FEATURE ARTICLE
Role of Integrins in the Development of the Cerebral Cortex

Spatial and temporal changes in expression and function of integrin receptors in the developing cerebral wall parallel neurogenesis, radial glial differentiation, neuronal migration and the emergence of neuronal layers in the cerebral cortex. The distinct outcomes of integrin and extracellular matrix ligand mutations underscore the dynamic role they play in these processes during corticogenesis. The changing patterns of adhesive interactions mediated by integrins and their ligands across the cerebral wall during embryogenesis may set in motion developmental programs needed for progressive acquisition of different neuronal or glial phenotypes in the cerebral cortex. Here we discuss the role of integrins during cortical layer formation.

Introduction
The functionally critical laminar organization of the cerebral cortex emerges as a result of appropriate migration and placement of neurons during cortical development. Post-mitotic neurons migrate radially from the ventricular zone towards the pial surface, past previously generated neuronal layers (Rakic, 1971, 1972) to reach the top of the cortical plate, where they terminate their migration and assemble into layers with distinct patterns of connectivity. Radial migration of cortical neurons can occur in two distinct modes: locomotion or somal translocation (Nadarajah et al., 2001, 2002). In contrast, populations of GABAergic interneurons, originating from the lateral ganglionic eminence, migrate tangentially into the neocortex (Anderson et al., 1971, 1972; Letinic and Rakic, 2001; Maricich et al., 2001). Some of these neurons migrate ventrally towards the cortical ventricular zone prior to radial migration towards the pial surface (Nadarajah et al., 2002). Specific cell–cell recognition and adhesive interactions between neurons, glia and the surrounding extracellular matrix (ECM) are likely to play an important role in distinct patterns of neuronal migration, placement and differentiation within the cortex.

The integrin family of cell surface receptors is a major mediator of cell–cell and cell–ECM interactions. Integrins can efficiently transduce signals to and from the external cell environment to the intracellular signaling and cytoskeletal compartments, while modulating signaling cascades initiated by other cellular receptors. Functional integrin receptors are formed by membrane spanning heterodimers of α and β subunits. There are at least 18 α and 8 β subunits that form >20 different integrin receptors (Juliano, 2002). The α subunits play a determinate role in ligand specificity and physiological response of the individual integrin receptor. ECM ligands and other cell surface molecules, such as receptor tyrosine kinases (RTKs), G-protein coupled receptors (GPCRs), growth factor receptors, L1-CAM, or members of the tetraspanin family of proteins, can bind to or associate with integrin receptors. These interactions activate, directly or indirectly, intracellular signal transduction cascades involving focal adhesion kinase (FAK), the Src family kinase Fyn, MAP kinase, protein phosphatases, SH2–SH3 adaptors, Rho-family GTPases and phospholipid mediators (Clark and Brugge, 1995; Boudreau and Jones, 1999; Giancotti and Ruoslahti, 1999; Juliano, 2002). The activation of these signaling cascades ultimately results in a number of changes of integrin characteristics, such as spatial localization, internalization, ligand affinity, intracellular association with signaling proteins, interaction with the cytoskeleton and, finally, transcriptional modulation.

Differential Distribution of Integrin Receptors and their Ligands in the Developing Cerebral Wall
During the course of cortical development, spatial and temporal expression of different integrin subunits and their ligands suggests a critical role in cortical layer formation and plasticity. As summarized in Table 1, the expression of integrin receptor subunits in the developing cortex can be grouped into three different categories: ubiquitous; spatially and temporally regulated; and cell-type specific.

