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# Intrinsic Polarity of Mammalian Neuroepithelial Cells

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**Progenitor cells in the mammalian forebrain can undergo either symmetric or asymmetric cell divisions by varying their cleavage orientation. In asymmetric divisions, cells distribute apically and basally localized proteins differentially to their daughters. Here we explore the intrinsic polarity of neuroepithelial cells in the developing telencephalon. Actin microfilaments are concentrated apically, forming beltlike structures that encircle spots of γ-tubulin immunoreactivity. Staining for N-cadherin, β-catenin, and the tyrosine kinase substrates pp120 and paxillin is also enriched at the lumenal surface, presumably due to the localization of these proteins at adherens junctions. Phosphotyrosine immunoreactivity is concentrated apically in rings, suggesting that adherens junctions are enriched for signaling molecules. In mitotic cells it appears that adherens junction proteins and phosphotyrosine immunoreactivity may be inherited either symmetrically or asymmetrically, depending on the cell's cleavage orientation during mitosis. The differential inheritance of junctional proteins may determine whether a daughter cell can respond to extrinsic signals after mitosis.**

## INTRODUCTION

During the development of the neural tube, progenitor cells undergo a series of divisions that expand and maintain the precursor pool and produce the young neurons that will ultimately populate the adult nervous system. Neurogenesis occurs at different times in different regions of the neural tube, and the number of neurons produced varies from region to region. Thus the temporal and spatial regulation of neurogenesis plays a key role in constructing a basic scaffold for CNS development. In examining the morphology of dividing cells in the ventricular zone, several authors have speculated that the mitotic cleavage orientation of dividing precursors may regulate the production of neurons (Langman *et al.,* 1966; Martin, 1967). Time-lapse imaging studies in living slices through the ferret telencephalon support this notion (Chenn and McConnell, 1995). When cleavage planes are oriented perpendicular to the ventricular surface (vertical divisions), the resultant daughters are morphologically and behaviorally identical, appearing to reestablish a progenitor cell morphology. Prior to the onset of neurogenesis, when the neocortical progenitor population is expanding (Caviness *et al.,* 1995), nearly all cleavages are oriented vertically (Chenn and McConnell, 1995). Thus we have hypothesized that at early times during cortical development, vertical divisions are symmetric and both daughters may reenter the cell cycle. Horizontally oriented cleavages, in contrast, give rise to basally situated daughters that behave like young migratory neurons and apical daughters that remain within the ventricular zone. During early cortical neurogenesis in the ferret, the frequency of horizontal divisions increases over time in rough correspondence to the increasing rate of neurogenesis during development (Caviness *et al.,* 1995). These observations support the hypothesis that horizontal divisions are asymmetric and neurogenerative.

The observation of asymmetric divisions in the mammalian neuroepithelium has prompted an interest in the mechanisms by which the two daughter cells adopt different fates after mitosis. One possible mechanism is that the progeny are distinguished from one another at the outset by the differential inheritance of distinct factors. The asymmetric partitioning of cytoplasmic or membrane-associated determinants to daughter cells plays a key role in generating distinct cell fates in a

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variety of animals including *Caenorhabditis elegans* (Priess, 1994) and *Drosophila* (Rhyu *et al.*, 1994; Spana *et al.*, 1995; Kraut *et al.*, 1996). Indeed, at least two proteins are localized asymmetrically in progenitors of the cortical ventricular zone: immunoreactivity for the Notch1 protein is localized to the basal pole of mitotic cells in ferret (Chenn and McConnell, 1995), while in similar cells in mouse, the Numb protein is found in an apical crescent (Zhong *et al.*, 1996). Both vertically and horizontally dividing cells show similar subcellular distributions of Notch1 and Numb; thus, the proteins will be distributed equally to both daughters following a vertical cleavage. In contrast, a horizontal cleavage will segregate the proteins unequally to each daughter, giving rise to molecularly distinct cells.

In addition to the asymmetric distributions of Notch1 and Numb, it has long been appreciated that neuroepithelial cells are morphologically polarized into apical and basolateral domains (Hinds and Ruffett, 1971; Shoukimas and Hinds, 1978; Astrom and Webster, 1991; Aaku-Saraste *et al.*, 1996), as are other epithelial cells. Cells of the ventricular zone are bipolar in shape. During interphase, each cell extends an apical process that abuts the lateral ventricle, into which it extends a single cilium, and a basal process that terminates in a flattened endfoot which contacts a basal lamina. (At later stages of development, when the ventricular zone is separated from the basal lamina and pial membranes by newly formed neurons, it is not clear whether the basal processes remain morphologically specialized.) Electron micrographs reveal that the apical processes of ventricular cells expand slightly at the ventricular lumen, where they are joined to one another by junctional complexes that correspond to the zonula adherens (Hinds and Ruffett, 1971; Shoukimas and Hinds, 1978; Aaku-Saraste *et al.*, 1996). While gap junctions are also found in the apical junctional complex, no tight junctions or desmosomes are seen after the time of neural tube closure (Aaku-Saraste *et al.*, 1996). During mitosis, when the cell collapses its bipolar morphology and rounds up at the ventricular surface, the junctional complex is retained (Hinds and Ruffett, 1971; Nagele and Lee, 1979; Astrom and Webster, 1991), similar to other polarized epithelial cells (Sandig and Kalnins, 1990; Woods and Bryant, 1993; Reinsch and Karsenti, 1994). In vertical divisions in the ventricular zone, the cleavage furrow progresses from the basal toward the apical cell surface, where it bisects the junctional apparatus between the two daughter cells. In contrast, in an electron micrograph from a horizontal cleavage, the apical daughter appears to inherit the entire junctional complex (Hinds and Ruffett, 1971). Thus vertical and horizontal cleavages differ in the distribution of the junctions and associated components to the resultant daughters, enabling cells to exploit their intrinsic polarity to create nonidentical daughters following horizontal divisions.

