Neural development: **Identical twins separated at birth?**

Christopher A. Walsh

A combination of time-lapse analysis of developing brain slices *in vitro* and the cloning of genes involved in determining cell fate has demonstrated the remarkable similarities between early neurogenetic events in vertebrates and invertebrates.

Address: Department of Neurology, Beth Israel Hospital, Harvard Medical School, 200 Longwood Avenue, Boston, Massachusetts 02115, USA.

Current Biology 1996, Vol 6 No 1:26–28

 ISSN 0960-9822

The development of the mammalian cerebral cortex requires the formation of a wide variety of neurons and glial cells in appropriate ratios and patterns. Unlike some invertebrates, in which the cells that generate the central nervous system can be individually identified and traced over time, the mammalian cerebral cortex develops from an extremely dense proliferative region that does not show obvious landmarks. Various studies of cell lineage *in vivo* and *in vitro* suggest that, ultimately, the cells of the cortex arise from undifferentiated cells that have the potential to form essentially any neural cell type, and that these cortical progenitor cells undergo some sort of progressive restriction of the types of cells they can produce. In other words, undifferentiated cells divide to form cells with more restricted potential, and usually also regenerate an undifferentiated cell. Such a cell division is called asymmetric because the two daughter cells of a given mother cell are different in their structure, fate, and/or mitotic behavior. But how does this loss of symmetry come about at a molecular level? In *Drosophila*, genetic pathways are known to regulate several well-defined asymmetric divisions, whereas understanding in vertebrates has been more limited. Some recent studies in vertebrates, however, promise to take the issue of asymmetric cell division from the realm of pure speculation to the beginnings of experimental analysis.

Drosophila neurogenesis

Several types of asymmetric cell division have been well studied in the nervous system of *Drosophila*. In different developmental systems, asymmetric divisions can result from the unequal inheritance of intracellular determinants, from extracellular factors that act differently on two initially equivalent cells, or from a combination of the two [1]. For example, neurons of the *Drosophila* central nervous system are derived from multipotential neural stem cells called neuroblasts. Each neuroblast can form from any one of four to six neuroectodermal cells at a given position. These cells are essentially equivalent, and are defined as a group by their shared expression of proneural genes, such as those of the *achaete-scute* complex (*AS-C*). However, once a given cell differentiates into a neuroblast, lateral inhibition (or lateral specification [2]), mediated by neurogenic genes including *Delta* and *Notch*, prevents adjacent cells within the group from also becoming neuroblasts. Activation of the Notch receptor protein (by binding of its ligand, Delta) in cells adjacent to the incipient neuroblast activates expression of genes such as *Enhancer of split*, whose protein products act in the nucleus to down-regulate expression of *AS-C* genes. Therefore, the formation of the neuroblast is due to lateral inhibitory cell–cell interactions that occur after cell division, and does not relay on a specified asymmetric cell division.

Once formed, the neuroblast undergoes a series of defined mitoses to generate ganglion mother cells (GMCs) and to regenerate a neuroblast. The GMCs differ morphologically from the neuroblasts and have a much more limited developmental potential, and they thus arise by asymmetric divisions. A major factor in determining the asymmetry of the neuroblast–GMC division is the asymmetric distribution of a protein called Numb [3]. Numb is physically segregated into the portion of the neuroblast cytoplasm that will give rise to the GMC. The neuroblast undergoes a highly stereotyped and polarized cell division such that the Numb protein is inherited strictly by the GMC, and strong genetic evidence suggests that this unequal inheritance is critical for distinguishing the two daughter cells. Another asymmetrically inherited factor, encoded by the *prospero* gene [4], also appears to determine the asymmetry of some neuroblast–GMC cell divisions. Finally, the GMCs themselves divide to form two or more daughters, again usually in an asymmetric fashion. However, analysis of a few GMC divisions suggests that, unlike neuroblast divisions, there are major effects of cell–cell interactions on the specification of the daughter neurons' fate.

