Mechanisms specifying area fate in cortex include cell-cycle-dependent decisions and the capacity of progenitors to express phenotype memory

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SUMMARY

Progenitor cells in the early developing cerebral cortex produce neurons destined for discrete functional areas in response to specific inductive signals. Using lineage analysis, we show that cortical progenitor cells at different fetal ages retain the memory of an area-specific inductive signal received in vivo, even though they may pass through as many as two cell cycles in the absence of the signal in culture. When exposed to inductive signals in vitro, only those progenitors that progress through at least one complete cell cycle alter their areal phenotype. Our findings suggest that induction of an areal phenotype is lineally inherited, with the phenotype specified prior to the final cell cycle.

Key words: cerebral cortex, LAMP, areal phenotype, cell cycle, neuron, lineage analysis, rat

INTRODUCTION

The differentiation of the cerebral cortex involves the formation of laminae in the radial domain, from ventricular zone to the pial surface, and the parcellation of functional areas in the tangential domain, both rostrocaudally and mediolaterally. Both cell-cell interactions and cell-autonomous restriction contribute to specification of laminar fate. For example, early in corticogenesis, the fate of neurons destined for different layers is regulated in the precursor population through a cell-cycle-dependent mechanism, with laminar phenotype specified during the late S or G2 phase of the final cell cycle (McConnell and Kaznowski, 1991); at later stages, when layers II/III are being generated, the cortical progenitors become restricted to an upper-layer fate (Frantz and McConnell, 1996). In contrast, the developmental processes governing the determination of features characteristic of different functional areas in the cortex are not known. One long-standing hypothesis proposes a relatively late specification of cortical areas that is driven by the interaction of a uniform cortical plate with thalamic input (O’Leary, 1989; Woolsey and Wann, 1976; Schlaggar and O’Leary, 1991). A growing body of evidence, however, indicates that the decision to generate neurons with phenotypes specific for discrete cortical areas occurs much earlier in corticogenesis, prior to subcortical interactions (Rakic, 1988; Barbe and Levitt, 1991; Arimatsu et al., 1992; Ferri and Levitt, 1993; Barth and Stanfield, 1994; Cohen-Tannoudji et al., 1994; Ebrahimi-Gaillard and Roger, 1996). At the cellular level, the precise timing of the specification and the heritability of the decision is unknown.

To address the mechanisms that specify areal fate, we have used a marker specific for limbic neurons and studied the regulation of its expression by extracellular signals. The limbic system-associated membrane protein (LAMP) is a cell adhesion molecule that is a member of the Ig superfamily (Pimenta et al., 1995). Analysis of the expression pattern of this protein (Levitt, 1984; Horton and Levitt, 1988) and its mRNA (Reinoso et al., 1996; Pimenta et al., 1996) in vivo demonstrates that LAMP is neuron-specific and restricted to regions of cortex, including perirhinal, cingulate and prefrontal, that classically are considered limbic; few or no neurons in non-limbic cortical regions, such as somatosensory and visual, express this protein. The restriction in LAMP expression is first detected at embryonic day (E)14-15 in the fetal rat and persists through subsequent developmental stages to the adult. Thus, the pattern of expression of LAMP coincides with limbic regions of the cerebral cortex in vivo. Transplant experiments indicate that extracellular signals can influence the initial decision among mitotically active progenitor cells to express LAMP; however, once a progenitor becomes postmitotic and expresses LAMP, the limbic phenotype is stable. Thus, at E12, when the cerebral wall is composed primarily of mitotically active cells, both presumptive limbic (perirhinal) and non-limbic (sensorimotor) progenitors, when transplanted into neonatal perirhinal cortex, express LAMP upon differentiation into neurons (Barbe and Levitt, 1991). Neither population, however, expresses LAMP after placement into a sensorimotor environment. In contrast, when LAMP-positive tissue is grafted from older animals (E14, E17), which contain mostly postmitotic neurons, into a sensorimotor locale, the neurons...
continue to express LAMP. Additional analysis of these transplants demonstrates that their patterns of thalamic and corticocortical innervation reflect their new molecular phenotype (Barbe and Levitt, 1992, 1995).