Adherens junctions play a structural role in maintaining the integrity of an epithelial sheet (Barth *et al.*, 1997), but more recently it has been appreciated that a variety of signaling molecules are concentrated at junctional complexes (Woods and Bryant, 1993; Barth *et al.*, 1997); for example, the receptor tyrosine kinase Sevenless and its ligand Boss are both localized apically in *Drosophila* imaginal disc epithelia (Banerjee *et al.*, 1987; Kramer *et al.*, 1991). Adherens junctions are beltlike junctions composed of cell adhesion molecules called cadherins, which are linked to a ring-like cytoskeleton of actin microfilaments by the catenins (β-catenin, α-catenin, and plakoglobin). In addition to their role in establishing and maintaining cell polarity (Cox *et al.*, 1996; Muller and Wieschaus, 1996), cadherins and catenins are actively involved in signaling by epithelial cells. Cadherins play a role in cell–cell adhesion and function as signal-transducing molecules that regulate proliferation and differentiation (Barth *et al.*, 1997). Cytoplasmic β-catenin appears to play an effector role in signaling by the Wnt/Wingless family of proteins, which is required for the proper determination of cleavage orientation in a number of asymmetric divisions in *C. elegans* (Rocheleau *et al.*, 1997; Thorpe *et al.*, 1997). In addition, the β-catenin found at adherens junctions is associated with other signaling molecules, including tyrosine kinases and phosphatases. Proteins containing PDZ domains, particularly the MAGUKs (membrane-associated guanylate kinase homologs), appear to play a central role in coupling receptors and signaling systems to the cytoskeleton through a common localization at junctional complexes (Ponting *et al.*, 1997). Mutation of the MAGUK protein LIN-2A in *C. elegans* both disrupts signaling through the LET-23 receptor tyrosine kinase and blocks its localization to junctions, suggesting that receptor localization is essential for signaling (Hoskins *et al.*, 1996; Simske *et al.*, 1996).

These studies raise the possibility that the differential inheritance of adherens junctions in asymmetrically dividing neuroepithelial cells may play an important role in regulating the ability of daughter cells to respond to extrinsic signals after mitosis. Such signals may regulate proliferation, differentiation, or other cellular behaviors. Little is known about the molecular components of adherens junctions in ventricular cells, although previous studies have shown that N-cadherin and the tight junction protein ZO-1 are localized api-

cally in neuroepithelial cells after neural tube closure in the chick, at a time when functional tight junctions have disappeared (Aaku-Saraste *et al.*, 1996). The present study explores the subcellular localization of a variety of other proteins, including cadherins, catenins, and substrates for kinase activity, and the organization of cytoskeletal elements in cells of the cortical ventricular zone. We find that progenitors segregate a variety of proteins to the apical side of the cell and provide evidence for the asymmetric inheritance of junctional proteins in horizontally oriented cell divisions.

## RESULTS

### *Polarized Distributions of F-Actin and γ-Tubulin Reveal Adherens Junctions and Centrosomes*

Adherens junctions are large protein complexes that are linked to a ring-like cytoskeleton of actin microfilaments (F-actin). Electron micrographs of mouse ventricular cells have shown that the apical processes of interphase cells contain microfilaments, approximately 60 Å in diameter, forming a circumferential bundle at the lumenal surface (Hinds and Ruffett, 1971; Nagele and Lee, 1979). Microfilaments are also contained in basal processes, but these show no apparent organization. In order to characterize further the normal distribution of actin in ventricular cells, sections through the embryonic day (E) 29 ferret cerebral wall were stained with rhodamine-conjugated phalloidin, a fungal protein that binds specifically F-actin. Examination of phalloidin-stained sections on a confocal microscope revealed that F-actin is highly concentrated in rings near the lumenal (apical) surface (Fig. 1A). This distribution is characteristic of the adherens junctions of many epithelia and corresponds to the position of the junctional complexes described previously in the ventricular zone (Hinds and Ruffett, 1971; Shoukimas and Hinds, 1978; Nagele and Lee, 1979; Aaku-Saraste *et al.*, 1996). There is little or no phalloidin staining elsewhere in the ventricular zone; however, the intensity and resolution of the staining may be insufficient to reveal the organization of small amounts of F-actin.

Electron micrographs have also revealed that the apical and basal processes of ventricular cells contain radially oriented microtubules and that paired centrioles are localized apically, adjacent to the cilium that extends into the lateral ventricle (Hinds and Ruffett, 1971). Centrioles are a common component of centrosomes, the major center of microtubule organization in eukaryotic cells (reviewed in Joshi, 1994). Centrosomes initiate the nucleation and assembly of microtubules at their minus ends, which remain anchored at the centrosome while the plus ends extend distally. Because γ-tubulin is thought to be responsible for microtubule nucleation and is physically associated with centrosomes (Stearns and Kirschner, 1994; reviewed in Joshi, 1994), the localization of γ-tubulin can provide an idea as to the overall polarity and organization of microtubules in cells. Sections through the ferret cortical ventricular zone were stained with a polyclonal rabbit antiserum against γ-tubulin (generously provided by Dr. T. Stearns; Stearns and Kirschner, 1994). In interphase cells, γ-tubulin is localized to discrete dots at the apical ventricular surface (Figs. 1B and 1D). In cells double-labeled for F-actin and γ-tubulin and viewed *en face* from the ventricular surface, each spot of γ-tubulin immunofluorescence at the lumen is encircled by a ring of F-actin (Fig. 1C). These results suggest that the

