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Cell lineage and cell migration in the developing cerebral cortex

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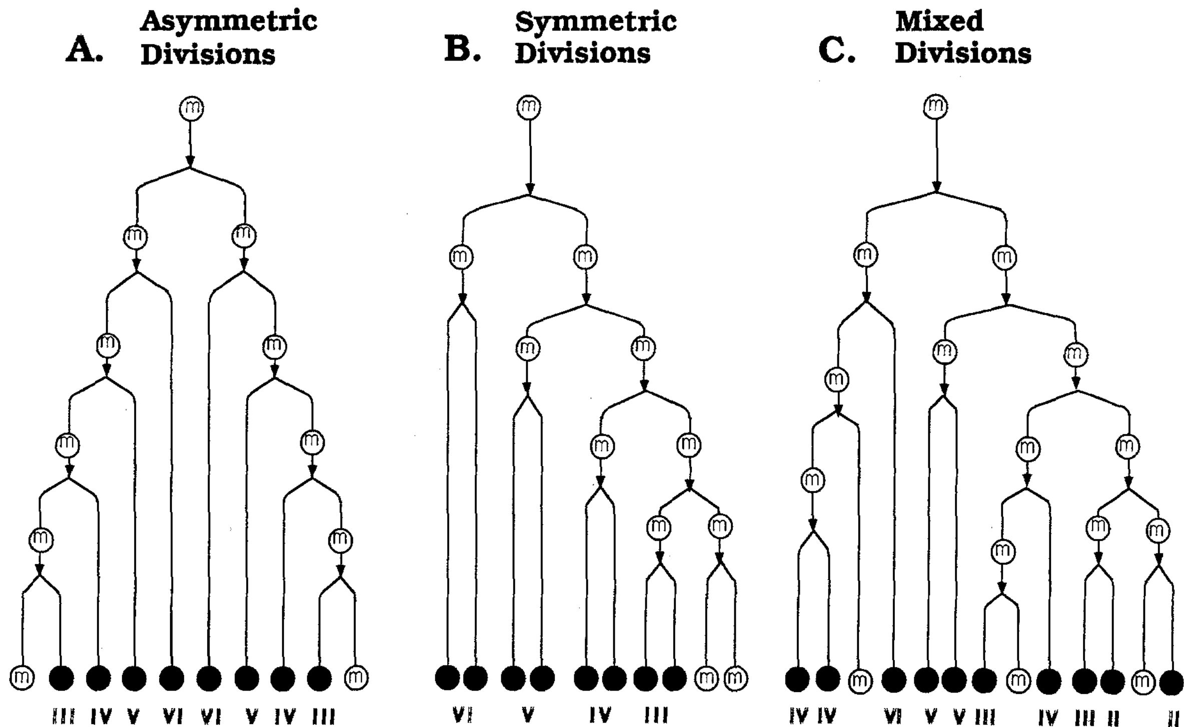
Summary. Modern techniques which trace lineages of individual progenitor cells have provided some clues about the processes that determine cell fate in the brain, and have also given us some information about migratory patterns of clonally related cells. In many parts of the central nervous system, progenitors are multipotent; single clones can contain multiple neuronal types or even mixtures of neurons and glia. In addition, one can observe a wide distribution in clone size, even when marking is done in a narrow time window. This suggests that progenitor cells may be fairly plastic and responsive to environmental signals. In the developing cortex, clonally related cells are initially grouped near each other, as in the retina and tectum. However, the subsequent migration of these cells from the ventricular zone to the cortex along glial fibers is accompanied by a progressive dispersion of clonally related neurons.
Key words. Cell lineage; cerebral cortex; neuronal development; glial development; radial glia.

The lineage relationships of neural cells can provide important information about factors influencing a cell's fate. These factors can be divided, roughly, into those that are intrinsic to the cell, on the basis of inherited patterns of gene activity, and those that reflect the cell's environment, presumably acting through intercellular signalling. Some invertebrates show highly 'determinate' patterns of cell cleavages, where identifiable cells give rise to a stereotyped set of progeny. Such patterns suggest that a cell's fate may be independent of its environment, although exceptions to this generalization are being revealed by careful experimental studies[16,17,92,103]. On the other hand, patterns of cell cleavages which are highly variable and unpredictable (also called 'indeterminate') immediately suggest an important role for intercellular signals in determining cell fate, and can provide clues to help locate those signals.

