We previously identified an interaction between collagen type IV and the EGF receptor that regulates the differentiation of a limbic cortical phenotype in vitro (Ferri and Levitt, 1995). During development, we map the expression of the EGF receptor and collagen type IV in the embryonic telencephalon of the rat. At embryonic day (E) 11, the earliest age examined, both proteins are coexpressed throughout the ventricular zone in the cerebral wall; this zone remains immunoreactive throughout corticogenesis (E14–E19). The cells comprising the subventricular zone are the most intensely immunoreactive for the EGF receptor, although little collagen type IV is detected in this region. In contrast, postmitotic neurons that leave the proliferative zones are negative for the receptor. Moreover, during the peak of neuronal migration, the intermediate zone lacks collagen type IV immunoreactivity. Neurons that settle in the cortical plate once again exhibit EGF receptor immunoreactivity; this same zone is devoid of collagen type IV. By E19, coexpression of both proteins is evident only in the rostral extension of the subventricular zone, the pathway of migrating cells leading to the olfactory bulb. The temporal and spatial overlap of the EGF receptor and collagen type IV in the cortical progenitor pool in vivo indicates that these molecules may participate in the initial decisions of neuronal differentiation. Their modified distribution during cortical maturation suggests a changing role for both proteins.

In mammals, the mature cerebral cortex is comprised of discrete functional areas that are distinguished anatomically by unique molecular phenotypes and patterns of connectivity. During development, an apparently homogeneous population of progenitor cells, located in the ventricular zone at the rostral end of the neural tube, generates all cortical cell types. The sequence of events that operates to specify each unique neuronal phenotype of the adult cortex is not well understood. Some phenotypic features appear to be acquired early in development, such as the commitment to differential gene expression among neurons in different cortical regions. For example, neurons located in limbic cortical areas are committed to express the limbic system-associated membrane protein (LAMP) at, or just after, their final cell division (Ferri and Levitt, 1995). Also, there is an early commitment by neurons in the lateral neocortex of the rat to express latexin, a protein localized only in neurons of the infragranular layers of the lateral cerebral cortex (Arimatsu et al., 1992, 1994). In addition, transplanted embryonic neurons of presumptive somatosensory cortex, harvested from an enhancer-trap transgenic mouse, express a reporter gene specific to this functional area, even when placed in heterotopic locations (Cohen-Tannoudji et al., 1994). Other phenotypes of the cortex, however, are expressed much later, such as the development of the barrel fields in the somatosensory cortex (Woolsey and Van, 1976; Schlaggar and O'Leary, 1991); the expression of these features depends on appropriate interactions with subcortical structures.

Our laboratory has focused on signals that operate early in development to regulate the molecular specification of a distinct cortical area, the perirhinal limbic cortex. One unique characteristic of limbic cortical neurons is the presence of LAMP on the membrane of their soma and processes (Levitt, 1984; Horton and Levitt, 1988; Zacco et al., 1990; Ferri and Levitt, 1993). LAMP, a member of the Ig superfamily (Pimenta et al., 1995), can specifically mediate the adhesion of limbic neurons (Zhukareva and Levitt, 1995) and is critical for the normal targeting of axons (Keller et al., 1989; Barbe and Levitt, 1992, 1995). During cortical development, neuronal progenitors are faced to a limbic or nonlimbic phenotype, defined by the expression of LAMP depending on their location in the ventricular zone (Ferri and Levitt, 1993). During a critical period, however, specific cues can alter the fate of these precursors. At E12, both presumptive limbic and nonlimbic progenitor cells, when transplanted into neonatal perirhinal cortex, express LAMP upon differentiation into neurons, regardless of their position in the donor (Barbe and Levitt, 1991). Neither population, however, expresses LAMP when placed in a sensorimotor environment. In contrast, by E14, postmitotic neurons from the presumptive perirhinal or sensorimotor cerebral wall are committed to express, respectively, the appropriate limbic or nonlimbic phenotype. To define the specific molecules that regulate LAMP expression, precursors from presumptive sensorimotor areas were dissociated and grown in vitro (Ferri and Levitt, 1995). LAMP expression is induced only when the nonlimbic population is plated on a collagen type IV substrate, in the presence of either epidermal growth factor (EGF) or transforming growth factor (TGF) α. An antibody generated against the EGF receptor blocks the induction of LAMP expression, indicating that the response occurs through this receptor. Moreover, neither fibronectin nor laminin are able to combine with the exogenous growth factors to induce LAMP expression; bFGF and PDGF also have no effect. Specific molecules were thus identified that are able to regulate precursor differentiation in vitro to produce a region-specific neuronal phenotype. It is not known, however, whether these molecules fulfill the same function in normal development in vivo. In fact, previous reports have suggested that collagen type IV expression is negligible in the CNS (Le-tourneau et al., 1988; Herken et al., 1990) and the specific cell types that express the EGF receptor in the embryo are not well defined. As a first step in demonstrating potential developmental interactions between collagen type IV and TGFα/EGF, we have mapped the expression of each component of the signaling system in vivo during the period when precursors become committed to a limbic or nonlimbic phenotype.