Of the β integrins, β1 and β3 integrin are expressed in all regions of the developing cerebral wall and their expression persists in the adult cortex (Cousin et al., 1997; Pinkstaff et al., 1999; Graus-Porta et al., 2001). β2, β3 and β4 integrins are not expressed in the cerebral cortex (Pinkstaff et al., 1999). β8 integrin was observed in the adult cortex primarily on neurons and oligodendrocytes (Cousin et al., 1997), whereas β1s is diffusely distributed throughout the neuropil (Nishimura et al., 1998). α1 and α2 integrins are expressed across the developing cerebral wall (Gardner et al., 1999; Dulabon et al., 2000), but in the adult cortex, α1 integrin appeared prominently only in layer V, whereas α2 integrin is expressed in layers II–VI (Pinkstaff et al., 1999; Rodriguez et al., 2000). α3 integrin is not detected in the mature brain (Pinkstaff et al., 1999). α4 integrin is expressed in layers II and III of the cerebral cortex (Pinkstaff et al., 1999). In contrast, α4 integrin can be found throughout the cortex on cell bodies and apical dendrites (Bi et al., 2001). α5 integrin is highly expressed in the ventricular zone and cortical plate of the developing cerebral wall (Georges-Labouesse et al., 1998) and its expression is restricted to layer VI in the mature cortex. α6 integrin is diffusely expressed, primarily in the adult cortex (Pinkstaff et al., 1999). α6 integrin appears in dendrites of layer V–VI of the cerebral cortex as early as embryonic day 16 (Einheber et al., 1996; Pinkstaff et al., 1999). α7 integrin is expressed on radial glia fibers of the developing cerebral cortex and in glial fibrillary acidic protein (GFAP) positive astroglial cells in mature cortex (Hirsch et al., 1994; Anton et al., 1999). Developmental changes in the cell surface integrin repertoire and functional modulation in the cell surface integrin behavior in the developing cerebral cortex by altering the strength and ligand preferences of cell–cell adhesion during development.

Expression of integrins occurs in a continuously changing ligand environment during corticogenesis. These ligands are ECM
components, such as fibronectin, tenascin, thrombospondin, glycosaminoglycans, laminins, reelin and integrin-associating molecules such as CD9 and L1-CAM (O'Shea et al., 1990; Sheppard et al., 1991; Dulabon et al., 2000; Schmid and Maness, 2001). Laminin, though expressed primarily in the basement membrane associated with the pia matter of the cerebral cortex, is also thought to be present in the ventricular zone, subplate and marginal zone of the developing cerebral wall. Its expression along routes of migrating neurons implies that glial laminin may serve as a substrate for neuronal attachment (Liesi, 1990; Hunter et al., 1992). Laminin-2 (merosin), which binds to β1 integrin and whose deficiency causes muscular dystrophy in humans, is distributed punctually on cortical neuronal processes. Fibronectin is initially found in the ventricular zone throughout the telencephalic vesicle, where it may serve to cell division and cell fate during neurogenesis. Eventually, fibronectin is expressed in radial glia, migrating neurons and cortical plate neurons during layer formation (Sheppard et al., 1991, 1995). Both laminin and fibronectin may associate with chondroitin-sulfate proteoglycans (CSPGs) and modulate neuronal adhesion (Snow et al., 1996). CSPGs are highly expressed in the ventricular zone, preplate and preplate derivatives during cortical development. In vitro assays with thalamic neurons suggest that CSPGs may constitute barriers for neuronal migration and neurite extension, with different CSPGs functioning either as attractants or repellants (Emerling and Lander, 1996).

Secondary deficits in CSPG expression in the developing cortex in mice deficient in MARCKS (a neural substrate for protein kinase C) result in widespread neuronal ectopia in the forebrain (Blackshear et al., 1997). Expression of both fibronectin and CSPGs declines rapidly as the cortex matures. In contrast, tenascins are not expressed in cortex until late in development, when radial glia start to differentiate into astrocytes (Sheppard et al., 1991). The significance of the expression of different ECM proteins in the basement membranes of the developing cortex is evident in the disrupted corticogenesis seen in mice deficient in the ECM components perlecan (Costell et al., 1999), laminin α5 chain (Miner et al., 1998), or laminin γ1 nitrogen binding site (Halfter et al., 2002). They are characterized by abnormal basal lamina assembly, altered radial glial development and dysplasia of neurons in the developing cortical plate. In humans, secondary deficiencies in basal lamina assembly may lead to cobblestone lissencephaly, where gaps in basal lamina enable neurons to migrate out of the developing brain to form ectopias (Buxhoeveden and Casanova, 2002; Moore et al., 2002; Olson and Walsh, 2002).

Integrins are also capable of synergizing with other cell surface receptor systems in order to finely modulate a cell's adhesive behavior in response to multiple environmental cues. Members of the tetra-membrane-spanning (tetraspanin) protein superfamily, including 5span-5, CD9, CD65, CD81, CD82 and CD151, can associate with integrins and regulate their activity (Berditchevski and Odintsova, 1999; García-Frigola et al., 2001). Low levels of CD9 are diffusely expressed in the developing brain in cell types including neuronal progenitor cells, astrocytes, microglia and oligodendrocytes. CD9 associates with β1 integrins to modulate cell motility and adhesion. CD63 is expressed on both CNS neurons and astrocytes, whereas CD81 is localized to the ependyma, choroid plexus, astrocytes and oligodendrocytes of the developing cortex (Kelic et al., 2001). CD151 is present only at very low levels in the developing brain (Hasegawa et al., 1997). β1 integrins can also interact with the membrane spanning neural cell adhesion molecule L1-CAM (Silletti et al., 2000), which is expressed on neurons in the intermediate and marginal zones and the subplate of developing cortex (Demyanenko et al., 1999). L1-integrin interactions are critical for modulation of neuronal migration during development (Thelen et al., 2002).

