell interactions and numb localization are required to specify SOP daughter cell fates. Inactivation of the Delta–Notch signaling between the daughters suppresses A cell fate (Parks and Muscavitch, 1993; Hartenstein and Posakony, 1990). In contrast, numb mutants result in both cells adopting the A cell fate; whereas ectopic expression of numb generates two B cells (Uemura et al., 1989; Rhyu et al., 1994). Thus, Delta-Notch-mediated cell interactions inhibit B cell development, whereas numb protein promotes B cell development. Although numb is asymmetrically localized during mitosis to one of the SOP daughter cells, it is not known which daughter cell inherits the protein (Rhyu et al., 1994).

Given the uncertainty about which SOP daughter cell contains numb protein, a number of models have been proposed to explain how the asymmetric localization of numb protein may trigger B cell fate. One model, numb is segregated to the cell that ultimately acquires the B cell fate and functions cell-autonomously to make sure this cell becomes resistant to the Delta-Notch mediated inhibitory signaling by its neighbor (for example, by down-regulating Notch function). This model would result in numb functioning to control the fate of the cell that inherits it. In an alternate model, numb is segregated to the cell that ultimately acquires the B cell fate and functions cell-autonomously to make sure this cell becomes resistant to the Delta-Notch mediated inhibitory signaling by its neighbor (for example, by down-regulating Notch function). This model would result in numb functioning to control the fate of the cell that inherits it. In an alternate model, numb is segregated to the cell that ultimately acquires the B cell fate and functions cell-autonomously to make sure this cell becomes resistant to the Delta-Notch mediated inhibitory signaling by its neighbor (for example, by down-regulating Notch function). This model would result in numb functioning to control the fate of the neighboring cell that does not inherit numb. In order to answer the question of autonomy, we have examined the role of numb in the Drosophila CNS instead.
of PNS. The CNS has the advantage of identified lineages which have stereotyped divisions and good molecular markers for each cell fate.

The CNS is formed by neural precursors (neuroblasts) that delaminate from the ventral neuroectoderm to form a stereotyped pattern in which each neuroblast can be uniquely identified (Doe, 1992). The formation and initial specification of neuroblast identity is governed by cell interactions (Goodman and Doe, 1993; Campos-Ortega, 1993; Chu-LaGraff and Doe, 1993). Subsequently, each neuroblast divides asymmetrically to 'bud off' a series of smaller ganglion mother cells (GMCs) from its dorsal surface; each GMC then divides equally to produce two postmitotic neurons or glia (Goodman and Doe, 1993). Each neuroblast produces an essentially invariant lineage (Udolph et al., 1993), and some aspects of their lineage occur normally in vitro (Huff et al., 1989). The autonomy and invariance of neuroblast lineages suggest that intrinsic factors may be involved in specifying GMC or neuronal determination.

Here we focus on the simplest CNS lineage: the MP2 precursor. Late in embryonic stage 8, a bilateral pair of MP2 precursors form adjacent to the ventral midline in each segment (Doe, 1992). (Embryonic stages according to Campos-Ortega and Hartenstein, 1985.) Although MP2 is morphologically identical to a neuroblast, it has a different cell division program. Whereas neuroblasts bud off an average of five smaller GMCs from their dorsal surface, the MP2 divides only once to produce two postmitotic neurons: a slightly larger dorsal cell, dMP2, and a slightly smaller ventral cell, vMP2. Over the next hour, these sibling neurons shift into the same dorsoventral plane, with the vMP2 always located anterior to the dMP2. By stage 13 each neuron has a distinctive axon projection: vMP2 projects anteriorly in the 'vMP2/pCC' fascicle and dMP2 projects posteriorly to pioneer the 'dMP2/MP1' fascicle (Lin et al., 1994). We find that numb protein is asymmetrically localized to dMP2 during MP2 mitosis. Loss of numb transforms dMP2 into vMP2 and ectopic expression of numb in vMP2 transforms that cell into the dMP2 fate. Thus numb protein is necessary and sufficient to specify autonomously the dMP2 fate in the MP2 lineage.