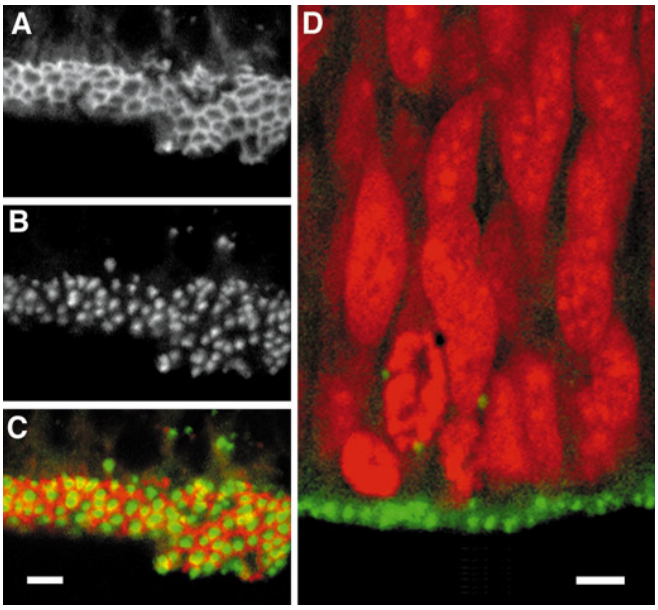


**FIG. 1.** Distribution of F-actin and γ-tubulin in the cortical ventricular zone of the E29 ferret. (A) Confocal image of rhodamine phalloidin staining to reveal F-actin. This *en face* view of the ventricular surface shows a ringlike distribution of F-actin, which presumably colocalizes with adherens junctions. (B) Confocal image of the same section using an antibody against γ-tubulin, which is concentrated in discrete puncta that mark the site of the centrosome. (C) Merged view of (A) and (B), with rhodamine phalloidin staining pseudocolored red and γ-tubulin staining in green. (D) Confocal image of a cross section through the ventricular zone (apical is to the bottom) showing immunostaining with an antibody against γ-tubulin (pseudocolored green) and nuclear morphology revealed with propidium iodide (pseudocolored red). Interphase cells appear to contain a single dot of γ-tubulin staining localized near the lumenal surface, but in mitotic cells two dots are visible and are located near the nucleus. Bar in (C) represents 4 μm for (A–C); bar in (D) represents 5 μm.

microtubules of interphase cells are oriented with their minus ends apical and emanate from microtubule organizing centers near the apical surface (Hinds and Ruffett, 1971; Mogensen *et al.*, 1997). In mitotic cells, two discrete dots of γ-tubulin staining were localized near the nucleus (Fig. 1D), consistent with the duplication and migration of centrosomes away from the lumen in preparation for mitosis (Karsenti, 1991).

### *Apical Localization of Proteins Associated with Adherens Junctions*

A variety of proteins colocalize to the adherens junctions of epithelial cells, including cadherins and catenins (Aberle *et al.*, 1996; Barth *et al.*, 1997), paxillin (Turner, 1994), pp120 (Shibamoto *et al.*, 1995), and focal adhesion kinase (Tani *et al.*, 1996). To assess the distributions of these and other proteins, sections of the cerebral wall from rats at E13, E15, and E17 were stained for immunohistochemistry using antibodies specific for each protein of interest. Western blot analysis confirmed the specificity of each antibody on E15 rat brain tissue (Fig. 2).

Apical concentrations of two key protein components of adherens junctions, N-cadherin and β-catenin, were apparent at all ages studied. N-cadherin staining was highly enriched at the apical ends of E13, E15, and E17 ventricular cells (Figs. 3A, 4A, and 4F), consistent with previous reports showing N-cadherin staining at the lumen of the neuroepithelium (Aaku-Saraste *et al.*, 1996). Similar results were obtained using a pan-cadherin antibody, but antibodies specific to E-cadherin failed to produce staining above background. Immunostaining with antibodies to β-catenin revealed a robust apical concentration of this protein at all three ages studied (Figs. 3B, 4B, and 4G). *En face* views of the ventricular surface revealed that β-catenin is enriched in ringlike structures at the lumen (Fig. 5A), reminiscent of the staining revealed by rhodamine phalloidin (Figs. 1A and 1C) that is inferred to localize to adherens junctions. These results suggest that N-cadherin and β-catenin are localized to adherens junctions in neuroepithelial cells. Although in many cell types β-catenin also functions outside of the junctional complex in a signaling pathway that transmits information from Wnt receptors to the cell nucleus (Rocheleau *et al.*, 1997; Thorpe *et al.*, 1997), we failed to see β-catenin localized to the nuclei of ventricular cells. Sections stained with antibodies against α- and γ-catenin failed to show staining above background (not shown) although γ-catenin was detectable in Western blots (Fig. 2). We did not detect staining using an antibody against ZO-1, a component of tight junctions that is concentrated apically in early neural tube cells (Aaku-Saraste *et al.*, 1996).

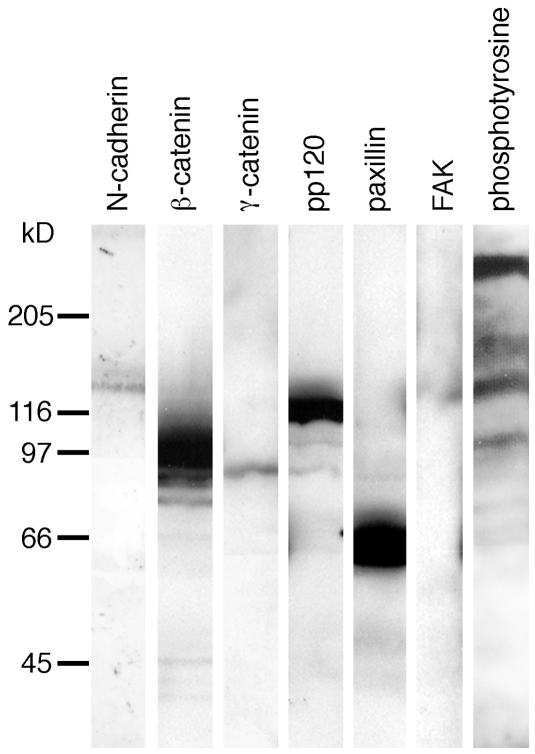


**FIG. 2.** Adherens junction proteins and phosphorylated proteins are present in the rat telencephalon. Western blots of ventricular zone extracts from E15 rat were probed with antibodies against various of adherens junction proteins and phosphotyrosine. Bands of N-cadherin (predicted molecular mass 135 kDa), β-catenin (92 kDa), γ-catenin (82 kDa), pp120 (120 kDa), paxillin (68 kDa), pp125FAK (125 kDa), and several presumably phosphorylated proteins are detected. The relatively low level of N-cadherin staining relative to that for β-catenin raises the possibility that other cadherins may also localize to the adherens junctions of ventricular cells.