The combination of distinct integrin receptor subunit expression and changing availability of types and levels of ligands may enable developing cortical neural cells to display different adhesive properties and activate different intracellular signal transduction pathways specific to particular integrin–ligand combinations. Distinct changes in neuronal function, shape, process extension, orientation, neuron–glia interactions and glial differentiation thus generated may lead ultimately to the emergence of neuronal layers in the cerebral cortex. This is evident in the cortical phenotypes of different integrin mutants.

### Cortical Abnormalities in Mice Deficient in Integrin Subunits

Different α integrin subunits dimerize preferentially or exclusively with β1 integrin, which is ubiquitously expressed in the developing cerebral cortex. Knockout mice have been created for nine of the α subunits that can dimerize with β1. Of these, early embryonic lethality (before E9) of α2 and α3 mutants precludes analysis of cortical development. Cortical phenotypes of α1, α4, α5, α6, α9 and α9 deficient mice are either normal or yet to be characterized carefully. However, distinct cortical malformations were found in α5, α6 and α9 knockout mice (Table 2).

Mice homozygous for a targeted mutation in the α5 integrin gene die soon after birth with severe defects in the development of the cerebral cortex, lungs, skin and kidneys (Kreidberg et al., 1996; Anton et al., 1999). In the cerebral cortex, laminar...
organization of neurons is lost and neurons are positioned in a disorganized pattern. \( \alpha_3 \) integrin modulates neuron–glia recognition cues during neuronal migration and maintains neurons in a gliophilic mode until glial-guided neuronal migration is over and layer formation begins (Anton et al., 1999). The gliophic to neurophilic switch in the adhesive preference of developing neurons and premature radial glia differentiation in the absence of \( \alpha_3 \) integrin were hypothesized to underlie the abnormal cortical organization of \( \alpha_3 \) integrin mutant mice. Reelin, an ECM protein released from the layer I cortical neurons, has been shown to interact with \( \alpha_3 \beta_1 \) integrin by several independent methods (Dulabon et al., 2000). During glial-guided migration to the cortical plate, neuronal \( \alpha_3 \) integrin may interact with glial cell surface molecules such as laminin-2 or fibronectin and at the top of the cortical plate, the ligand preference of \( \alpha_3 \) integrins may change from radial glial cell surface ECM molecules to reelin. Proteolytic activity of reelin may also degrade fibronectin or laminin at the top of the cortical plate (Quattrrochi et al., 2002). Different ligands or ligand concentration can determine the surface levels of integrins by regulating the rate at which integrin receptor is removed from the cell surface. Ligands can also regulate polarized flow of integrins towards or away from growth cone membranes (Lawson and Maxfield, 1995; Condic and Letourneau, 1997; Grabham and Goldberg, 1997). Thus, changes in the availability, function and ligand preference of \( \alpha_3 \) integrins may trigger the decrease in a migrating neuron’s bias for gliophilic adhesive interactions and promote neurophilic interactions needed for neurons to detach from radial glial guides and organize into distinct layers. Interestingly, deficiencies in \( \alpha_3 \) integrin ligands, laminin-2 and reelin lead to cortical anomalies such polymicrogyria or lissencephaly in humans (Sunada et al., 1995; Hong et al., 2000).

In contrast to \( \alpha_3 \) integrin, \( \alpha \nu \) integrins appear to provide optimal levels of basic cell–cell adhesion needed to maintain neuronal migration and differentiation. Substantial disruption of cellular organization in cerebral wall and lateral ganglionic eminence is seen at E11–12 in \( \alpha_3 \) null mice. Extensive intracerebral hemorrhage in \( \alpha_3 \) deficient mice, beginning at E12–13, prevents further evaluation of cortical development in late surviving (until birth) \( \alpha_3 \) null mice (Bader et al., 1998). \( \alpha_3 \) integrins expressed on radial glial cell surface can potentially associate with at least five different subunits: \( \beta_1 \), \( \beta_3 \), \( \beta_5 \), \( \beta_6 \) and \( \beta_8 \). Adhesive interactions involving fibronectin, vitronectin, tenascin, collagen, or laminin, ECM molecules that are found in the developing cerebral wall, can be mediated through these \( \alpha_3 \) containing integrins (Moyle et al., 1991; Hirsch et al., 1994). Both transient cell–matrix interactions and cell anchoring mechanisms that are mediated by different \( \alpha_3 \) containing integrins and their respective ligands are likely to medulate the process of glial development, neuronal translocation and differentiation in cerebral cortex.