We established an in vitro system to identify specific environmental signals that may influence LAMP expression (Ferri and Levitt, 1993). Most progenitors isolated from the presumptive perirhinal (limbic) portion of the cerebral wall express LAMP upon differentiation in vitro. Under similar culture conditions, few precursors from the presumptive sensorimotor (non-limbic) region express this protein. Thus, cortical progenitors differentiate similarly in vitro as they do in vivo with respect to LAMP expression, indicating that this protein is a suitable molecular marker of limbic fate in this culture system. We recently found that the combination of TGF\(\alpha\)/collagen type IV can induce a limbic fate in non-limbic progenitor cells in vitro (Ferri and Levitt, 1995). The expression patterns of the EGF receptor, which is activated by TGF\(\alpha\), and collagen type IV in the cerebral wall are consistent with their participation in these initial decisions of neuronal differentiation in vivo (Eagleson et al., 1996). During fetal development, these molecules are co-expressed primarily in the ventricular and subventricular zones, laminae containing all the cortical neuronal precursors, consistent with the idea that they influence early cell fate choices made by cortical progenitors. Indeed, in vitro analysis suggests that the TGF\(\alpha\)/collagen type IV signaling system can only affect the choice of an area phenotype while the cortical progenitors are mitotically active (Ferri et al., 1996). Thus, the addition of TGF\(\alpha\) after 60 hours, a time corresponding to an almost complete absence of cell proliferation, fails to induce LAMP in neurons arising from precursors obtained from the presumptive sensorimotor region of the wall (Ferri and Levitt, 1995). In contrast, if TGF\(\alpha\) is present for only the first 10-20 hours, when mitotic activity is greatest, LAMP is induced in the majority of non-limbic precursors (Ferri et al., 1996).

Based on population assessment, therefore, induction of a limbic area phenotype is an early event in corticogenesis. The specific timing and dependence of the inductive signaling on the cell cycle, and the heritability of the decision to express a limbic phenotype, remain unknown; the present study addresses these mechanisms directly by utilising cell-lineage-based paradigms to monitor the behaviour of cortical progenitors in inductive and non-inductive environments. The results show an almost invariable response of individual precursor cells at any age to LAMP-inductive signals, with evidence for expression of phenotype memory that lasts through several cell cycles following signaling.

MATERIALS AND METHODS

Timed pregnant Holtzman Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, Indiana) were used for this study. The day a vaginal plug was observed was designated E0. All chemicals are from Sigma and culture media and supplements from Gibco unless otherwise stated.

Neuronal cultures

Cultures of dissociated E12 and E14 rat cortical precursors, isolated from a region of the cerebral wall that included both presumptive limbic (perirhinal) and non-limbic (sensorimotor) areas, were prepared as previously described (Ferri and Levitt, 1993). Briefly, pregnant rats were anesthetized with an overdose of sodium pentobarbital, and embryos were removed and placed in a modified Earl’s Balanced Salt Solution (EBSS) on ice. Tissue from 2-3 litters was pooled and incubated in 0.35% collagenase/dispase (Boehringer Mannheim) in Ca\(^{2+}\)/Mg\(^{2+}\)-free EBSS at 37°C for 30 minutes. After three rinses in EBSS, cells were dissociated by mechanical trituration with a fire-polished pipette and plated at a density of 5 x 10^4 cells/cm\(^2\) onto coverslips coated with either 20 μg/ml laminin, a non-inductive matrix, or 200 μg/ml collagen type IV (Collaborative Biomedical Products), the inductive matrix. Cells were cultured for 5 hours at 37°C in 5% CO\(_2\) in a medium composed of a 1:1 mixture of Dulbecco’s Modified Eagle’s Medium and Ham’s F12 nutrient mixture, supplemented with 10% fetal calf serum, 50 units/ml penicillin, and 50 μg/ml streptomycin. After 5 hours, by which time the cells had adhered to the substratum, the medium was replaced with defined N2 medium (Bottenstein, 1985) and the cultures maintained for 4 days. In some experiments, 10 ng/ml TGF\(\alpha\) (Collaborative Biomedical Products), a dose shown to be effective in stimulating LAMP expression (Ferri and Levitt, 1995), was added to the cell suspension prior to plating, and with the N2 medium at 5 and 48 hours.