The development of the cerebral cortex illustrates several problems common to the whole brain[51], as well as others that are unique. For example, the cortex contains neurons and glia, and one of the oldest questions in developmental neurobiology is how these two neural types diverge. His[34] first suggested that neurons arise from Keimzellen ('germ cells'), located along the ventricular lining of the brain, while glia derive from 'spongioblasts', or radially oriented epithelial cells. He compared these cell types to the germ cells and Sertoli cells or the testis – an analogy that explicitly implied a true genetic divergence of progenitors at an early stage. This model has influenced the design of experiments and even the terms of discussion ever since. While the 'lineage' of a cell strictly refers to its parentage and progeny, the terms 'neuronal and glial lineages' are often used more loosely, with this very usage implying that the two represent sep-



This drawing illustrates schematically three hypothetical modes of cell division. In *A*, an initial symmetrical division produces two mitotic progenitors (m). In the second division, each progenitor adopts an asymmetrical, 'stem cell' pattern of cleavage, producing one postmitotic daughter and one mitotic cell in each generation. Each stem cell produces four postmitotic neurons, which can schematically be thought of as ultimately reaching layers VI, V, IV, III, etc. of the cerebral cortex. This would match the observed sequential formation of neurons in these layers seen in ^{3}H-thymidine studies[1,4,7,31,47,65,69,78,87,89,96].

In *B*, mitotic progenitors always divide symmetrically – that is, giving rise to two products that are equivalent in their mitotic potential, either both mitotic or both postmitotic. This mode of cell division can still generate neurons in the cortical layers in an 'inside-out' sequence.

In *C*, a more complicated pattern shows a mixture of symmetrical and asymmetrical cell cleavages, where two progeny from one cell division may or may not differ in mitotic potential and/or cell type determination. These three possibilities represent only a few of the many possible patterns of cell division. Note that in each of these figures, neurons of different layer (or different type, whatever the case may be) can be generated in sequence.

arable paths of a developmental hierarchy. As we shall see below, there is mounting evidence that neuronal and glial development do not fit this simple hierarchy.

Beyond the differences in neuronal and glial development, other aspects of early development can also provide insights into its biological mechanisms. For example, both neurons and glia are present in a huge variety of subtypes which have been defined by their morphology or by their neurochemical properties. Are there separate precursors for distinct functional classes of neurons? Do the rules suggested for glial development in the optic nerve, where one cell type can produce both oligodendrocytes and astrocytes[66,67], also hold for the cerebral cortex? What are the patterns of cell division that characterize these neural precursors? In principle, cell divisions may be symmetric, asymmetric, or some combination (fig.). While a simple, 'stem cell' lineage, where each division is asymmetric, has often been invoked to explain the sequential formation of neurons of different type in the cortex, symmetrical divisions, or some combination of symmetrical and asymmetrical divisions, can produce a similar overall pattern of neurogenesis, but would suggest greater complexity in its regulation.

In addition to these general questions, other fascinating developmental problems in the cortex arise from its uniquely complicated laminar structure and the wholesale cellular migration necessary to generate it[48,50,73]. The neocortex is essentially a folded sheet of several cellular layers, usually six. Neurons which reside in the cortex are not formed there; instead, they undergo their final cell division deep in the brain, along the inner surface of the cerebral ventricles, and must migrate radially to their final positions[68,71–73]. Furthermore, neurons in the cortical layers are formed in an 'inside out' sequence: the oldest neurons occupy the deepest layers of the cortex, and newer neurons need to migrate past the older ones to reach progressively more superficial layers[1,4,7,31,47,65,69,78,87,89,96].