**RESULTS**

**numb is localized to the dMP2 neuron during the MP2 division**

numb protein is observed in the cytoplasm of all neuroectodermal cells and in ‘crescents’ at the dorsal side of each neuroblast at mitosis (Rhyu et al., 1994). Just as every neuroblast segregates numb protein to the dorsal cortex, so too is numb localized to the dorsal cortex of the MP2 precursor. We used confocal microscopy to document numb protein localization in the dividing MP2 precursor; MP2 was unambiguously identified by expression of the MP2-specific enhancer trap line AJ96. The stage of the cell cycle was assayed by staining for DNA. At stage 10, before MP2 mitosis, numb is uniformly distributed around the cortex of MP2 (data not shown). At late stage 10, during MP2 mitosis, numb protein is localized to the dorsal cortex of the precursor (Fig. 1A-C). At stage 11, following MP2 division, all detectable numb protein is segregated into the dorsal dMP2 neuron and excluded from the ventral vMP2 neuron (Fig. 1D-F). The asymmetric localization of numb into dMP2 led us to investigate whether numb is required for the specification of dMP2 or vMP2 cell fate.

**Loss of numb transforms dMP2 into vMP2**

A good marker for the mature dMP2 neuron fate is the odd protein. odd is detected in the MP2 precursor (Fig. 2A) and in the new-born dMP2 and vMP2 neurons (Fig. 2B); it is extinguished in vMP2 during stage 11 but maintained in dMP2 through stage 13 (Fig. 2C). odd is also expressed in the nearby MP1 neurons. In embryos lacking zygotic numb function (hereafter called ‘numb embryos’), the MP2 precursor forms normally, expresses odd (Fig. 2D) and divides unequally to produce a larger dMP2 and a smaller vMP2 (Fig. 2E). Although MP2 still divides unequally, both neurons extinguish odd expression during stage 11 and completely lack odd expression at stage 13 (Fig. 2F); this is precisely the time odd is normally down-regulated in vMP2, suggesting that both neurons have adopted the vMP2 fate.
numb embryos show no change in the expression of odd in the MP1 neurons.

To characterize further the loss-of-function numb phenotype in the MP2 progeny, we scored the direction of their axon projections. dMP2 and vMP2 are among the earliest neurons to extend axons, and the 22C10 antibody can be used to selectively label their axons (plus the aCC motoneuron) at early stage 13. In wild-type embryos, the vMP2 axon projects anteriorly and the dMP2 axon projects posteriorly (Fig. 2C). In numb embryos, both MP2 daughter neurons differentiate as vMP2, as determined by their anterior axon projections (Fig. 2F). The numb phenotype is fully penetrant: in every segment, dMP2 is transformed into a vMP2 neuron that lacks odd expression and has an anterior axon projection.

**Ectopic numb transforms vMP2 into dMP2**

Since numb protein is necessary for the dMP2 fate, we tested whether it is sufficient to transform vMP2 into a dMP2 fate. We ectopically expressed numb using a heat-shock promoter-numb transgene (Rhyu et al., 1994) just after the MP2 precursor division at early stage 11, and assayed dMP2 and vMP2 cell fate at stage 13. We find that ectopic expression of numb is sufficient to transform vMP2 into the dMP2 cell fate. In 29/100 of hemisegments scored, both MP2 siblings maintain odd expression and have posterior axon projections, characteristic of dMP2 neurons (Fig. 3B). Control embryos exposed to the same heat-shock conditions have normal dMP2 and vMP2 neurons (Fig. 3A). As with the loss of numb function, the physical asymmetry of the MP2 division is not altered, only the fate of the daughter cells.