Retrovirus-mediated lineage analysis

The IZAP retrovirus vector, which transduces the bacterial lacZ gene, was used to infect mitotically active cortical progenitor cells in vitro. The IZAP vector expresses lacZ from the Moloney leukaemia virus long terminal repeat promoter and was made in the lab of Dr C. Cepko. Prior to plating, 100 μl of viral supernatant (determined empirically to produce 10-15 clones per coverslip) and polybrene (8 μg/ml) were added to the cell suspension and incubated at 37°C in 5% CO\(_2\) for 20 minutes, with gentle agitation at 5 minute intervals. The retroviral integration requires that a cell first pass through M phase, in which the nuclear membrane breaks down allowing viral genome access to the host cell genome (Hajihosseini et al., 1993; Roe et al., 1993). During the subsequent S phase, retroviral DNA integrates into one strand post-replication fork of host DNA. Assuming a cell cycle time of 8-12 hours (Takahishi et al., 1995), a cortical progenitor infected during G1 or S phase may not integrate the viral genome until the S phase of the following cell cycle.

Immunocytochemistry

A double-staining method was used to visualize LAMP and β-glucosidase immunoreactivity in the same population. Coverslips with cultured cells were placed in 10% formalin fixative for 10 minutes. After 5 rinses in phosphate-buffered saline (PBS, pH 7.2) and 1 rinse in Blotto (4% Carnation dried milk in PBS), a cocktail of mouse anti-LAMP (1:750) and rabbit anti-β-glucosidase (1:100, 5’-3’) in Blotto was applied. After a 3 hour incubation at 37°C, the cells were washed five times with PBS and incubated with Cy3-conjugated donkey anti-mouse IgG (1:800, Jackson ImmunoResearch) and FITC-conjugated donkey anti-rabbit IgG (1:200, Jackson ImmunoResearch) in Blotto for 1 hour at room temperature. Coverslips were then rinsed with PBS and mounted onto glass slides in PBS/glycerol/propyl gallate. Immunoreactivity was visualized using a Leitz microscope with the appropriate fluorescence filter cubes.

Analysis

Individual clones were identified under a 10× objective and the number of cells per clone determined. To examine the effects of the inductive and non-inductive environments on the phenotypic composition of the clone, the percentage of β-glucosidase-positive cells that were LAMP-positive was determined under a 100× objective. Multicell clones were scored as either homogeneous (all LAMP-positive or all LAMP-negative) or heterogeneous. For each condition, at least 150 clones from 15 coverslips over 3 separate experiments were analysed.
RESULTS