Both EGF and TGFα are expressed pre- and postnatally in the CNS (Fallon et al., 1984; Rall et al., 1985; Schauies et al., 1989; Lazar and Blum, 1992) and, although both are ligands for the EGF receptor, TGFα appears to be the more abundant in most parts of the brain (Kaser et al., 1992; Lazar and Blum, 1992; Seroogy et al., 1993). To define the specific population of cells capable of responding to these ligands, we examined the distribution of the EGF receptor, using a specific antisera (Tucker et al., 1993). Several studies have demonstrated EGF receptor immunoreactivity in selected regions of adult rat neocortex, basal forebrain, olfactory tubercle, caudate-put...
tamen, and hippocampus (Gomez-Pinilla et al., 1988; Werner et al., 1988), as well as the early postnatal hippocampus (Tucker et al., 1993). Recently, in situ hybridization studies demonstrated the presence of mRNA for the EGF receptor in the subventricular zone of the neonatal rat (Serogy et al., 1995). Collagen type IV, a major component of epithelial basement membranes, is thought to be expressed mainly in association with the cells of the blood vessels and pia. It is present, although very sparsely, in the embryonic neuroepithelium of the hindbrain (Letourneau et al., 1988). In the present study, we utilized polyclonal antibodies directed against the EGF receptor and collagen type IV (Yurchenco and Ruben, 1987) to define the anatomical distribution of these proteins during embryonic development of the telencephalon in the rat. We demonstrate that both the EGF receptor and collagen type IV are expressed in the cortical progenitor pool in vivo, indicating that, potentially, they are able to affect the initial decisions in differentiation.

Materials and Methods

Animals
Timed-pregnant Holtzmann albino rats were obtained from Harlan-Sprague-Dawley and maintained under a 12 hr light/dark cycle with free access to food and water. Detection of the sperm plug was considered embryonic day (E)0. Pregnant dams were anesthetized with an intraperitoneal injection of Nembutal (50 mg/kg). The uterine horns were dissected from the abdominal cavity and placed in Hank's Balanced Salt Solution. Embryos were released from the extraembryonic membranes and staged according to crown-rump length (Olson and Seiger, 1972).

Antibodies
The spatiotemporal distribution of EGF receptor and collagen type IV through the entire rostrocaudal extent of the telencephalon was mapped from E11 to E19. EGF receptor was mapped by means of a rabbit polyclonal antibody, Ab 41 (courtesy of Dr. R. M. Rosner, University of Chicago), generated against the cytoplasmic domain of the protein. On Western blots, Ab 41 specifically recognizes a 170 kDa band in membranes from adult and neonatal hippocampus; it does not react with HER2 or the rat homolog, NEU (Tucker et al., 1993). Collagen type IV was mapped by means of a rabbit polyclonal antibody (courtesy of Dr. P. Yurchenko, University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School) generated against collagen type IV purified from lathyritic murine EHS tumor (Yurchenco and Ruben, 1987).