**numb does not affect all sibling neuron fates**

Since numb is expressed in all neuroblasts and plays such a critical role in the specification of the dMP2 neuron, we looked for defects in other sibling neurons in the CNS. We find no change in the RP2/RP2sib neurons produced from the first GMC of neuroblast 4-2 (Doe, 1992). One eve-positive RP2 is found per hemisegment (Fig. 4); if numb is required to specify sibling fates in this lineage, either zero or two RP2s should be observed. The first GMC of neuroblast 1-1 produces the sibling aCC and pCC neurons (Doe, 1992). In grasshopper, the fates of these neurons are specified by cell interactions (Kuwada and Goodman, 1985). In both wild-type and numb embryos, only one aCC neuron is found per hemisegment (data not shown). The only CNS phenotype that we have observed is a reduction in the number of eve-expressing EL neurons from 10 to about 4 in each abdominal hemisegment. The lack of a strong phenotype in the CNS is not likely due to perdurance of maternal numb protein, since maternally contributed protein cannot be detected by late stage 8, the beginning of CNS development (E. P. S. and C. Q. D., unpublished results).

**DISCUSSION**

The expression and function of numb in the MP2 lineage is a striking example of a determinant necessary and sufficient to specify cell fate (Fig. 5), numb is asymmetrically localized during mitosis of the MP2 precursor into the dMP2 daughter cell and excluded from the vMP2 daughter cell. Absence of numb transforms dMP2 into a vMP2 neuron, whereas ectopic numb protein in vMP2 gives the opposite transformation of vMP2 into a dMP2 neuron.

Although we observe a completely penetrant dMP2/vMP2 phenotype in numb embryos, we find no global perturbation of GMC development, despite the asymmetric localization of numb protein into each newborn GMC. Perhaps in neuroblast lineages there are other proteins that provide a similar function in the absence of numb. This redundancy may not be present in the unique MP2 lineage, perhaps due to its unusual division pattern. One gene that shows potential to fulfill this role is prospero (pros). Similar to numb, pros protein is asymmetrically localized into the budding GMC during neuroblast mitosis (Spana and Doe, 1995). Unlike numb, pros is localized to the GMC nucleus where it required for correct GMC specification. numb; pros double mutant embryos show an additive eve phenotype (not synergistic), suggesting that pros and numb have independent functions in the CNS (E. P. S. and C. Q. D., unpublished results).

Loss of numb does not affect the unequal cleavage of the MP2 precursor; rather, numb protein is localized in response to a pre-existing cellular asymmetry. This asymmetry is shared between the MP2 precursor and the neuroblasts despite their different division patterns. MP2 divides once to produce two cells of almost the same size, the slightly larger one is born dorsally and the smaller, ventrally. Neuroblasts divide asymmetrically to bud off a small GMC on the dorsal side while the larger neuroblast remains ventral. In both MP2 and neuroblasts, numb is localized to the dorsal cell. One potential source of this cellular asymmetry may lie in the neuroectoderm from which these cells arise. The apical-basal polarity of the neuroectoderm is dependent on the products of the crumbs and star dust genes (Tepass and Knust, 1993). This polarity may be maintained by the delaminated neuroblasts.

Since we see a requirement for the numb protein in the cell that receives it, we propose that numb acts in a cell-autonomous fashion in the MP2 lineage. Although autonomy is usually determined using genetic mosaics, traditional genetic mosaics in the sibling neurons would not be useful since it is the numb protein that is required, not the genetic background. We believe that, in this case, the simplest model of numb functioning in the cell in which it is observed should be used. Given these results, we would predict that numb also functions in a cell-autonomous fashion in the ES organ lineage (Jan and Jan, 1995), thus placing numb protein in the B cell following SOP division. The ES organ lineage requires both asymmetric numb localization as well as cell interactions mediated by the Delta-Notch signaling pathway (Parks and Muskhavitich, 1993 and Hartenstein and Posakony, 1990). numb acts antagonistically to the Delta-Notch signaling pathway and our results support a model in which numb inhibits Notch function (or targets of Notch signaling) in the cell that assumes the B cell fate in the ES lineage. The parallel function of numb protein in the ES and MP2 lineages leads to the prediction that the Delta-Notch signaling pathway might also function in the MP2 lineage. Indeed, preliminary data show that Delta mutants have a phenotype opposite that of numb mutants in the MP2 lineage (E. P. S. and C. Q. D., unpublished results).