Retrovirus-labeled clones

Cortical progenitor cells were dissected from the frontoparietal region of the embryonic rat cerebral wall. This includes areas that would normally not express LAMP as well as limbic regions of the cortex. We focused upon two stages of corticogenesis: E12, when neurons destined for the preplate are being generated, and E14, at which time neurons destined for layers VI through IV are being produced (Bayer and Altman, 1991). To follow the fate of individual progenitor cells, the cortices were infected immediately following dissociation with a replication-incompetent retrovirus vector that transduces the bacterial lacZ gene encoding β-galactosidase. Such retroviruses integrate into post-replication DNA of dividing precursor cells; thus, β-galactosidase is expressed in only one of the daughter cells following mitosis (Hajihosseini et al., 1993; Roe et al., 1993). If this daughter cell enters G0 and becomes postmitotic, a single-cell clone is produced; progression through at least one additional, complete cell cycle, which takes at least 8-10 hours (Takahashi et al., 1995), results in a multicell clone. Expression of LacZ and LAMP can be detected immunocytochemically within the same cells (Fig. 1), allowing the areal phenotype of cells to be determined within clones grown in the absence or presence of inductive signals. After 4 days in vitro, by which time all neuronal progenitors are no longer mitotically active (Ferri et al., 1996), the number of clones per coverslip ranged from 10 to 15. This sparse number facilitated identification of individual clones, as demonstrated previously in other in vitro studies (Hall and Landis, 1991; Gotz et al., 1995).

Cortical clones exhibit phenotype memory

After 4 days in vitro, individual clones were clearly separated from one another and contained between one and six cells. Cell clusters were considered clonal if cells were within 100 μm of each other. When grown in the presence of the inductive signals, on collagen type IV with TGFα, there was no effect on the size distribution of the clones (Fig. 2) or the number of labeled clones per coverslip. This result highlights the inductive nature of the TGFα/collagen type IV signal. Given the consistency in clone size distribution, it is unlikely that either selective proliferation or cell death plays a significant role in defining unique aspects of clone composition under inducing or non-inducing conditions. TGFα has no effect on the proliferative behaviour of early cortical progenitors, as we and others showed previously (Kilpatrick and Bartlett, 1995; Ferri et al., 1996), or on the number of neurons present at 2 or 4 days in vitro (Ferri and Levitt, 1995). In addition, we showed that, under a variety of culture conditions, including the limbic-inductive environment, mitotic activity is always highest during the first 24 hours in culture, with little cell division occurring after 48 hours (Ferri et al., 1996). The majority of clones, therefore, should be composed of 1 or 2 cells (generated during the first 10-20 hours in culture), with very few containing more than 4 cells (generated after 30 hours in vitro). We found the size distribution to be consistent with the predictions from the proliferative behaviour (Tables 1, 2).

At both embryonic ages, clones were almost entirely homogeneous (>90%), containing LAMP-positive or LAMP-negative cells only (Fig. 3); mixed clones containing both LAMP-positive and LAMP-negative cells were rare and may represent the small percentage of neuron-glial mixed clones reported under similar conditions (Williams et al., 1991). Consistent with this suggestion, at E12, approximately 10% of clones are heterogeneous with respect to MAP2 expression in sister cultures. The lineage-based analysis revealed a surprising feature of area specification. There were numerous multicell, LAMP-positive clones in the absence of inductive signals, indicating that the decision to express a limbic phenotype must have been made in vivo and the daughter cells are able to exhibit phenotype memory in vitro. Moreover, the largest clones are equally, if not more, likely to be homogeneous compared to the smaller clones (Tables 1, 2), indicating that the phenotypic memory of a cell is able to extend for as many as two cell cycles (3- to 4-cell clones) or three cell
cycles (5- to 6-cell clones) in the absence of the inductive signal in culture.

**Limbic phenotype induction is cell-cycle dependent**

To determine when cells are induced to adopt a limbic fate, we compared the phenotypes of single-cell clones with multicell clones cultured with the inductive signals. When grown in the presence of TGFα/collagen type IV, the percentage of LAMP-positive, multicell clones significantly increased from 39.8±3.2% to 76.2±0.2% in cultures of E12 cerebral wall and 26.7±7.2% to 73.9±7.8% in cultures of E14 cerebral wall (Fig. 4). Even in the inductive environment, we found that the multicell clones were composed again of predominantly one molecular phenotype (Fig. 3). Thus, when the progenitors from non-limbic regions respond to the LAMP-inducing signal prior to withdrawing from the cell cycle, they generate neurons of an identical areal phenotype. The percentage of MAP2-positive multicell clones was determined at E12 and found to correspond to the percentage of clones (~75%) exhibiting LAMP induction in sister cultures.