pp120 (also known as p120cas) is a relatively new member of the catenin family that binds directly to the cadherin / β-catenin complex; it was identified originally as a substrate of the cytoplasmic tyrosine kinase pp60src (Reynolds *et al.*, 1994; Shibamoto *et al.*, 1995; Staddon *et al.*, 1995). Antibody staining of E13, E15, and E17 rat cortical tissue revealed that pp120 is concentrated apically at all three ages (Figs. 3C, 4C, and 4H). *En face* visualization of the ventricular surface reveals that pp120 immunoreactivity is enriched in rings at the lumenal surface (Fig. 5B), presumably due to the localization of pp120 at adherens junctions.

Paxillin is a cytoskeletal protein that is concentrated at sites of cell adhesion, where it can be phosphorylated by tyrosine kinases such as focal adhesion kinase

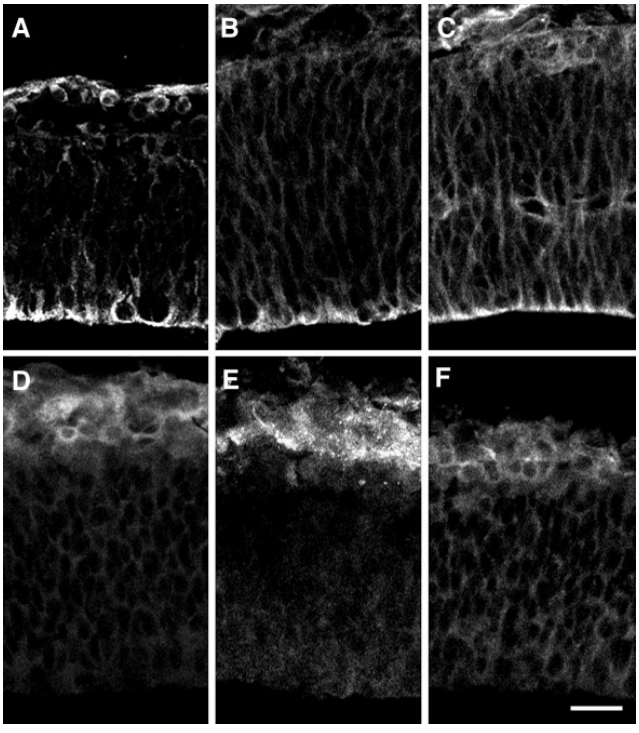


**FIG. 3.** Immunostained sections through the E13 rat ventricular zone reveal the distributions of (A) N-cadherin, (B) β-catenin, (C) pp120, (D) paxillin, (E) phosphotyrosine, and (F) pp125FAK. The pial surface of the brain is at the top and apical is at the bottom. Confocal images reveal that at E13, N-cadherin, β-catenin, and pp120, immunoreactivities are concentrated apically. Little staining is seen with antibodies against paxillin or phosphotyrosine, while pp125FAK has a diffuse cytoplasmic distribution in this section. Staining of cells at the pial (upper) surface of the brain is also seen in control sections (not shown). Bar represents 25 μm.

(pp125FAK) (Turner, 1994). pp125FAK is activated in response to signaling mediated by integrins and growth factor receptors (Schaller and Parsons, 1994). We found that paxillin is highly enriched at the apical surfaces of ventricular cells in E15 and E17 rat cerebrum (Figs. 4D and 4I). Interestingly, paxillin staining did not appear to be concentrated apically in the ventricular zone of E13 rats (Fig. 3D). Sections were also stained with an antibody against pp125FAK; although occasional sections suggested an apical distribution, most staining in ventricular cells appeared diffusely cytoplasmic (e.g., Fig. 3F).

### *Phosphotyrosine Immunoreactivity Is Enriched Apically in the Ventricular Zone*

In a number of polarized epithelial cell types, many signal transduction pathways appear to be highly localized to apical regions of the cell. To explore the possibility that signaling is localized in ventricular cells, we examined the distribution of molecules that might suggest the presence or activity of localized signal transduction pathways. Previous studies have shown that in epithelial cells of both vertebrates and *Drosophila*, tyrosine phosphorylation is highly localized to adherens junctions (reviewed in Woods and Bryant, 1993). In order to determine the distribution of phosphorylated tyrosines, and thereby localize regions of potentially high tyrosine kinase activity, sections of embryonic rat and E29 ferret ventricular zone were stained with one of two monoclonal antibodies which recognize phosphotyrosine residues. In both species, phosphotyrosine immunoreactivity is highly concentrated at the lumenal surface of the ventricular zone; in rat this staining is present at E15 and E17 (Figs. 4E and 4J). *En face* views of the ventricular surface reveal that phosphotyrosine staining forms rings (Fig. 5C), once again highly reminiscent of the distribution of F-actin (Figs. 1A and 1C). This ringlike distribution of phosphotyrosines suggests that signaling via tyrosine phosphorylation may be highly localized to the adherens junctions of ventricular cells. Interestingly, little phosphotyrosine immunoreactivity is visible in E13 rat brain sections, and there is no evidence for preferential staining at the lumenal surface (Fig. 3E). These data suggest that the number or the stability of signaling events involving tyrosine phosphorylation at the lumen becomes more prevalent at later embryonic ages. In epithelial cells, pp120 and paxillin are common targets of tyrosine phosphorylation at adherens junctions (Reynolds *et al.*, 1994; Turner, 1994). Western analysis of proteins containing phosphotyrosine residues in the E15 rat cortex reveals a number of bands (Fig. 2), but the identities of these proteins are not known.