Immunocytochemistry
A total of 16 embryonic rats were used in this study and at least two embryos of each age (E11, E12, E14, E15, E17, and E19) were analyzed. Either whole embryos (E11 to E14) or the brain alone (E15 to E19) were immersion fixed first in 4% paraformaldehyde (pH 6.5) for 2 hr, then 4% parafomaldehyde (pH 9) overnight at 4°C. All fixed tissue was equilibrated in two 12 hr changes of 10, 20, and 30% sucrose in phosphate-buffered saline (PBS, pH 7.2) and subsequently incubated in 30% hydrogen peroxide in methanol for 30 min to quench endogenous peroxidase activity. After two 5 min washes in PBS, the sections were blocked with 0.3% Triton X-100 in Blotto (4% carnation dried milk in PBS), washed four times with Blotto alone, and incubated in one of the primary antibodies (1:50 in Blotto for EGF receptor; 1:100 in Blotto for collagen type IV) overnight at room temperature. Following six washes in PBS, the sections were incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (1:100 in Blotto) for 1 hr. Controls included primary and secondary antibodies alone, all of which resulted in the absence of immunostaining. Sections were then washed extensively in PBS, and the HRP visualized using a standard reaction with 3,3-diaminobenzidine tetrachloride as the substrate. The reaction was complete in 4 min, after which the sections were rinsed in PBS, dehydrated through a graded series of alcohols and Hemo-De (Fisher Scientific), then coverslipped with DPX mounting media (BDH Ltd.). Immunoperoxidase-stained sections were photographed in bright-field illumination with a Leica photomicroscope on Kodak Technical Pan black and white film.

Sections from the third series were stained with cresyl violet for determination of different telencephalic regions, as well as the specific layers of the developing cortex, in the different-aged embryos. The atlas of Altman and Bayer (1995) was used as an aid in identifying these regions. The terminology is according to the Boulder Committee (1970).

Results

Characterization of EGF Receptor and Collagen Type IV Antibodies
Both the EGF receptor (Tucker et al., 1993) and collagen type IV (Yurchenco and Ruben, 1987) antibodies used in the present study have been characterized previously in neonatal and adult animals. We analyzed antibody specificity in embryonic tissue used for the immunocytochemical analysis. In membranes isolated from E14 rat cortices, Ab 41, the EGF receptor antibody, recognizes an appropriate size band with an apparent molecular mass of 170 kDa (Fig. 1A). In homogenates of E14 cortices, the collagen type IV antibody recognizes two bands of appropriate size that migrate at approximately 180 and 165 kDa (Fig. 1B). In addition, collagen type IV immunoreactivity in the adult kidney (data not shown) shows a staining pattern consistent with collagen al, 2(IV) (Miner and Sanes, 1994). These data demonstrate that both antibodies only recognize the appropriate bands in extracts of embryonic forebrain, indicating that these antibodies do not cross-react with additional molecular species in the developing nervous system.

EGF Receptor and Collagen Type IV Immunoreactivity in the Developing Telencephalon
At the earliest age examined (E11), both EGF receptor- and collagen type IV-immunoreactivity are already expressed at high levels within the ventricular zone of the prosencephalon. At all ages, EGF receptor staining is characterized by punctate profiles that surround cell somata in the neuroepithelium (Fig. 2). Occasionally, cytoplasmic staining is seen. Collagen type IV staining is evident as a ring around the sur-
Figure 2. A series of low-magnification photomicrographs illustrate the overall distribution of EGF receptor (e) and collagen type IV (c) immunoreactivity at rostral (A, D, and G), middle (B, E, and H), and caudal (C, F, and I) levels of the telencephalon. At E11, both proteins are localized throughout the ventricular zone, although collagen type IV exhibits more intense staining dorsally (large arrows in A–C). Note the intense EGF receptor staining of the olfactory placode (small arrowheads in A and B). At E14, the differential distribution of the EGF receptor and collagen type IV becomes apparent. At E19, collagen type IV exhibits a bilaminar pattern (arrows in F). The EGF receptor immunoreactivity is expressed heavily in the cortex and cortical plate (large arrowheads in G–I), and the rostral subventricular zone (small arrowhead in G). LV, lateral ventricle; R, retinal neuroepithelium; LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence; A amygdala; Hip, hippocampus. Scale bar, 400 μm for A–C, 800 μm for D–F, 1600 μm for G–I.

faces of cell somata and extracellularly in cell-poor regions as punctate profiles. The following is a description of the expression of these two proteins in the telencephalon throughout embryogenesis.