In addition to its laminar structure, the cortex is also organized vertically, perpendicular to the laminae, into 'columns'. The vertical organization of neuronal connections in the cortex was first noted by Lorente de No[45]. Physiological studies of somatosensory cortex[58,59], visual cortex[36,37], and more recently other areas of the cortex have shown that these vertical interconnections reflect a columnar organization of functional properties.

There have been various attempts to define a fundamental processing unit of the cortex, for example the 'minicolumn' of Mountcastle[59], which he defined as a vertically oriented cord containing about 110 cells in all species and all areas (except primate striate cortex), and occupying a cylinder with a diameter of about 30 μm[59]. Functional subunits are dovetailed into larger 'macrocolumns'[59] or 'hypercolumns'[37], whose diameters range from 300 to 1000 μm in different areas of the brain, and which often are most clearly defined in terms of their thalamic afferents[37, 59, 60, 70].

Since columns emerge after neurons migrate radially into the cortex from the ventricle, there might be an important ontogenetic relationship between neuronal migration and later functional organization. Postmitotic neurons migrate in relation to a scaffolding of radial glial fibers, which extend from the ventricular to the cortical surface[52, 53, 68, 71–73, 77, 85]. These radial cells are present during the time of neurogenesis[52, 53, 68, 71, 72, 84, 85], cease mitosis during the time of maximal neuronal migration[84] (but see Misson et al.[53]), and disappear from the cortex, apparently by differentiating into astrocytes[84, 97] after neuronal migration is complete. Furthermore, migrating neurons are often seen in close apposition to these radial fibers[53, 68, 74], suggesting that they use the fibers as guides (see Rakic, Hatten and Mason, this issue). This has prompted the suggestion that 'minicolumns' contain neurons deposited from a limited number of radial glial cells[52, 59, 71] and the further speculation that radial glial fascicles may maintain a mapping of the neuronal progenitors at the ventricular surface on to the cortical surface[52, 71, 77]. More recent work has suggested that the situation is not simple[72, 73], but the possible role of clonal relationships in establishing functional columns can only be addressed by directly showing clonal relationships in the mammalian cerebral cortex. Several new techniques have given us some preliminary data about cell lineages in the brain, and relationships between lineage patterns and migration patterns.

Approaches to vertebrate lineage mapping

One powerful technique for mapping lineages has been the refinement of intracellular injections to allow delivery of tracer dyes to single cells. A tagged cell can then be allowed to divide normally, and its progeny can be visualized and analyzed. The method was originally used in invertebrates, but more recently has been applied to several vertebrate systems, especially *Xenopus*[27, 33, 35, 38, 39] and zebra fish[41]. Injections are only possible where ventricular cells (or blastomeres, as the case may be) are relatively large and accessible, so that injections have not yet been feasible in mammals. There is the additional drawback that most injected labels are degraded or diluted with time and cell divisions, so that there are time limits on the length of an experiment. However, cell injections have great power in that the experimentor has a direct view of the cell which has been injected, and with some dyes, the progeny of an injected cell can be followed through sequential cell division.

Chimeras, composed of tissue from two species or genetically distinct strains, can give indirect information about cell lineages. Chimeras can be made either by fusion of primitive cells from two embryos ('tetraparental' chimeras) or by transplantation of specific cells from chick to quail or vice versa. Cells from distinct sources can later be visualized using histochemical assays[29, 30], in situ hybridization[22, 23, 79], or nucleolar morphology[3]. Although these studies are important in suggesting broad patterns of neurogenesis and migration, they do not generally provide lineage information about individual progenitors. Furthermore, some studies are complicated by the fact that cells of some species grow at slightly different rates[23].