Asymmetric cell divisions in mammalian neocortical development

The pattern of cell divisions that gives rise to the neurons of the vertebrate cerebral cortex is much less clearly worked out. The cortex ultimately derives from proliferating cells in two regions that lie deep in the brain, the ventricular zone and the subventricular zone. Studies of cell proliferation using markers of DNA synthesis have shown that the proportion of mitotic cells that generate postmitotic daughter cells destined for the cortex changes systematically during development: early in development many of the cell divisions serve to replenish the supply of progenitor cells, whereas later in development the majority

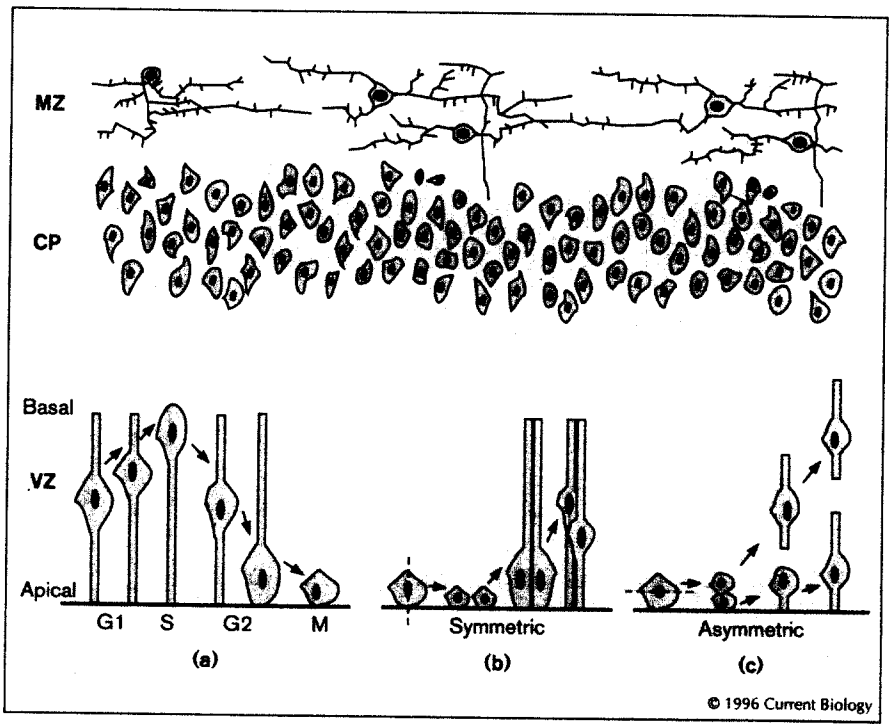
of cell divisions produce postmitotic neurons and so deplete the pool of progenitors. But although some asymmetrical divisions must occur, and cell lineage studies in the cortex have provided clues that both symmetrical and asymmetrical cell divisions may take place (see, for example, [5,6]), such studies have not been able to show whether there are systematic patterns to the symmetry/asymmetry of these divisions [7]. Studies in vertebrates suffer from the inherent difficulty of tracing individual cell divisions over time.

Recently, Chenn and McConnell [8] have addressed the problem of symmetric and asymmetric cell divisions in the mammalian cerebral cortex by developing an *in vitro* system for directly observing cell division in real time. They sought to test earlier suggestions that the orientation of the mitotic cleavage plane predicts the fates of daughter cells in the cortex (see Fig. 1). They determined the orientation of mitotic spindles in the developing ventricular zone and found that the majority of them were oriented approximately perpendicular to the ventricular surface, with 'vertical cleavage planes.' At the earliest stages of cortical development, before neurogenesis begins, 90 % of cleavages are vertical, with the number decreasing gradually to 50 % by a few days after birth, when neurogenesis is nearly complete. The remainder of cleavage planes were oriented either horizontally or at intermediate angles. When observed with time-lapse video microscopy, most of the vertical cleavages generated two daughter cells that went on to display similar behavior: they separated to form two cells, side by side, with very similar, bipolar morphology. The sibling cells remained in close proximity to one another, apparently in contact, as their cell bodies slowly migrated away from the ventricular lumen in preparation (presumably) for another round of DNA synthesis. These divisions were termed symmetric.