E11
At E11, the telencephalic vesicles that give rise to the cerebral cortices first appear (Bayer and Altman, 1991). At this age, the telencephalic wall is comprised solely of the ventricular and marginal zones, the former a homogeneous-appearing pseudostratified epithelium containing only dividing cells. Generally, most cells within this zone are EGF receptor positive, with no obvious gradient in the intensity of immunoreactivity throughout the rostrocaudal extent of the vesicles (Fig. 24–C). At all levels, however, the most intense EGF receptor-positive cells are concentrated along the ventricular surface (Fig. 3A), in the mitotic zone, where cells are actively undergoing division (Sauer and Chittenden, 1959; Sidman et al., 1959). There does appear to be a dorsoventral gradient within the telencephalon, with the more intensely immunoreactive cells located in the dorsal wall compared to the ventral wall (Fig. 24–C). In addition, particularly heavily labeled cells were noted in two nontelencephalic areas: the olfactory placode (Fig. 2B), which gives rise to the olfactory epithelium, and the ventricular zone of the dorsal diencephalon (data not shown), which gives rise to the anterior thalamus.

Like the EGF receptor, the pattern of collagen type IV immunoreactivity within the ventricular zone is uniform throughout the rostrocaudal extent of the telencephalon (Fig. 24–C). However, there is no dorsoventral gradient of labeling around the precursor cells adjacent to the ventricular surface. In sharp contrast, there is a gradient in the portion of the ventricular zone away from the lumen, known as the synthetic zone, where DNA synthesis occurs (Sauer and Chittenden, 1959; Sidman et al., 1959); the intensity of collagen type IV is much higher in the dorsal part of this region compared to that observed more ventrally (Fig. 24–C). Perhaps most striking, there is a virtual absence of collagen type IV staining in the olfactory placode (Fig. 2B), demonstrating that not all embryonic epithelial tissues express high levels of collagen type IV; the dorsal diencephalon, however, shows a similar pattern to that seen in the cortex.
EGF receptor

Collagen IV

Figure 3. Photomicrographs of coronal sections through the cerebral wall at E11 (A and B), E12 (C and D), E14 (E and F), and E15 (G and H), demonstrating the changes in EGF receptor and collagen type IV expression during early corticogenesis. Intense EGF receptor immunoreactivity is observed in the ventricular and subventricular zones (VZ and SVZ) at all ages. In contrast, although collagen type IV staining is prominent around the cells at the ventricular surface, there is a marked reduction in intensity in the outer region of the VZ and the SVZ as development proceeds. At E14/15, postmitotic neurons in the preplate (PP) and intermediate zone (IZ) do not express the receptor, although both regions are positive for the matrix molecule. Neurons that have ceased migrating to form the cortical plate (CP) reexpress the EGF receptor, while this region is essentially devoid of collagen type IV staining. Scale bar, 50 μm.

E12

This is the first age at which the dorsolateral portion of the telencephalic vesicles that form the cerebral cortices is clearly distinguishable from the invaginations of the ventrolateral ventricular zone that produce the basal telencephalic structures (Bayer and Altman, 1991). Differences in the intensity of EGF receptor immunoreactivity between these two regions of the ventricular zone, however, are not as pronounced as at earlier stages. In addition, the intensity of EGF receptor immunoreactivity is more uniform across the width of the ventricular zone compared to E11 (Fig. 3C). This stage marks the onset of neurogenesis in the basal telencephalon; those cells that have exited the cell cycle and move out of the ventricular zone are no longer EGF receptor immunoreactive (data not shown). In contrast to the EGF receptor, the pattern of collagen type IV immunoreactivity is essentially unchanged between E11 and E12 (Fig. 3D).