Retroviral marking exploits the fact that retroviruses integrate a DNA copy of their genome into the DNA of the host as an obligatory part of their life cycle. By engineering retroviruses so that they are incapable of replication, any gene of interest (in principle) can be transferred into dividing cells[10–12]. A virally transmitted β-galactosidase gene can be used as a stable, histochemical marker which is not diluted, and is passed on to all progeny of infected cells[10, 11, 62, 64, 81, 82] (see also Gray, Leber and Sanes, this issue). However, the individual marked progenitor cells are not directly observed and cannot be directly followed, and so the number of clones labeled in a given experiment cannot be directly determined. Assignment of clonality rests on statistical analyses using dilutions, a method best applied to situations where clonally related cells remain clustered. When labeled cells are not clustered, retroviral studies are difficult to interpret. In studying the cerebral cortex, where migration is extensive, this becomes a severe limitation, which is discussed in more detail below. Despite their individual limitations, together these several new techniques tend to provide data which are complementary, and which have cast new light on lineage relationships in the developing brain.

Single progenitors can produce multiple neuronal types

In many parts of the nervous system, individual ventricular cells can give rise to a wide range of morphological types of neurons. In the retina, single progenitors can give rise to all neuronal types[95, 100], and the determination of neuronal type seems to be unrelated to cell lineage[35, 94, 95, 100]. In the chick optic tectum, individual progenitors also give rise to a variety of neuronal types[24]. In the *Xenopus* spinal cord, individual progenitors seem to produce either the 'primary' or 'secondary' neurons of the spinal cord, which are formed at different stages and function at different ages; however, within each group, neuronal progenitors give rise to many neuronal types[27], and similar multipotentiality has recently

been shown in the chick spinal cord[44]. A potentially important exception may be the chick hindbrain, where preliminary reports[40] suggest that individual progenitors in the rhombomeres can give rise to large clones consisting almost exclusively of one cell type.

In the cerebral cortex, clusters of labeled neurons contain a wide variety of phenotypes and can include cells in any combination of layers[46,63,99]. There has been recent preliminary data to suggest that some early progenitors preferentially produce cells in deep or superficial layers, based on chimera analysis[30,18] and retroviral marking[42]. Perhaps the situation here is similar to that of the *Xenopus* spinal cord. However, unlike the spinal cord or chick hindbrain, there are clearly progenitors that are truly multipotential for neurons in both deep and superficial layers. Therefore, it is unclear whether a partial segregation of progenitors for different sets of layers, if confirmed, would have any mechanistic significance.

Neurons and glia can arise from common precursors during neurogenesis

Although ultimately neurons and glia must derive from a common precursor, it came as a surprise to find that retinal neurons and Müller glial cells can have a common progenitor, even in the final cell division[94,100]. A common progenitor for neurons and glial cells has since been seen in the chick optic tectum[21], spinal cord[27,44], and dorsal root ganglia[19], and may be a general rule throughout the nervous system. Immunohistochemical analysis using a monoclonal antibody that stains neural epithelial cells have suggested that radial neuroepithelial cells themselves may be bipotential[20].

There is some evidence that neurons and glia have a common precursor in the cerebral cortex, too, although it is too early to be certain. Single cell cultures of rat forebrain using conditioned media[93] have shown that neuronal progenitors divide well in culture, and that a substantial fraction (22%) of ventricular cells give rise both to neuronal and non-neuronal cells. In other in vitro studies of cortical cells, such mixed clones made up only 1% of the total[46], although in these latter studies clones that contained neurons seem to have been small, perhaps reflecting the absence of conditioned medium. Using the retroviral method, some clusters of labeled cortical cells contain both neurons and glial cells[2,46,63,99]. However, glial clones are sometimes quite large, containing hundreds of glial cells stretching over 500 μm or more[80]. Because of the wide spread of glial clones, mixed clusters are hard to interpret; they have been interpreted as suggesting a common lineage[63,99] or separate lineage[46] by different groups. Resolution of this issue will require improvement in current retroviral technology, such as the use of two retroviruses with distinct labels[21] (also Fields-Berry, Halliday and Cepko, unpublished), or the application of alternative mapping strategies to the cortex.

Symmetrical cell divisons (non-'stem cell') are surprisingly common during neurogenesis

In its simplest form, a model proposing asymmetrically dividing stem cells, as presented in figure A, makes explicit predictions about the size of neuronal clones. During the period of neurogenesis, clones should increase by one postmitotic cell with each cell division. The cell cycle length in the retina[104] and the cortex[98] has been shown to be 8–20 h using ^{3}H-thymidine, and bromodeoxyuridine experiments in hippocampus have given similar results[26].Therefore, if asymmetrical divisions are the rule, neuronal clones should contain small numbers of cells, increasing by 1–2 cells for each day of neurogenesis.