In addition to \( \alpha_3 \) integrin, laminin isoforms in the developing cerebral cortex can also interact with \( \alpha_6 \) integrin dimers (Georges-Labouesse et al., 1998). \( \alpha_6 \) null mice die at birth.
(Georges-Labouesse et al., 1996), with abnormal laminar organization of the cerebral cortex and retina (Georges-Labouesse et al., 1998). Chain migration of neurons in the post-natal rostral migrational stream, from the subventricular zone to the olfactory bulb, also depends on \( \alpha _{6} \) integrin signaling (Jacques et al., 1998). Analysis of \( \alpha _{6} \) integrin deficient embryos revealed ectopic neuronal distribution in the cortical plate, protruding out to the pial surface. The cortical plate was further disorganized by wavy neurite outgrowth of ectopic neuroblasts. Coinciding abnormalities of laminin synthesis and deposition also occur in the mutant brain. Persistence of glial laminin throughout development may have prevented neuroblasts from appropriately arresting their migration in the developing cortical plate in \( \alpha _{6} \) null mice. Since cerebral cortical stem cells still exist in \( \alpha _{6} \) mutants, albeit abnormally, other integrin dimers may have overlapping functions with \( \alpha _{6} \) integrins during early cortical development. The similarities in the ligand preferences of \( \alpha _{6} \) and \( \alpha _{4} \) integrins are suggestive of potential functional overlap. The severe and novel cortical abnormalities in \( \alpha _{3}\alpha _{6} \) double knock-out mutants, i.e. disorganization of cortical plate with large collection of ectopias, aberrant basal lamina organization and abnormal choroid plexus, support a synergistic role for \( \alpha _{3} \) and \( \alpha _{6} \) integrins during cortical development (De Arcangelis et al., 1999). Deficiency in \( \beta _{1} \) integrin, which only associates with \( \alpha _{6} \), leads to an identical cortical phenotype. Mutations in either \( \alpha _{6} \) or \( \beta _{1} \) integrin in humans result in skin blistering (epidermolysis bullosa). However, the brain phenotype of the affected patients is unknown. \( \beta _{1} \) integrin in the cerebral cortex can dimerize with at least 10 different \( \alpha \) subunits, including \( \alpha _{3}, \alpha _{6} \) and \( \alpha _{4} \). Most of the cortical specific \( \alpha \) subunits seem to dimerize only with \( \beta _{1} \) integrin and \( \beta _{1} \) integrin deficiency leads to lethality around E5.5 (Fassler and Meyer, 1995; Stephens et al., 1995). In an attempt to study the role of \( \beta _{1} \) integrin in the developing cortex, \( \beta _{1} \) integrin-floxed mice were crossed with nestin-cre mice, resulting in widespread inactivation of \( \beta _{1} \) integrins in cortical neurons and glia from E10.5 (Graus-Porta et al., 2001). Cortical layer formation is disrupted in these mice, in large part as a result of defective meningeal basement membrane assembly, marginal-zone formation and glial end feet anchoring at the top of the cortex. BrdU birthdating studies suggest that glial-guided neuronal migration is not significantly impaired. However, perturbed radial glial end feet development may contribute to the defective placement of neurons in the cortex. Determination of the onset of radial glial abnormalities in \( \beta _{1} \) integrin deficient cortex (i.e. whether they occur prior to E18) and the use of quantitative bioassays for neuron–radial-glia interactions may clarify whether lack of pial anchoring of radial glial cells in \( \beta _{1} \) deficient cortex affects their ability to function concurrently as neuronal precursors and neuronal guides and contributes to the observed cortical phenotype. Furthermore, cortical neurons in \( \beta _{1} \) deficient mice invade the marginal zone in areas devoid of reelin producing Cajal–Reitzius (CR) cells and in regions with CR cell ectopias, accumulate underneath them. Invasion of neurons only into areas devoid of reelin producing CR cells supports a role for reelin in normal termination of neuronal migration. Since \( \alpha _{6}\beta _{1} \) integrin has been shown to regulate reelin mediated detachment from glial guides, it was expected that \( \beta _{1} \) deficient neurons would continue to migrate past CR cell ectopias, instead of accumulating underneath them (Graus-Porta et al., 2001). However, the absence of this phenotype indicates redundant functions for other known or novel reelin receptors in neuronal placement in the \( \beta _{1} \) deficient cortex. Alternatively, since \( \beta _{1} \) integrin is thought to modulate the gliophilic–neurophilic adhesive balance in vitro (Galileo et al., 1992; Anton et al., 1999; Hatten, 1999) and reelin mediated radial glial differentiation (Forster et al., 2002), \( \beta _{1} \) deficient neurons may have accumulated under CR cell ectopia due to an inability to regulate appropriate neuron–neuron or neuron–glia adhesion in response to reelin in the absence of \( \beta _{1} \) integrin. Given the varied cortical phenotypes of \( \alpha _{6}, \alpha _{3} \) and \( \alpha _{4} \) null mice and the ability of \( \beta _{1} \) to associate with multiple other cortical \( \alpha \) integrins, it is surprising that the \( \beta _{1} \) conditional phenotype is not more severe. This may reflect the transdominant, transnegative, or compensatory influences distinct integrin receptor dimers exert over each other and the ECM ligands in the developing cerebral cortex. For example, an increase in fibronectin and collagen IV activity is seen in \( \alpha _{6} \) null keratinocytes (Hodivala-Dilke et al., 1998). In vitro, binding of a ligand to a signal transducing integrin can initiate a unidirectional signaling cascade affecting the function of a different target integrin in the same cell (Simon et al., 1997; Blystone et al., 1999). Elucidation of whether such integrin crosstalk regulates patterns of neuronal development and interactions with specific ECM molecules in the developing cortices of various integrin null mice will be informative in understanding the role of integrins in corticogenesis.