### *Asymmetric Inheritance of Junctional Proteins*

The apical localization of cadherin, catenin, paxillin, and phosphotyrosine immunoreactivity in mitotic cells suggests that these proteins may be inherited differentially by daughter cells depending on the plane of a mitotic division. To visualize the distribution of these proteins directly in mitotic cells, immunostained sections were also stained with the vital nucleic acid binding dye Syto-11 or with propidium iodide to reveal cell nuclei. Figure 6 illustrates several mitotic cells undergoing cleavage at a variety of orientations. Although we cannot distinguish which junctional complex belongs to the mitotic cell(s) in each field, all of the proteins under study appear to retain an apical distribu-

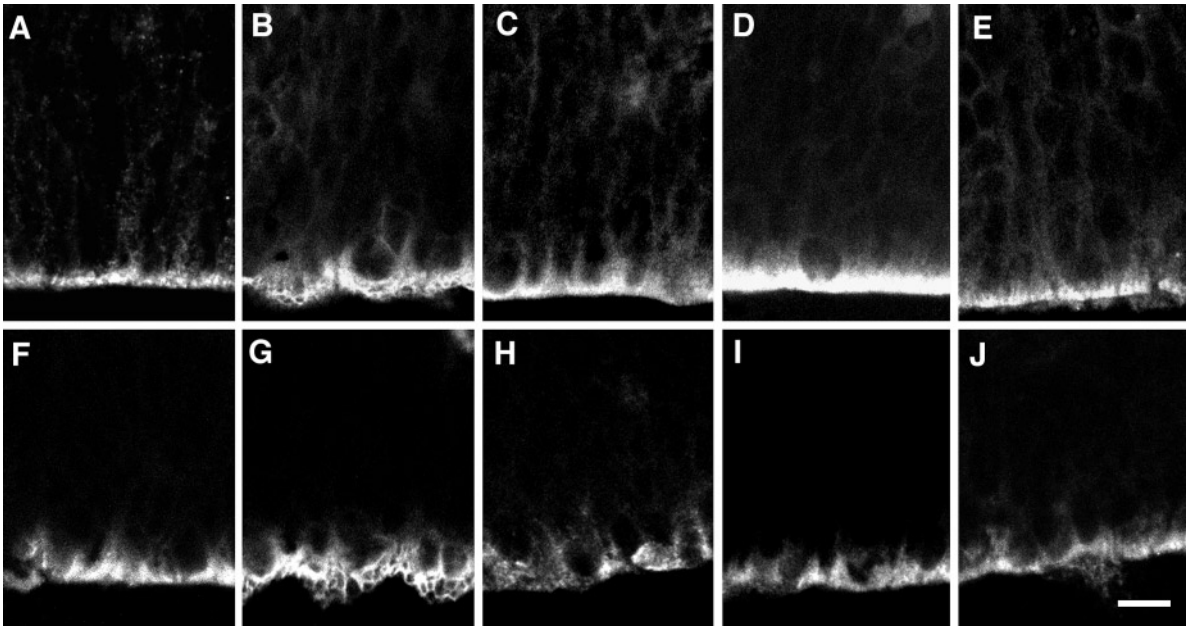


**FIG. 4.** Sections through the rat ventricular zone stained to reveal the distributions of (A,F) N-cadherin, (B,G) β-catenin, (C,H) pp120, (D,I) paxillin, and (E,J) phosphotyrosine at two different ages: E15 (A–E) and E17 (F–J). Apical is down. These confocal images show that all four proteins are concentrated at the lumenal surface of the ventricular zone at both ages. Ringlike staining for β-catenin is visible in (B,G), and is even more obvious in Fig. 5A. Bar represents 10 μm.

tion during mitosis. Thus in vertical cell cleavages, proteins such as β-catenin (Fig. 6A) and phosphotyrosine immunoreactivity (Figs. 6D and 6E) appear destined to be inherited equally by the two daughter cells. In horizontal divisions (Fig. 6C) the apical proteins are presumed to distribute preferentially to the apical daughter, consistent with the direct observation of the adherens junctional complex in electron micrographs (Hinds and Ruffett, 1971). It is not clear how junctional proteins would be distributed to daughters of cell divisions with intermediate cleavage orientations (Fig. 6B). If the inheritance of the junctional complex plays a role in determining the fates of daughter cells, the exact angle of cell cleavage would be expected to affect the distribution of these proteins. We therefore looked more closely at the cleavage orientations of mitotic cells in the ventricular zone.

### *Mitotic Orientations of Dividing Ventricular Cells*

The localization of α-tubulin in the ventricular zone reveals the mitotic spindles of dividing cells located near the lumenal surface (see Fig. 2 in Chenn and McConnell, 1995). The projected cleavage angles of metaphase cells in the E29 ferret ventricular zone were measured and tabulated for 102 mitotic cells (Fig. 7). When broken down into 10° bins, only about 50% were oriented within 10° of vertical (80°–90°); the remaining orientations were distributed fairly uniformly among all other orientations. Approximately 15% were oriented within 30° of horizontal, as reported previously (Chenn and McConnell, 1995). If the unequal inheritance of junctional proteins plays an important role in determining the fates of daughter cells following mitosis, it is interesting to consider the angle at which a progenitor must divide in order to allocate the entire junctional complex to one of the two daughters. This is not a simple calculation, because the ring of junctional proteins broadens in mitosis, compared to the smaller rings seen in interphase cells (Nagele and Lee, 1979). However, inspection of electron micrographs of ventricular cells in anaphase or telophase (Hinds and Ruffett, 1971, Figs. 25–28) suggests that cleavages at angles greater than ~30° away from vertical should generate one daughter that inherits the entire junctional apparatus and another daughter that fails to inherit this structure.