In the rodent retina, neuronal clones number up to 234 cells when labeling is done on E14 (about 14 days from the end of neurogenesis), and up to 22 cells when labeling is done on P0 (about 7 days from the end of neurogenesis)[94,95], suggesting that symmetrical divisions occur throughout neurogenesis. In *Xenopus* spinal cord[27], there is a strikingly nonrandom distribution of cell number even in small neuronal clones, with an overrepresentation of powers of two: 2, 4, 8... This suggests that symmetrical divisions in *Xenopus* spinal cord are greatly favored even during the final divisions of neuronal precursors.

Preliminary data suggest that some cortical progenitors undergo symmetrical divisions, even during the period of neurogenesis. Individual, mitotic forebrain progenitors in culture can show both symmetrical and asymmetrical divisions[93]. Retroviral marking shows many cortical clones to be small, which would be consistent with a stem cell lineage[46]. However, some clusters of labeled cells contain up to thirty cells, even after survival times of 5 days or less when cell clustering makes clonal boundaries clear, and before formation of most glia[2,99]. Thus, a proportion of cortical progenitors may undergo symmetrical divisions even during neurogenesis. Also, small clones may also result from symmetrical divisions which are few in number, as has been seen in the frog spinal cord. This issue also remains unresolved though, again reflecting interpretational limits to present retroviral lineage tracing technology.

Migration patterns of clonally related cells vary widely

Results of microinjection of lineage tracers into single blastomeres have shown an indeterminate pattern during early development in zebra fish[41]. For example, injection of the same, identified blastomere in different zebra fish produced a different pattern of label each time[41]. In the nervous system, labeled cells were not clumped, and generally were distributed over about half of the nervous system. This extensive and unpredictable intermixing of products of individual blastomeres seems to reflect both

intermingling of neighbor cells, as well as wholesale cell migrations particularly during gastrulation and neurulation[41]. In chick-quail transplants, transfer of a tiny piece of presumptive neural tube to a region near Hensen's node produces wide spread of its progeny, while similar transplants to adjacent sites show less spreading; thus, cell migrations which accompany morphogenetic events are not always random[86]. Early cell divisions in mice also seem to share similar indeterminacy[79,90]. In contrast, mixing of cells of neighboring blastomeres in *Xenopus* is more gradual, and there are generally reproducible patterns of distribution of descendents of a labeled blastomere[33,38,39,55,88,101]. The significance of these differing patterns is unclear.

During neurogenesis, some regions of the CNS show remarkably sharp maintenance of clonal boundaries. In the retina, clonally related cells remain in columnar arrays[35,94,95,100]. Similarly in the chick optic tectum, most cells in a clone show a striking radial arrangement; larger clones, produced by labeling at earlier stages, form several columnar arrays that are nearly adjacent[24,25] (see Gray, Leber and Sanes, this issue). A similar pattern has been seen in the chick retina after retroviral labeling at very early stages (Guillemot, Fekete and Cepko, unpublished observations).

In contrast, the mammalian cerebral cortex does not show simple columns of clonally related cells. This was first suggested by chimera experiments in the mouse[22,23,30] which showed extensive mixing of cells between columns. Retroviral marking studies suggest the same conclusion; however, before discussion of these data it is important to emphasize several points of interpretation.

In retroviral experiments, inferences about clonal relatedness of labeled cells assumes that clonally related, labeled cells remain clustered. If the number of clusters is proportional to the number of injected viral particles, while the average number of cells in each cluster is independent of the number of viral particles injected, this suggests that each cluster represents the progeny of a single infected progenitor[81,94]. However, this statistical analysis becomes inapplicable if clusters are not unambiguously definable, as discussed below.