The role of numb and possibly the Delta-Notch signaling pathway in the MP2 lineage makes the MP2 lineage an excellent model system for specification of neuronal fates.
**Fig. 1.** numb protein is asymmetrically localized to the dorsal cortex of MP2 at mitosis and subsequently segregated to the dMP2 neuron. Embryos containing the AJ96 enhancer trap insert (which specifically labels MP2) were immunolabeled to reveal the expression of β-galactosidase in MP2 (red), the distribution of numb protein (green), and DNA to identify mitotic cells (not shown). Anterior is to the left and dorsal is up. (A-C) MP2 precursor in mitosis in a stage 10 embryo. (A) β-galactosidase fills the MP2 cell. (B) numb protein is asymmetrically localized to the dorsal cortex of MP2. (C) Merged image of A and B. (D-F) The dMP2 and vMP2 progeny immediately after division. (D) β-galactosidase in the larger dorsal dMP2 (large arrow) and the smaller ventral vMP2 (small arrow). (E) numb protein is specifically localized in dMP2 and excluded from vMP2. (F) Merged image of D and E.

**Fig. 2.** dMP2 is transformed into vMP2 in numb embryos as shown by odd expression and axonal projection. Wild-type (A-C) and numb<sup>1</sup> (D-F) embryos were stained for odd protein expression. (C,F) Embryos were double labeled with odd (red) and 22C10 (green). See Materials and Methods for how the digital montages in C and F were created. (A,B,D,E) Lateral views with dorsal up and anterior to the left; (C,F) ventral views with anterior up. (A) A wild-type stage 10 embryo expresses odd in the MP2. (B) In early stage 11, MP2 divides to produce two neurons that both express odd. dMP2 is the larger cell in the more dorsal position (large arrow) and vMP2 is the smaller ventral cell (small arrow). (C) At stage 13, odd is still found in dMP2 (large white arrow), but is no longer expressed in vMP2 (small white arrow). The adjacent MP1 neurons (arrowheads) also express odd and mark the position of the ventral midline. The dMP2 axon projects posteriorly (large gray arrow) and the vMP2 axon projects anteriorly (small grey arrow). (D) In numb embryos, odd is expressed normally in MP2. (E) Both siblings express odd normally (arrows). (F) At stage 13, the larger ‘dMP2’ neuron does not express odd (large white arrow) and has an anterior axon projection (large gray arrow), reflecting a transformation to the vMP2 fate. The smaller vMP2 neuron is normal: it lacks odd expression (small white arrow) and has an anterior projection (small gray arrow). The MP1 neurons (arrowheads) express odd normally.
Fig. 5. numb protein is an asymmetrically localized determinant necessary and sufficient to cell-autonomously specify dMP2 neuronal identity. (A) In wild-type embryos, numb (dark gray) is asymmetrically localized to the dorsal side of MP2 during mitosis and is subsequently inherited by the dMP2 neuron. odd (light gray) is detectable in MP2 and both sibling neurons after cytokinesis; it is maintained in the dMP2 neuron but repressed in the vMP2 neuron. vMP2 projects its axon anteriorly and dMP2 projects its axon posteriorly. (B) In numb mutant embryos, both odd expression and anterior axon projections reveal a transformation of dMP2 into the vMP2 fate. (C) In hs-numb embryos, the heat shock is applied at or just after MP2 division. This numb localization in MP2 is unaffected, but both neurons receive the numb protein. Both odd expression and the posterior axon projections reveal a transformation of vMP2 into the dMP2 fate.
Genes with phenotypes similar to numb or Notch in the ES lineage, genes involved in the Delta-Notch signalling pathway and genes already known to be expressed in the MP2 lineage can now be examined in the MP2 lineage for a role in cell fate specification. It will be interesting to see whether any of these genes function with numb in the specification of the dMP2 and vMP2 neurons.

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REFERENCES


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