## DISCUSSION

Ventricular zone progenitor cells are intrinsically polarized cells that can undergo either symmetric or asymmetric divisions. To identify molecules that may

show a polarized distribution within ventricular cells, sections through the developing telencephalon were stained to reveal a variety of proteins that were likely to localize apically, due to their association with adherens junctions. Rhodamine phalloidin staining revealed that actin filaments form a beltlike structure at the cells' apical surface; in interphase cells these filaments encircle a spot of γ-tubulin immunoreactivity, marking the centrosome. Immunostaining with antibodies to cadherins, β-catenin, and tyrosine kinase substrates such as pp120 and paxillin showed that these proteins also form rings at the apical surface, presumably due to their colocalization with adherens junctions. Consistent with the suggestion from previous work that adherens junctions are enriched for signaling molecules (Woods and Bryant, 1993), phosphotyrosine immunoreactivity is concentrated at

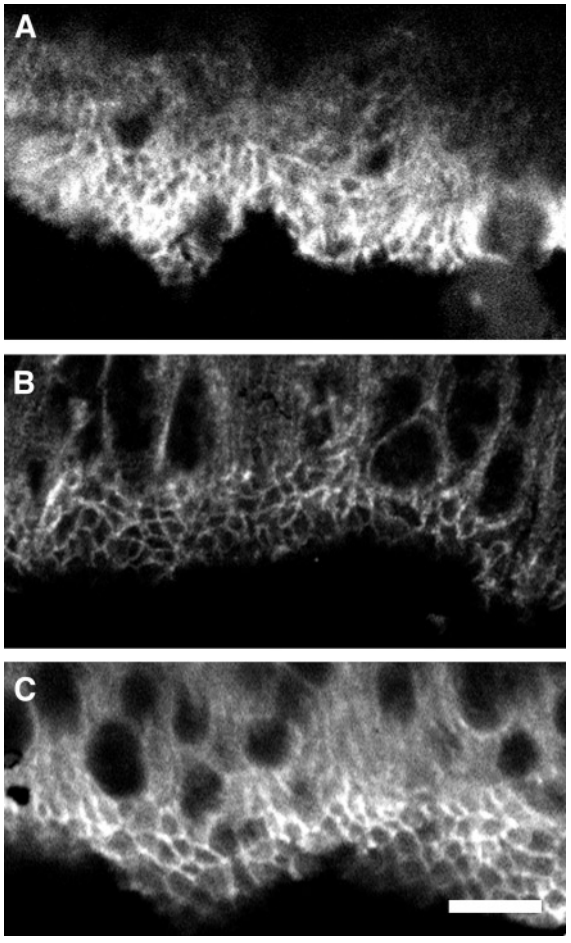


**FIG. 5.** Ringlike distributions of adherens junction proteins. These are confocal images of sections through the rat ventricular zone, viewed *en face* to reveal the ringlike distribution of (A) β-catenin at E15, (B) pp120 at E17, and (C) phosphotyrosine immunoreactivity at E15. Bar represents 10 μm.

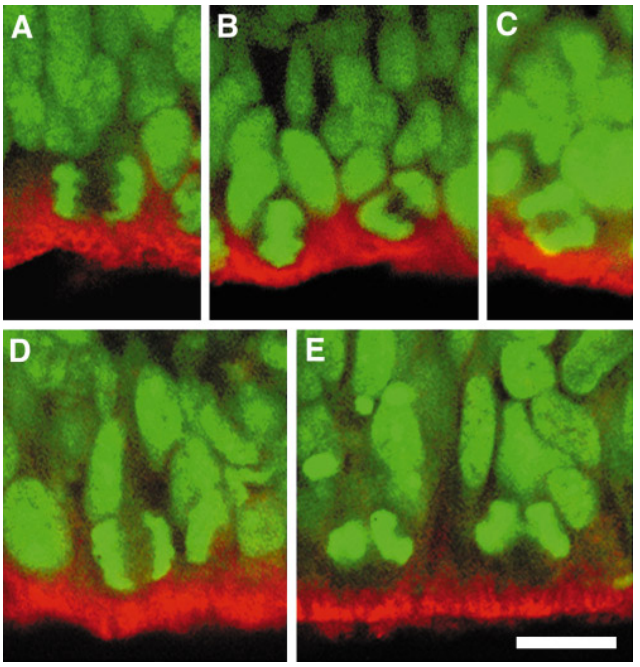


**FIG. 6.** β-Catenin and phosphotyrosine immunoreactivities remain localized apically regardless of cleavage orientation. Confocal images of sections through the E15 rat ventricular zone stained with antibodies against β-catenin (A–C) or phosphotyrosine (D–E), pseudocolored red, and with Syto-11 (A–C) or propidium iodide (D–E), pseudocolored green, to reveal the nuclei of cells in anaphase or telophase of the cell cycle. (A,D,E) Vertically oriented divisions. (B) A cell undergoing cleavage at an intermediate orientation is visible on the right, with a vertically oriented metaphase plate on the left. (C) A near-horizontal division. Bar represents 10 μm.

the lumenal surfaces of ventricular cells. Visualization of adherens junction proteins and phosphotyrosine in mitotic cells revealed that these molecules are present throughout the cell cycle and that they can be inherited either symmetrically or asymmetrically, depending on the cell's cleavage orientation during mitosis. These studies raise the possibility that the differential inheritance of adherens junction proteins in asymmetrically dividing neuroepithelial cells may determine the ability of a daughter cell to respond to extrinsic signals after mitosis. Alternatively, the junctional complex may have a structural role in anchoring apical daughters within the ventricular zone; basal daughters might not benefit from the inheritance of adhesive junctions that could impede their mobility as they initiate migration.