Following retroviral injections into mouse or rat cortex during the period of neurogenesis, short (3–6 days) survival times produces labeled cells which are mostly clustered[2,46,63,99], though some cells, especially in lateral parts of neocortex, are not[2,99]. As in the retina and tectum[24,94], the number of these clusters reflects viral titer, while the number of cells per cluster is independent of viral titer[2,63]. This suggests that retroviral labeling follows familiar rules, with each of these clusters representing a clone. These clusters are generally oriented radially, occasionally precisely so. However, some clusters contain cells which seem to be following pathways leading to destinations in the cortical plate which are substantially separated tangentially[2,99].

In the adult cortex, labeled neurons are never grouped as nearest-neighbor clusters. Because of this scatter, a complete statistical analysis is not feasible, since any attempt to group cells will necessarily involve assumptions about how far apart clonally related cells can be (50 µm? 200 µm? 1 mm?). However, even if all clonal boundaries cannot be drawn with certainty in the adult cerebral cortex, the overall distribution of labeled cells differs strikingly from that seen in the retina or tectum. For example, cortical clones do not form simple columns. Some labeled cells are arranged in roughly radial clusters up to 200 µm across in the mouse[46] (Austin and Cepko, unpublished observations) or 500 µm across in the rat, especially in dorsomedial regions of cortex[99]. In figures 1 a and 3 b of Luskin et al.[46], the sharp curve of the cortical outline suggests that the cells in these roughly radial groups are located in dorsomedial cortex as well. However, in more lateral areas of the cortex, including much of somatosensory cortex and auditory cortex, labeled neurons are frequently even more widely scattered[2,99]. The simplest means of reconciling the loss of clustering which was seen after shorter survival times is that clonally related neurons are initially near one another, but during their subsequent radial migration sibling cells become tangentially displaced from one another.

Tangential displacement of clonally related cortical cells can occur at several levels, and may still be consistent with the hypothesis that radial migration is guided by radial glial fascicles. Since radial glia diverge as they emanate from the ventricular zone[53,85], a tiny tangential separation between cells that are near the ventricle produces a large displacement after radial migration is complete. Indeed, there is some evidence for nonradial movement of daughter cells in the ventricular and subventricular zones, both from retroviral work[2,99] and from confocal microscopy of labeled cells[13]. Retroviral labeling studies[2,46,54,63,99] have confirmed pioneering morphological studies[68,74] in showing that, once postmitotic neurons leave the subventricular zone, they seem to be closely apposed to radial glial fibers. However, three-dimensional reconstructions from serial electron micrographs have shown that individual migrating cells can contact several glial fibers[74], leading to the suggestion that there may be some exchange of migrating cells among different radial glial fascicles[72,73]. Retroviral labeling studies using short survival times seem to show clonally related cells diverging to migrate along different glial fascicles[2,99]. In addition, radial glial fibers themselves are exchanged between fascicles in the intermediate zone[53], so that migrating cells in a clone may become tangentially displaced from one another even if they follow glial fibers originally in a common fascicle. Lastly, as the radial glial cells enter the cortical plate they defasciculate and become separated tangentially[53]. Other, smaller nonradial displacements of migrating cells may occur at this level.

The notion that radial migration may be subject to small, but cumulative, tangential displacements offers a simple explanation for the way that distribution patterns of neurons within a clone differ between dorsomedial and lateral neocortex. In the dorsomedial cortex, the radial path is short and straight. However, in the more lateral areas of the rodent cortex, radial glial fibers are quite elongated and curved [53]. A displacement of only one or two cell diameters early in the course of migration could produce sibling cells that are very widely separated in the cortex.

The differences in clonal patterns between the cerebral cortex on the one hand, and the retina and optic tectum on the other, may reflect differing rules of cell migration. In the early retina and tectum, radial pathways are quite short and many postmitotic neurons may maintain attachments to the inner and outer surfaces of the neural epithelium until migration of the cell body is almost complete [14, 15, 32]. This contrasts to the ameboid migration of postmitotic neurons in the cortex [68], where cells may migrate radially for days or weeks with no insertions into either epithelial surface; this latter mode of migration may be less precise. Furthermore, the cortex itself may manifest differing modes of migration at different stages [8, 48]: studies of migration at early stages have shown migrating neurons with little relation to radial glia [56, 89], and these oldest cortical neurons still migrate normally in the reeler mutation, which seems to disrupt radial glia-mediated migration selectively [8].