Pathways that are hypothesized to be activated downstream of integrins in developing cortical neurons include the CDK5/p35 complex and dab1. Mutant mice deficient in p35, CDK5, or dab1 exhibit major defects in laminar organization of the cerebral cortex (Feng and Walsh, 2001; Olson and Walsh, 2002). CDK5 is a neuron-specific cyclin dependent kinase, whose activation is dependent on association with its p35 cofactor (Tasi et al., 1994). The p35/CDK5 complex has been shown to interact with Rac, a member of the Rho family of GTPases, the Lis1-interacting protein NUDEL and the microtubule-associated protein tau (Nikolic et al., 1998; Feng et al., 2000; Niethammer et al., 2000), thus providing several avenues through which it may affect cytoskeletal reorganization involved in distinct aspects of neuronal development. dab1 is a cytoplasmic adapter protein containing a PTB domain, which may interact with the NPXY motif in the cytoplasmic domains of \( \beta _{1} \) receptors. Filamin1, a cytoskeletal protein whose mutation results in periventricular heterotopia, can also bind to \( \beta _{1} \) and \( \beta _{3} \) integrins (Sharma et al., 1995; Loo et al., 1998). Furthermore, integrin signaling can also switch distinct responses of migration modulating growth factors, such as neuregulin (NRG) (Colognato et al., 2002). At present, the mechanisms of activation and regulation of these signaling pathways downstream of integrins during cortical development remain incompletely understood.

**Concluding Remarks**

Development of cortical cells occurs as they adhere to a diverse array of ECM matrix ligands via multiple integrins. Glial progenitors and neurons undergo distinctly different sets of adhesive interactions with their environment during corticogenesis. This may serve as an essential mechanism for the acquisition of distinct cortical neuronal or glial phenotypes. The functional hierarchy and diversity observed within integrins during the process of laminar organization in the cerebral cortex may have resulted from their effects on ECM organization, differences in their modes of association with the cytoskeleton, interactions with non-ECM neuronal cell surface molecules such as tetraspan proteins or EGF receptors, integrin crosstalk and the distinct intracellular signaling cascades induced in response to ligand binding. Molecular analysis of human and mouse cortical malformations has uncovered several critical links between ECM, integrins and downstream signaling molecules during corticogenesis. Further profiling of developmental distribution...
of integrin receptors using BAC-mediated transgenic approaches (Heintz, 2001), generation of mutant lines lacking multiple, functionally related integrin receptors and the use of cell type specific or inducible gene manipulation methods will enable the determination of the relative contributions of different integrins to the assembly of the cerebral cortex.

Notes
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