E14

The ventricular zone can now be distinguished from the superficial outer edge of the developing cortex, the preplate. The latter region is comprised of the first cortical cells that leave the cell cycle, primarily the Cajal-Retzius cells, that will reside in layer I of the cortex, and the subplate neurons (Marín-Padilla, 1978; Luskin and Shatz, 1985; Chun and Shatz, 1989). Virtually all the cells located within the developing preplate are EGF receptor negative, while those in the ventricular zone remain EGF receptor positive (Figs. 2D-F, 3E). In contrast, collagen type IV immunoreactivity is evident in the preplate at this stage (Fig. 3F). In the same tissue sections, however,
there is a dramatic decrease in the intensity of collagen type IV staining within the superficial layers of the ventricular zone itself. The most intensely EGF receptor-immunoreactive cells are those of the emerging subventricular zone (Fig. 3E), although little collagen type IV is present in this region. The cells in this zone are mitotically active and are primarily the progenitors for the late-generated neurons and glia of the cortex. The most dense collagen type IV staining remains at the ventricular surface surrounding actively dividing cells (Fig. 3F).

**E15**

At this age, the subventricular zone has expanded and remains the region that exhibits the most intense immunoreactivity for the EGF receptor (Fig. 3G); as at E14, there is little collagen IV immunoreactivity in this region (Fig. 3H). The intermediate zone, now visible between the subventricular zone and the preplate, contains migrating neurons destined for the cortical plate and cortical afferent and efferent axons. Cells in this zone are EGF receptor negative (Fig. 3G), although the region is immunoreactive for collagen type IV (Fig. 3H). In the more lateral parts of the cortex, deep layer neurons have reached the cortical plate, dividing the preplate into the marginal zone and subplate. The neurons within the cortical plate and subplate once again express the EGF receptor (Fig. 3G). Interestingly, the marginal zone and subplate are becoming more densely immunoreactive for collagen type IV, while the cortical plate is less intense (Fig. 3H).

**E17**

By this stage, the ventricular zone constitutes a much smaller proportion of the developing cerebral wall. The cells within the ventricular zone remain both EGF receptor and collagen type IV positive, as do the cells of the subventricular zone (Fig. 4A–C). The cells of the intermediate zone, however, are negative for both proteins. Neurons within the cortical plate, now comprised of several layers, and the subplate are also EGF receptor positive (Fig. 4A). Outside the ventricular zone, the distribution of collagen type IV immunoreactivity is not uniform (Fig. 4B), with light staining in the subventricular zone and more dense immunoreactivity in the subplate, deep layers of the cortical plate and marginal zone. Little, if any, collagen type IV immunoreactivity is observed in the upper layers of the cortical plate, except around the developing vascular bed.
EGF receptor

Collagen IV

Figure 5. Photomicrographs of sagittal sections through the E19 telencephalon illustrating the coexpression of EGF receptor and collagen type IV in the rostral extension of the subventricular zone (indicated by arrows in C and D). The boxed areas in A and B are shown at higher magnification in C and D. Scale bar, 600 μm for A and B, 100 μm for C and D.

E19

At this late stage of embryonic development, the ventricular zone is no longer a dominant feature of the cerebral wall, and there has been a noticeable expansion of the cortical plate. The pattern of both EGF receptor and collagen type IV immunoreactivity is similar to that at E17 (Fig. 4D-F), although occasional, fine projections of collagen type IV immunoreactivity extend upward from the subplate into the cortical plate. An additional area of EGF receptor immunoreactivity appears at this time: olfactory neurons, originating at the anterior portion of the subventricular zone, migrate to their final destination in the olfactory bulb in the rostral migratory stream (Luskin, 1993). These cells, more clearly identified in sagittal sections, are intensely immunoreactive for the EGF receptor (Fig. 5A,C). In addition, the pathway that these cells follow to the olfactory bulb is heavily stained for collagen type IV (Fig. 5B,D).