### *Adherens Junction Proteins and Cellular Asymmetry*

The present study has revealed that the apical region of ventricular cells is ringed by F-actin microfilaments

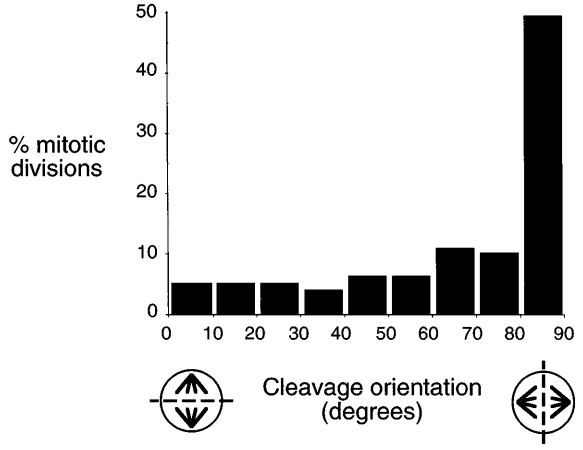


**FIG. 7.** Histogram showing the relative frequencies of different cleavage orientation in fixed sections of the E29 ferret cortical ventricular zone stained to reveal mitotic spindles. 49% of all divisions were oriented between 80° and 90°, and the remainder of the cleavages were distributed relatively evenly among all other orientations.

and a variety of proteins known in other systems to colocalize to adherens junctions, including N-cadherin, β-catenin, pp120, and paxillin. Adherens junctions have been described in the mammalian ventricular zone both histologically and by electron microscopy (Sauer, 1936; Sauer, 1937; Hinds and Ruffett, 1971; Shoukimas and Hinds, 1978). Adherens junctions form a thick belt around the apical ends of epithelial cells and are thought to play important roles in both cell adhesion and intracellular signaling. Calcium-dependent adhesion molecules, the cadherins, and the associated catenins are localized to the adherens junctions in other epithelia where they play important roles in controlling cell proliferation (reviewed in Barth *et al.*, 1997). Interestingly, adherens junctions appear to be involved with cell–cell interactions not only via cadherins and their associated catenins, but also through protein tyrosine kinase pathways. In epithelial cells of both vertebrates and *Drosophila*, most phosphotyrosine immunoreactivity is localized to adherens junctions (Woods and Bryant, 1993; Woods *et al.*, 1997). Furthermore, in *Drosophila*, a number of important signaling proteins are concentrated apically, including the receptor tyrosine kinase Sevenless, its ligand Bride of Sevenless (Boss), E-cadherin, the β-catenin homolog armadillo, and others (Woods and Bryant, 1993; Woods *et al.*, 1997). These observations suggest that adherens junctions form signaling centers in epithelial cells, possibly concentrating receptors and their target proteins in a common location to facilitate the efficient transmission of signals between cells.

Here we too have found that phosphotyrosine staining is concentrated in rings at the apical surfaces of ventricular cells, apparently colocalizing with the junctional complex. We do not know whether these phosphotyrosine residues were generated through the interaction of extracellular signaling molecules with cell surface receptors, the action of cytoplasmic tyrosine kinases, or a combination of both. However, it is appealing to speculate that at least some of this immunoreactivity results from the activation of cell surface receptors by mitogens that control proliferation or by inducers that control cell fate decisions. Studies of dividing cells suggest that adherens junctions in ventricular cells, as well as tight junctions, adherens junctions, and septate junctions in dividing intestinal epithelium and *Drosophila* epithelia, are maintained throughout the cell cycle (Hinds and Ruffett, 1971; Woods and Bryant, 1993). Thus, in the ventricular zone, a horizontal division results in the inheritance by the apical daughter of the entire junctional complex and associated signaling apparatus, while its basal sibling inherits none of these proteins. As a result of this unequal inheritance, it is plausible that basal daughters of asymmetric divisions are unresponsive to extrinsic factors, such as mitogens or inducers, and exit the cell cycle to enter the pathway for neuronal differentiation. Alternatively, apical and basal daughters may inherit autonomously acting determinants (such as Numb: Zhong *et al.*, 1996) that direct their subsequent behavior. We do not know the exact cleavage orientation that is required for the asymmetric inheritance of adherens junctions. However, inspection of electron micrographs (Hinds and Ruffett, 1971) suggests that cleavage angles as small as 30° away from vertical might be sufficient for one daughter to inherit the bulk of the junctional complex, at the expense of the other daughter.

### *The Control of Cleavage Orientation*

The existence of symmetric and asymmetric divisions raises the question of what mechanisms might control cleavage orientation. In both the budding yeast *Saccharomyces cerevisiae* and the early divisions of the nematode *C. elegans*, a site on the cell cortex appears to mark the location of the previous mitosis. In haploid budding yeast, subsequent budding occurs adjacent to the previous site of division (reviewed in Chant, 1996). In *C. elegans*, the cortical site marking the position of the first zygotic cleavage is recognized by the mitotic apparatus of the posterior (P1) cell (reviewed in Priess, 1994; Guo and Kemphues, 1996). Rotation of the mitotic apparatus appears to involve tension between the centrosome and

the cortical site. In contrast, the spindle of the anterior cell (AB) does not rotate; the mitotic apparatus apparently ignores the cortical cue. In mammalian neural progenitors, Rhyu and Knoblich (1995) have speculated that a cue localized to the lateral cell cortex could similarly direct a ventricular cell to divide with a vertical cleavage plane, while a cue localized in the apical–basal axis could direct horizontal divisions. Alternatively, there might be only a single cue to direct vertical cleavages; all other orientations would result from a masking of this cue and the consequent randomization of cleavage orientation. When the cleavage orientations of ferret ventricular cells were examined closely, only ~50% of divisions fell within 10° of vertical. The remaining cleavages were distributed roughly equally among all other orientations. These results support the second hypothesis that cleavage orientation is organized around cues that specify vertical division, but that the masking or removal of such cues results in the randomization of division planes. Although the large number of precisely vertical cleavages suggests that the orientation of cleavage planes is regulated tightly around 90°, neither morphological nor molecular evidence has suggested that a ''birth scar'' is left at the site of previous division in ventricular cells, as there is in yeast.