A smaller proportion of cell divisions (at the early stage of neurogenesis analyzed) appeared to be manifestly asymmetric. These had their cleavage planes oriented horizontally and generated two daughter cells that appeared to behave differently from one another. Horizontal cleavages created a basal and an apical cell; the apical cell usually stayed near the ventricular surface, while the basal cell seemed to migrate away from the ventricular surface, apparently showing the features of a migrating neuron. The authors suggest that these asymmetric cell divisions may be responsible for forming a postmitotic neuron directly from a dividing progenitor cell. Finally, Chenn and McConnell [8] showed that protein constituents of the dividing ventricular zone cells are concentrated at the basal surface, so that horizontal cleavages will segregate most proteins unequally between the two daughters. They used an antibody to vertebrate Notch1, a vertebrate homologue of the *Drosophila* Notch receptor, to show that the basal daughter cell retained all or most of the Notch1 immunoreactivity after horizontally oriented cleavages. They suggest that the unequal distribution of Notch1 relates to the asymmetrical fates of the daughter cells of horizontal cleavages.

Figure 1

Early development of the cerebral cortex. All cortical cells are ultimately derived from the ventricular zone (VZ). **(a)** Proliferating cells translocate their nuclei as the cells progress through the cell cycle, so that nuclei are located apically, adjacent to the ventricular surface, while in M phase, and then move further away during S phase. Postmitotic neurons are found in the marginal zone (MZ), immediately at the outer surface of the brain, and beneath that in the cortical plate (CP). The cortical plate contains many immature neurons and eventually produces the bulk of cortical neurons. Postmitotic neurons exit the VZ and must migrate up to the cortex over an ever-increasing distance. **(b)** Symmetric cell divisions show a vertically oriented cleavage plane, giving rise to two adjacent daughter cells. **(c)** Alternatively, cell divisions can display a horizontal cleavage plane, producing two asymmetric daughter cells (adapted from [8]).



Asymmetric cell divisions and cell-cycle exit

What is the role of the asymmetric distribution of the Notch1 immunoreactivity? Chenn and McConnell [8] are cautious to distinguish the asymmetric distribution of Notch from the asymmetric distribution of Numb in the neuroblast–GMC division of *Drosophila*, as the significance of the asymmetric Notch1 localization is far less clear. Whereas there is strong genetic evidence that Numb directs the asymmetry of the division in which it is asymmetrically distributed, there is no such genetic evidence for Notch1 in vertebrates. In fact, increasing *Drosophila* Notch activity biases undifferentiated precursors to adopt a nonneural fate [2], so Chenn and McConnell [8] point out that it is actually paradoxical that Notch1 should be localized to the presumptive postmitotic neuron.

How can the paradox of Notch1 being expressed in the apparent postmitotic neuronal products of asymmetric cell divisions be resolved? One possibility is that vertebrate Notch1 functions rather differently from *Drosophila* Notch. However, several recent studies suggest that the negative regulation of neurogenesis by Notch or its homologues is amazingly well conserved between *Drosophila* and vertebrates. For example, in chicks and *Xenopus*, a gene homologous to *Delta* has recently been identified which, when overexpressed, inhibits neuronal differentiation in adjacent neural precursors [9,10], just as *Delta* appears to do in *Drosophila*. Like *Drosophila* Notch, vertebrate Notch1 also appears to inhibit neurogenesis in a cell-autonomous fashion, with increased levels of Notch1 expression or constitutively activated forms of Notch1 inhibiting neurogenesis [11,12], and decreased levels of Notch1 promoting neurogenesis [12]. More recently, Notch1 has been shown to activate the expression of *HES-1*, a mammalian homologue of *Enhancer of Split*, in myogenic precursor cells [13]. *HES-1*, in turn, directly inhibits neurogenesis in the cerebral cortex when overexpressed with retroviral vectors [14]. Finally, mutations in vertebrate *HES-1* produce mice with severe defects in the development of the central nervous system, coupled with precocious expression of the mouse homologues of the *Drosophila* proneural *AS-C* genes (such as *MASH-1*) [15], also suggesting that *HES-1* normally inhibits expression of mammalian *AS-C* homologues. The available evidence, therefore, at least suggests that the Notch signaling pathway in vertebrates has many similarities to that in *Drosophila*.