Discussion

The present study represents the first detailed description of the expression patterns of collagen type IV and the EGF receptor protein in fetal brain development. The EGF receptor signaling system has been an increasing focus of developmental studies in vitro (Anchan et al., 1991; Casper et al., 1991; Lillien and Cepko, 1992; Reynolds et al., 1992; Tucker et al., 1993; Ferri and Levitt, 1995), but other than in the retina (Lillien and Cepko, 1992) there has been no information on the embryonic distribution of the receptor in vivo. Additionally, only one study has been published previously on collagen type IV expression in developing CNS (Letourneau et al., 1988), with negligible immunodetection reported. We demonstrate here that both the EGF receptor and collagen type IV are expressed by progenitor cells in the proliferative zones of the developing telencephalon throughout embryonic development. Moreover, the changing spatiotemporal distribution of these proteins within the cortex is consistent with our previous transplantation and culture studies (Barbe and Levitt, 1991; Ferri and Levitt, 1995). First, neurons derived from E12 presumptive perirhinal cortex express LAMP in vitro, even in the presence of an antibody to the EGF receptor, suggesting that they have been exposed to the appropriate signals in vivo prior to culturing (Ferri and Levitt, 1995). We observed intense immunoreactivity for both the EGF receptor and collagen type IV in the cortical neuroepithelium as early as E11. Second, E12 sensorimotor precursors differentiate into neurons that express LAMP when grown in the presence of EGF/TGFα (Ferri and Levitt, 1995), indicating that these cells have a functional EGF receptor. Indeed, at E12, progenitors located in the dorsal cerebral wall, that gives rise to sensorimotor cortex, are EGF receptor immunoreactive. Third, both in vitro (Barbe and Levitt, 1991) and in vitro (Ferri and Levitt, 1995), postmitotic neurons from the sensorimotor region are unable to alter their fate and become LAMP positive, even when exposed to the appropriate environmental signals; for example, when TGFα is added to cultures of sensorimotor precursors 60 hr after plating, when almost no cells are mitotically active, there is no induction of LAMP expression. The present study demonstrates that postmitotic neurons in the preplate and intermediate zone are no longer immunoreactive for the EGF receptor, and thus probably have lost their ability to respond to EGF/TGFα. Finally, nonlimbic neurons, transplanted at E17, fail to express LAMP when placed in the host perirhinal cortex (Barbe and Levitt, 1991), even though these cells are EGF receptor immunoreactive. When considered in light of our in vitro data (Ferri and Levitt,
1995), it appears that the absence of collagen type IV in the cortical plate at later embryonic stages may limit the ability of older neurons to respond to signals from the host environment to alter their molecular phenotype. Alternatively, postmitotic neurons, once they are committed to a particular phenotype, may downregulate certain genes, such as notch, that had previously allowed them to respond to differentiative signals (Fortini et al., 1992; Coffman et al., 1995). It will be important to examine the behavior of older neurons (E17/E19) in vivo, in the presence of both TGFs and collagen type IV, to distinguish between these two possibilities.

Potential Mechanisms for Specifying a Limbic Phenotype

The distribution of the EGF receptor and collagen type IV alone is not sufficient to define specific populations of cells within the ventricular zone that will give rise only to limbic cortical areas at later stages in development. Although the present study allows us to distinguish gross differences in the intensity of EGF receptor immunoreactivity within different regions of the cortex, it is not possible to determine the absolute number of receptors present on any one cell. If the number of EGF receptors expressed by each progenitor reflects that potential of that cell to respond to EGF/TGFs, it may be that precursors located in presumptive sensorimotor cortex have fewer receptors than those located in presumptive perirhinal cortex. The ability of a cell to regulate the number of EGF receptors that it expresses appears to be an important mechanism in controlling cellular differentiation (Lilien, 1995). Alternatively, other molecules may be required in vivo to specify limbic cortical neurons. For example, a restricted distribution of EGF and/or TGFs would be one means of generating molecular heterogeneity in the microenvironment of the cortical precursor cells. To date, the anatomical distribution of either growth factor has not been examined at the early, critical stages of cortical development, although the mRNA for both ligands can be detected by Northern blot analysis as early as E14 in the mouse (Lazar and Blum, 1992).