Further studies will need to define more clearly the relation of clonal organization to the functional architecture of the cortex. Although cells in a 'minicolumn' are not simple clones, they nevertheless may enter the cortical plate along common radial glial fibers, as originally suggested [59, 68]. Moreover, although the tangential spread of clonally related neurons in the cortex is certainly large compared to any definition of a functional column, it remains to be shown directly whether clonal boundaries in fact cross functional or cytoarchitectonic boundaries.

Possible mechanisms for determination of cortical cell class

If the patterning of cell division ultimately proves not to be a major determinant of cell fate and functional order in the cortex, what can we say about the mechanisms which might be responsible? Development of the vertebrate brain may ultimately fit a model similar to that of the *Drosophila* retina, where extensive analysis has failed to show any consistent clonal relationships. Instead, functional retinal subdivisions are constructed by the progressive recruitment of undetermined cells, apparently in response to positional cues [43, 75]. Retinal cell type determination then involves activation of proteins which regulate DNA transcription, apparently in response to these positional cues [16, 17, 57]. Recently, several genes containing 'homeoboxes', or other DNA binding domains, have been shown to have striking regional- or cell-specific patterns of activation during vertebrate development [28, 102]. Such patterned activation of genes does not require determinate cell cleavages.

Any presumptive mechanism of cell type determination must explain the reproducible finding that the time at which a neural progenitor ceases mitosis is still clearly a very strong factor in the determination of that cell's ultimate cortical position and cell type. As mentioned, there is a clear sequence to the formation of neurons in the cortical layers. Moreover, in the *reeler* mouse, the cortical layers are essentially upside down although the cells maintain their lamina-specific connections [5, 6, 8]. During development, cells of the cortical layers are still born in the same sequence [7], suggesting here, too, that a cell's 'birthdate' contributes to the determination of its fate, and that this determination may occur independently of, and perhaps prior to, radial migration. This conclusion has been extended by an elegant series of transplantation experiments by McConnell [49], which are described elsewhere in this issue. Perhaps whatever factors influence cell location and cell fate, whether they are intrinsic or environmental (as they seem to be in the vertebrate retina [76], as well as in *Drosophila*), differ at different stages of neurogenesis.

Although cells may receive information about laminar location, and perhaps morphological subtype, near the time of their final cell division, other properties of neurons are determined later. For example, neurons in the same lamina of cortex, but located in different functional areas, show different properties and patterns of axonal connection. These 'area-specific' properties of the cortex seem to be highly dependent on intercellular interactions, and to be quite plastic. If portions of visual cortex are transplanted to sensorimotor cortex, the transplanted layer V cells have normal intrinsic connections, but their extrinsic, area-specific connections are characteristic of sensorimotor, not visual cortex [61, 91]. Furthermore, transplantation of visual cortex into somatosensory cortex causes the visual neurons to assume a cytoarchitectonic organization characteristic of somatosensory cortex, complete with 'barrels' that characterize this region [83]. These results have led to the suggestion that the cortex initially shows little regional specification, and that this specification is critically affected by developing inputs to the cortex [60, 61].

Thus, while the lineage of a cortical cell may limit its developmental choices, other factors, especially interactions with cortical afferents, act later to define important aspects of cell fate. This sort of a program in which cell number is regulated at an earlier stage, with aspects of cell function critically regulated by the periphery at a later stage, produces potential evolutionary advantages. Fast, partially independent evolution of the cortex and the body plan could occur, while keeping the two always coordinated: sudden changes in body shape could be accommodated by a flexible cortex, since changes would be signalled early in development. Conversely, changes in

cortical size could occur in the context of a cortex still functionally integrated with the periphery. Such a flexible mechanism may have contributed to the rapid evolution of mammals following the appearance of the neocortex.

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