Chenn and McConnell [8] suggest several other possibilities. First, Notch is a complex, multifunctional protein with many ligands, so it may be over-simplistic to assume that its role at any given place and time is simply to inhibit neuronal differentiation. Notch1 is by no means limited to nervous tissue; instead, it seems to be involved in lateral specification events in many tissues [2]. Another possibility comes from recent retroviral studies of cell lineage in the cerebral cortex. These studies suggest that progenitor cells of the ventricular zone can produce postmitotic neurons but can also produce mitotic precursors that may divide outside the ventricular zone [5–7], either in the subventricular zone or elsewhere. These secondary progenitors may produce glia, or particular types of neurons. Notch1 immunoreactivity in progenitor cells would appear to be more consistent with the role it appears to play in other systems, namely maintaining cells in an undifferentiated state. Finally, Chenn and McConnell [8] suggest that Notch1 may 'freeze' postmitotic cells until they migrate out of the ventricular zone, while making them competent to respond to signals that may come from outside the ventricular zone. So, while it is too early to know exactly what the asymmetric distribution of Notch1 does, the key role of this protein suggests that its asymmetric distribution will have major functional significance. What sets up the asymmetry of cell divisions in the ventricular zone? Chenn and McConnell [8] point out that that question remains unanswered, for now. However, genetic analysis will hopefully contribute greatly in the near future, particularly when combined with the careful analysis of living tissues.

Acknowledgements

I thank C.P. Austin, J. Gonzalez, F. Guillemot, C.B. Reid, and M. Shankland for comments on the manuscript. Work in the author's lab is supported by the NINDS (KO8 NS01520 and RO1 NS33769), and by the Klingenstein Foundation. C.A.W. is a scholar of the Rita Allen Foundation.

References

1. Jan YN, Jan LY: **Maggot's hair and bug's eye: role of cell interactions and intrinsic factors in cell fate specification.** *Neuron* 1995, **14**:1–5.
2. Artavanis-Tsakonas S, Matsuno K, Fortini ME: **Notch signaling.** *Science* 1995, **268**:225–232.
3. Knoblich JA, Jan LY, Jan YN: **Asymmetric segregation of numb and prospero during cell division.** *Nature* 1995, **377**:624–627.
4. Hirata J, Nakagoshi H, Nabeshima Y, Matsuzaki F: **Asymmetric segregation of the homeodomain protein prospero during *Drosophila* development.** *Nature* 1995, **377**:627–630.
5. Kornack DR, Rakic P: **Radial and horizontal deployment of clonally related cells in the primate neocortex: relationship to distinct mitotic lineages.** *Neuron* 1995, **15**:311–321.
6. Reid CB, Liang I, Walsh C: **Systematic widespread clonal organization in cerebral cortex.** *Neuron* 1995, **15**:299–310.
7. Takahashi T, Nowakowski RS, Caviness VS: **Mode of cell proliferation in the developing mouse neocortex.** *Proc Natl Acad Sci USA* 1994, **91**:375–379.
8. Chenn A, McConnell SK: **Cleavage orientation and the asymmetric inheritance of Notch1 immunoreactivity in mammalian neurogenesis.** *Cell* 1995, **82**:631–641.
9. Chitnis A, Henrique D, Lewis J, Ish-Horowicz D, Kintner C: **Primary neurogenesis in *Xenopus* embryos regulated by a homologue of the *Drosophila* neurogenic gene *Delta*.** *Nature* 1995, **375**:761–766.
10. Henrique D, Adam J, Myat A, Chitnis A, Lewis J, Ish-Horowicz D: **Expression of a *Delta* homologue in prospective neurons in the chick.** *Nature* 1995, **375**:787–790.
11. Austin CP, Feldman DE, Ida JA Jr, Cepko CL: **Vertebrate retinal ganglion cells are selected from competent progenitors by the action of *Notch*.** *Development* 1995, **121**:3637–3650.
12. Dorsky RI, Rappaport DH, Harris WA: ***Xotch* inhibits cell differentiation in the *Xenopus* retina.** *Neuron* 1995, **14**:487–496.
13. Jarriault S, Brou C, Logeat F, Schroeter EH, Kopan R, Israel A: **Signaling downstream of activated mammalian Notch.** *Nature* 1995, **377**:355–358.
14. Ishibashi M, Moriyoshi K, Sasai Y, Shiota K, Nakanashi S, Kageyama R: **Persistent expression of helix–loop–helix factor HES-1 prevents mammalian neural differentiation in the central nervous system.** *EMBO J* 1994, **13**:1799–1805.
15. Ishibashi M, Ang S-L, Shiota K, Nakanashi S, Kageyama R, Guillemot F: **Targeted disruption of mammalian *hairy* and *Enhancer of split* homologue-1 (*HES-1*) leads to up-regulation of neural helix-loop-helix factors, premature neurogenesis and severe neural tube defects.** *Genes Dev* 1996, **9**:3136–3148.