Recently, a family of ligands in the EGF/TGF family superfamily, variously named heregulins (Holmes et al., 1992; Marchionni et al., 1993), Neu differentiation factor (Peles et al., 1992; Orr-Urtreger et al., 1993), glial growth factors (GGF; Shah et al., 1994), ARIA (Corfas et al., 1995), and neuregulins (Meyer and Birchmeier, 1994), have been implicated in early phenotypic decisions in the PNS. For example, culture studies suggest that these proteins can suppress neuronal differentiation while promoting or allowing glial differentiation by neural crest stem cells (Shah et al., 1994). A similar role in CNS development has been suggested by in situ analysis that reveals that, as early as E14, members of this family are expressed in unique patterns in the developing cerebral wall, including the ventricular and subventricular zones (Marchionni et al., 1993; Orr-Urtreger et al., 1995; Corfas et al., 1995). It is not clear whether these molecules are involved in the signaling system we have identified, because studies to date have not demonstrated that members of the heregulin family of proteins will activate the EGF receptor (Plowman et al., 1993a). Instead, the receptors activated in response to this family of ligands, p180\textsuperscript{mem}/HER4 and p185\textsuperscript{mem}/HER2 (Bargmann et al., 1986; Plowman et al., 1993a,b; Tzahar et al., 1994), may represent alternate signaling pathways that specify other area-specific phenotypes.

Heterogeneity of signaling within the ventricular zone could also be established through a differential distribution of a variety of receptors. In such a scheme, the full complement of receptors present on each precursor would influence its response to a particular ligand. For example, neuregulin is not able to affect all cell types that express p185\textsuperscript{mem}/HER2 (Plowman et al., 1993b); thus, the anatomical distribution of this receptor component alone does not define the neuregulin-responsive population. There is now evidence that, in the absence of p180\textsuperscript{mem}/HER4, no interaction between p185\textsuperscript{mem}/HER2 and neuregulin can occur (Plowman et al., 1993b). It is the overlapping distribution of these two receptors that defines the neuregulin-responsive cells. Recently, a similar scenario has been demonstrated for the ciliary neurotrophic factor/leukemia-inhibitory factor (CNTF/LIF) family of ligands. CNTF and LIF share two receptor components that are widespread throughout the body. The addition of the CNTF component to the LIF receptor complex is sufficient to convert a functional LIF receptor into a functional CNTF receptor (Ip et al., 1993). In this way, the CNTF component, which is mostly restricted to the nervous system, uniquely characterizes a subset of LIF-responsive cells that can also respond to CNTF. It is possible that, in a similar manner, the EGF receptor comprises only one component of a larger receptor complex in certain precursors within the ventricular zone. The broad distribution of the EGF receptor, as seen in this study, would permit the widespread action of EGF/TGFs throughout the cerebral cortex. The limited distribution of another component would then restrict the action of an EGF-related protein to a subset of all EGF/TGF-responsive cells. This subpopulation would then give rise to a distinct phenotype.

Role of EGF Receptor Outside the Ventricular Zone

The importance of both the EGF receptor and collagen type IV in the induction of LAMP expression is perhaps best illustrated by two mitotically active populations of precursors in the telencephalon that arise at later embryonic stages. Although both populations express the EGF receptor, only one gives rise to LAMP-positive cells. This may reflect differential exposure to an environment containing collagen type IV. The first population represents the progeny of the anterior part of the subventricular zone that migrate along a highly restricted pathway to their final destination in the olfactory bulb (Altman and Das, 1966; Luskin, 1993; Lois and Alvarez-Buylla, 1994). Collagen type IV is distributed along this same migratory route, suggesting a role for this molecule in the development of these cells, for example, as a guidance factor. Recent observations indicate another potential role for collagen type IV in the differentiation of this population. First, unlike other migrating neurons, these cells continue to divide while progressing to the olfactory bulb (Luskin and Boone, 1994; Menezes et al., 1994). Thus, they are still able to respond to the environmental signals required for LAMP expression. Second, the environment through which these cells migrate is rich in cells synthesizing TGFs (Seroogy et al., 1993; Kornblum et al., 1994); this molecule may interact with the collagen type IV to induce LAMP expression. Indeed, mRNA encoding LAMP is first detected in the olfactory bulb at birth (B. S. Reinoso, personal communication), a time at which these cells are first reaching their final position.