Most remarkably, in contrast to the multicell clones, single-cell clones were not induced to express a limbic areal phenotype when exposed to TGFα/collagen type IV (Fig. 5). These clones represent progenitors that were already progressing through their final cell cycle at the time of plating. The data show that specification of a limbic fate requires that progenitors be exposed to inductive signals for at least one complete cycle.

**Table 1.** Over 90% of clones from E12 cerebral wall are comprised of a single areal phenotype (LAMP-positive or LAMP-negative)

<table>
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<tr>
<th>Condition</th>
<th>1</th>
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<tr>
<td>Collagen IV</td>
<td>156</td>
<td>79</td>
<td>34</td>
<td>14</td>
<td>7</td>
<td>2</td>
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<tr>
<td>Collagen IV + TGFα</td>
<td>149</td>
<td>87</td>
<td>27</td>
<td>13</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Laminin</td>
<td>105</td>
<td>47</td>
<td>27</td>
<td>12</td>
<td>11</td>
<td>2</td>
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<tr>
<td>Laminin + TGFα</td>
<td>161</td>
<td>77</td>
<td>49</td>
<td>19</td>
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The number of homogeneous clones/total number of clones. For each culture condition, at least 15 coverslips over 3 separate experiments were analysed.

*Clone size.*
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...cell cycle; our previous population analysis was unable to identify this requirement (Ferri et al., 1996).

Phenotype memory and limbic inductive responses are independent of birth order

Analysis of neuronal clones derived from E12 and E14 cerebral wall indicates that birth order does not define the responsiveness of progenitors to the LAMP-inductive signal. Moreover, a progenitor cell from E14 that is born after 2 days in culture (>4-cell clone) is just as likely to respond to the inductive signal as a cell from E12 that is born during the first day in culture (2-cell clone) (Fig. 4). These extremes represent the production of preplate and layer IV in fronto-parietal cortex (Bayer and Altman, 1991). These data, however, do not address whether a neuronal progenitor arising at the latest stages of neuronogenesis, when layers III and II are being generated, might lose the ability to alter its areal fate. We therefore performed the same clonal analysis on progenitors derived from the fronto-parietal region of the E16 cerebral wall, in the presence and absence of TGFβ/collagen type IV. At this age, most neuronal progenitors in the wall are located dorsomedially in non-limbic cortex and, thus, only a small proportion of clones express LAMP. As shown for E12 and E14, single-cell clones do not respond to TGFβ/collagen type IV (Fig. 6). Thus, progenitors at all ages require a complete cell cycle in responding to limbic inductive signals. Of the multicell clones, only the 2- and 3-cell groups contained neurons. The larger clones, therefore, were not analysed further, because LAMP is only expressed by neurons (Zacco et al., 1990). In the 2- and 3-cell clones, there was a 3.5-fold induction of LAMP expression in progenitors grown in TGFβ/collagen type IV. Not all E16 neuronal progenitors responded, however, because the maximum level of induction reached approximately 25%, about half of the percentage of 2- and 3-cell neuronal clones present in sister cultures. These data, together with the analysis of E12 and E14 progenitors, demonstrate a consistent pattern of inductive behaviour through the entire period of neuronogenesis.