## EXPERIMENTAL METHODS

### *Immunohistochemistry*

F-actin and γ-tubulin were visualized after E29 and E36 ferret brains were fixed by perfusion with 4% paraformaldehyde. Sections were cut at 20 μm on a cryostat. The sections were postfixed by immersion in 70% ethanol (−20°C) for 10 min and blocked with 1% Triton X-100, 1 mg/ml bovine serum albumin (BSA), and 15 mg/ml goat serum in phosphate-buffered saline (PBS; 30 min, room temperature). To visualize γ-tubulin, sections were incubated in the rabbit polyclonal antiserum XG-1-4 (gift of T. Stearns; 1:500 in PBS with 1 mg/ml BSA and 1% Triton X-100 at room temperature overnight; the XG-1-4 serum was generated against *Xenopus* γ-tubulin and shows substantial cross-species recognition). Sections were then incubated with a Bodipy-conjugated goat anti-rabbit monoclonal antibody (The Jackson Laboratories; 1:200, 1 hr, room temperature) diluted in either rhodamine phalloidin (Sigma; 50 U/ml in PBS) to reveal F-actin or propidium iodide solution (Sigma; 5 mg/ml in PBS) to reveal cell nuclei.

For phosphotyrosine staining, E29 and E36 ferret embryos and E15 rat embryos were perfused with 4% paraformaldehyde. Twenty-micrometer cryostat sections through the cerebral wall were blocked for 10 min in PBT (0.3% Triton X-100, 0.5% BSA in PBS) at room temperature, incubated in anti-phosphotyrosine antibody (4G10, Upstate Biotechnology; or PY-20, Transduction Laboratories; 1:1000 in PBT; 2 h at room temperature), and then incubated with a FITC-conjugated goat anti-mouse secondary antibody (The Jackson Laboratories; 1:200 in PBT, 1 h at room temperature).

For all other staining, E13, E15, and E17 rat brains were perfused with 4% paraformaldehyde; 20-μm cryostat sections were blocked with 1% Triton X-100, 1 mg/ml BSA, and 15 mg/ml goat serum in PBS (30 min, room temperature), incubated with mouse monoclonal antibodies to β-catenin, γ-catenin, pp120, paxillin, or pp125FAK (Transduction Laboratories; 1:50 to 1:500 in PBT for 2 h at room temperature), and then incubated with a Cy-2-conjugated goat anti-mouse antibody (The Jackson Laboratories; 1:200, 1 h at room temperature). N-cadherin staining employed a rabbit antiserum (NcadEC1, 1:250, gift of Dr. David Colman) and a Texas-red-conjugated goat anti-rabbit antibody (The Jackson Laboratories; 1:200). Some sections were counterstained for nuclear morphology by incubation with 5 mM Syto-11 (Molecular Probes) for 5 min at room temperature.

Immunostained sections were visualized on a laser-scanning confocal microscope (Bio-Rad MRC 600), and images were compiled using the COMOS software package. Confocal images using different fluorochromes were merged with Adobe Photoshop. In all cases, controls with no primary antibody showed no staining in the ventricular zone.

### *Western Blots*

Caps of dorsal telencephalon were removed from embryonic rat brains and suspended in lysis buffer (10 mM Hepes, pH 7.5, 10 mM NaCl, 0.1 mM EDTA, and 0.1 mM EGTA) containing the Sigma Protease Inhibitor Cocktail. The tissue was quickly homogenized in Kontes Pellet Pestles (Fisher) before being mixed with an equal volume of 2× SDS sample buffer and being boiled for 2–5 min. The resulting protein samples were applied to 8% SDS–PAGE and transferred to nitrocellulose filters. The blots were probed with various antibodies (those used above for immunohistochemistry, all at a 1:1000 dilution) and then incubated with HRP-conjugated goat anti-mouse or anti-rabbit antibody (The Jackson Laboratories, 1:1000). The signal was visualized by the ECL Western blotting system (Amersham).

### *Mitotic Orientation of Dividing Ventricular Cells*

Microtubules were visualized as described previously (Chenn and McConnell, 1995). Briefly, E29 ferret brains were fixed in either 90% methanol (−20°C) or microtubule stabilizing buffer (80 mM Pipes, 5 mM EGTA, 1 mM $MgCl_2$, pH 6.8; Schulze and Kirschner, 1986) followed by 0.5% glutaraldehyde in the same buffer. Brains were embedded in paraffin and sectioned at 10 μm. Sections fixed with glutaraldehyde were immersed in PBS with 1 mg/ml $NaBH_4$ for 7 min to quench the glutaraldehyde. Sections were blocked with 1% BSA in PBS (1 h, room temperature), incubated with the mouse monoclonal antibody DM1α (Sigma; 1:500 in PBS with 1% BSA, 1 h, room temperature), and then incubated with a Texas-red-conjugated donkey anti-mouse secondary antibody (Jackson ImmunoResearch; 1:200 in PBS with 1% BSA, 1 h, room temperature). DM1α staining revealed microtubules, including those of the mitotic spindle. The orientation of each metaphase or anaphase spindle was measured relative to the ventricular surface, with an angle of 0° corresponding to a horizontal cleavage plane (spindle perpendicular to lumenal surface) and an angle of 90° corresponding to a vertical cleavage plane (spindle parallel to lumenal surface). Previous studies have revealed an excellent concordance between measurements of cleavage orientation in fixed cells using the DM1α antibody and in living cells using either DiI or Syto-11 to visualize mitotic divisions directly (Chenn and McConnell, 1995).

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