The second population comprises cells of the subventricular zone, from which collagen type IV is virtually absent. These precursors primarily generate glia and do not express LAMP at any stage of development (Zacco et al., 1990). For this population, in the virtual absence of collagen type IV, EGF/TGFs may act primarily as a mitogen and not as a differentiation signal. In fact, these cells can be induced to proliferate in vitro in response to EGF, even when harvested from the adult (Reynolds and Weiss, 1992; Reynolds et al., 1992), and under appropriate conditions, can differentiate into neurons and glia.
Role of the Extracellular Matrix in Development

In the adult CNS, components of the extracellular matrix are generally associated only with blood vessels and the pial membranes. During development, some components, such as laminin (Letourneau et al., 1988; Liedi and Silver, 1988), fibronectin (Stewart and Pearlman, 1987; Chun and Shatz, 1988), and glycosaminoglycans (Nakanishi, 1983; Snow et al., 1990, 1991), are expressed transiently and their spatiotemporal distribution suggests a role in axonal elongation and guidance as well as neuronal migration. There is increasing evidence, however, that an interaction between extracellular matrix molecules and a variety of growth factors is critical in regulating the proliferation and differentiation of neural precursor cells. For example, the ability of acidic FGF to induce the stable differentiation of type 2 astrocytes from the 0-2A progenitor cell in vitro requires the presence of extracellular matrix (Lilien et al., 1990). Perhaps the best characterized matrix/growth factor interaction is that between fibroblast growth factor (FGF) and the heparan sulfate proteoglycans (HSPG); this interaction modulates the subsequent binding of FGF to its signal transducing receptor (Rapraeger et al., 1991; Yayon et al., 1991) and thus regulates the activity of the growth factor. Interestingly, in vitro studies indicate that the FGF-responsive cells themselves often synthesize and deposit the appropriate molecules into the extracellular matrix. For instance, endothelial cells secrete a distinct HSPG that modulates the ability of acidic FGF to stimulate their mitotic activity (Gordon et al., 1989). The neuroepithelium of the mouse, by altering the level of posttranslational glycosylation of a unique species of HSPG at different stages of development, regulates the species of FGF that will be specifically bound by the surrounding extracellular matrix. Thus, at E9, FGF-2 is preferentially bound while at E11, FGF-1 is the preferred species (Nurcombe et al., 1993). The recent colocalization of FGF and HSPG in the neuroepithelium of the mouse (Ford et al., 1994) and the lens capsule of the rat (Lovicu and McAvoy, 1993) suggests that the binding of FGF to a specific HSPG may immobilize the factor to a particular region of the developing embryo, thus limiting spatially the sites of action of the growth factor.

No comparable interaction between EGF/TGFβ and collagen type IV has so far been demonstrated, although collagen type IV is able to bind TGFβ1 (Paralkar et al., 1991). However, in addition to a possible role in ligand immobilization and presentation, the matrix may regulate receptor expression on the progenitor cells. The loss of EGF receptor immunoreactivity on the migrating cells coincides spatially with decreased levels of collagen type IV in the intermediate zone (Fig. 6). Reexpression in the cortical plate, where collagen type IV is virtually absent, could be triggered by the passage of cells through the subplate, where type IV collagen is very high (Fig. 6). This model could be tested in vitro, for example, where the expression of the EGF receptor can be monitored in relation to matrix proteins in the environment. The dynamic changes in receptor and matrix expression during cortical neurogenesis indicate that, at distinct stages of development, signaling through the same components may lead to unique physiological responses.

Notes

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