DISCUSSION

The present study shows that specification of an areal fate in the mammalian cerebral cortex occurs within the progenitor cell population during all stages of neuron production by signals that regulate choice rather than proliferation or survival. Using lineage analysis, we show that this specification is dependent upon precursor cells receiving inductive signals while progressing through at least one complete cell cycle. We also demonstrate that there may be a lineage-dependent mechanism controlling regional identity in the cerebral cortex, with cortical progenitor cells retaining the memory of an area-specific inductive signal for as many as two

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<td>Collagen IV</td>
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<td>76/76</td>
<td>17/17</td>
<td>9/9</td>
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<td>0/0</td>
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<tr>
<td>Collagen IV + TGFβ</td>
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<td>78/79</td>
<td>35/35</td>
<td>19/19</td>
<td>4/4</td>
<td>1/1</td>
</tr>
<tr>
<td>Laminin</td>
<td>130/130</td>
<td>76/77</td>
<td>22/23</td>
<td>9/9</td>
<td>3/3</td>
<td>0/0</td>
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<td>Laminin + TGFβ</td>
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<td>62/63</td>
<td>27/27</td>
<td>6/6</td>
<td>2/2</td>
<td>0/0</td>
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The number of homogeneous clones/total number of clones. For each culture condition, at least 15 coverslips over 3 separate experiments were analysed.

*Clone size.

Fig. 4. Percentage of LAMP-positive, multicell clones (+ s.e.m.) after 4 days in culture, grouped in relation to clone size. Cortical progenitors from E12 or E14 rats were grown on either collagen type IV or laminin, in the presence (black bars) or absence (white bars) of TGFβ. In the presence of the LAMP-inducing signal (collagen type IV/TGFβ), almost 80% of the multicell clones express LAMP, irrespective of their size.
cell cycles. This is not an irreversible commitment, however, as placing cortical progenitors in an environment different from their normal one can alter their fate. Finally, during their last cell cycle, the precursors become refractory to the LAMP-inductive signals, reflected by the total lack of responsiveness of single-cell clones at all fetal ages examined.

**Phenotypic memory and limbic inductive responses are cell-cycle related**

The clonal analysis presented here shows that, in the absence of LAMP-inductive signals, even progenitors that progress through 2 and up to 4 cell cycles are capable of expressing the protein upon differentiation. This shows that cells receiving the inductive signal in vivo retain their fate in a non-inductive environment in vitro and thus do not automatically reset as they reenter each cell cycle, as suggested from studies of laminar fate (McConnell and Kaznowski, 1991). Thus, cortical progenitors are able to retain the memory of an area-specific inductive signal through several rounds of division in the absence of the signal, resulting in the lineal inheritance of the decision to express LAMP. This resembles the regulation of cell fate in the chick hindbrain, where it has been suggested that the decision to produce a particular neuronal phenotype may be remembered through as many as four rounds of mitotic expansion (Lumsden et al., 1994).

The data also address the temporal aspects of the TGFα/collagen type IV induction. While our previous transplant and cell culture studies demonstrated that postmitotic neurons are unlikely to respond to the signals that induce a limbic fate (Barbe and Levitt, 1991; Ferri and Levitt, 1995), definitive cell-cycle dependence could only be inferred. Because retrovirally labeled clones represent actively proliferating cell populations, we expected all to respond to the limbic inductive signal. In fact, LAMP induction does not occur when TGFα/collagen type IV is first presented during only part of the final cell cycle, as represented by the group of single-cell clones. Progenitors that progress through at least one complete cell cycle in the presence of the inductive signal respond by producing LAMP+ daughter cells. The stage of the cell cycle in which the progenitors first become competent is unknown, but the analysis shows that, once induced, all daughters respond identically, with greater than 90% of LAMP-positive clones being homogeneous.

**Specification of cortical regional phenotype**

Previous studies addressing the mechanisms underlying the commitment to a particular neuronal phenotype in the cerebral cortex have assayed features, such as laminar position and neu-
rotransmitter fate, that are common to most cortical areas (McConnell and Kaznowski, 1991; Gotz et al., 1995; Frantz and McConnell, 1996). While there are some parallels between the results of these studies and the present one, there are two features of area specification that our present results indicate are rather unique. First, as mentioned above, exposure to the LAMP-inducing signal during part of the last cell cycle is insufficient to induce expression by non-limbic progenitors. Thus, the decision to generate neurons with a phenotype specific for a cortical region appears to occur prior to the final cell cycle. This same phenomenon has been demonstrated in the chick hindbrain by lineage tracing methods using dye injection (Lumsden et al., 1994). In contrast, the decision to generate a neuron destined for a particular cortical layer occurs during late S/early G2 of the final cell cycle (McConnell and Kaznowski, 1991). Second, cortical progenitors, isolated at three different fetal ages that span the entire period of neuron production in the rostral cerebral wall, remain competent and respond to the LAMP-inductive signal. This finding is consistent with expression patterns of LAMP in the cortex, where most deep layer and many superficial neurons produce lamp transcript (Reinoso et al., 1996; Pimental et al., 1996) and protein (Levitt, 1984; Zacco et al., 1990). The group of 2- to 3-cell clones, derived from the E16 progenitors, that did not respond to TGFβ/collagen type IV may represent superficial neurons that normally do not express LAMP in limbic cortex. In the case of laminar fate, the developmental potential of progenitor cells becomes restricted over time, such that precursors derived from older animals are unable to respond to the deep-layer-inductive signals (Frantz and McConnell, 1996). This invokes a biological feature in which progenitor cells change over developmental time, shown previously for the retina (Lillien and Cepko, 1992; Lillien, 1995); in the cortex, the change is related to its inherent organization, where different neuronal classes constitute each layer. Thus, as we have shown here in relation to areal specification, responses of early or late progenitors need not change, since expression of the area phenotype is mostly uniform throughout the layers.

We propose that, during development, early regionalization of the cerebral cortex occurs through interactions among the cells of the progenitor pool within the developing neuroepithelium (Levitt, 1994; Levitt et al., 1997). Selective inductive and supportive signals operate over the entire time period of neuronogenesis to produce neurons with molecular features unique to specific cortical regions. TGFβ, for example, may represent one such signal; the ganglionic eminence and developing striatum produce high levels of TGFβ(Lazar and Blum, 1992) and are therefore a potential source of this factor for the adjacent presumptive limbic cortices that express LAMP. Current investigations of LAMP expression in TGFβ(Luetchte et al., 1993; Mann et al., 1993) and EGF receptor (Threadgill et al., 1995) knockout mice are underway. The inductive interactions rely on both the spatial distribution of signals and the intrinsic capacity of cells to respond to the available cues. The capacity of progenitors to respond may be regulated by genes that control cell-cycle progression. A similar scenario was observed during differentiation of vulval precursor cells (VPC) in C. elegans (Eusling and Ambrose, 1996). In this system, the mutation of heterochronic genes controlling cell-cycle progression caused significant changes in the phenotype adopted by the VPC upon differentiation. The effect appears to be due to altered competence of the precursors to respond to the inductive signals, because this mutation did not alter the timing of signal production or the expression of the signal transduction machinery. In the context of cortical development, spatially restricted inductive signals could readily respecify progenitors that move to a different molecular domain prior to their final cell cycle, as suggested by our previous transplantation studies (Barbe and Levitt, 1991, 1992, 1995) and by recent experiments indicating that progenitor cells migrate tangentially as well as radially (Walsh and Cepko, 1992, 1993; Fishell et al., 1993; Reid et al., 1995). The present study highlights what may be a conserved mechanism of specification that occurs in a cell-cycle-dependent fashion (McConnell, 1995; Eusling and Ambrose, 1996).

We thank Drs Susan McConnell and Richard Nowakowski for helpful discussions of the experiments and manuscript. We also gratefully acknowledge Dr Liel Jones for assistance with the confocal microscopy. This work was supported by NIMH Grant MH45507 (P.L.), March of Dimes Basic Research Grant 1-FY95-1023 (K.L.E., P.L.), a Windisch Investigator Award from NARSAD (P.L.) and a George H. Cook Rutgers Undergraduate Honors Award (A.V.C.).

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(Accepted 5 February 1997)