

Neurogenesis at the brain-cerebrospinal fluid interface

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Introduction

The complexity of the human brain is due in part to the flexibility of fundamental processes that govern neural progenitor proliferation (Rakic 2009). The challenge that evolution faced, and has solved so elegantly, is to provide developmental mechanisms that vary the size of the cortex phylogenetically, while assuring that changes in cortical size still provide a functionally integrated structure. The solution involves separating proliferating cells from postmitotic neurons. Cortical size is controlled by the proliferating cells, while independent mechanisms control the functional architecture of the cortex, acting in progenitors as well as through interactions between cortical cells and the neurons that interconnect with them. The flexibility in the control of cortical size seems to be provided in part by the presence of multiple types of progenitor cells (**Figure 1**) (Boulder Comm. 1970, Fietz & Huttner 2010, Kriegstein & Alvarez-Buylla 2009), whose patterns of cell division and cell specification are also controlled in interacting, but partially separable ways. Although our understanding of the mechanisms underlying mammalian cerebral cortical development has certainly benefitted from studies in lower vertebrates and invertebrates, humans have also proven a robust genetic system for the identification of key genes underlying cortical neurogenesis, many of which subserve the mitotic spindle. The mechanisms underlying the action of these “microcephaly” genes have been the subject of several recent reviews (Manzini & Walsh 2011, Thornton & Woods 2009). Here, we focus on how cell-extrinsic cues may help regulate neurogenesis by interacting with or complementing the mechanisms that regulate the mitotic spindle.

Proliferative elements in the developing cerebral cortex

Apical progenitors have cell bodies adjacent to the ventricular surface, defined as the apical surface. They include the initial population of neuroepithelial progenitors making up the early neuroepithelium, as well as later-appearing radial glial cells. Apical progenitors share many features with other epithelial cells, such as apical-basal polarity and side-to-side contacts with neighboring cells via adherens junctions. Neuroepithelial and radial glial cell processes generally extend from the apical ventricular surface to the basal lamina at the pial surface, though a population of short apical neural precursors with basal processes of more variable length have also been described in the ventricular zone (VZ) (Gal et al. 2006, Stancik et al. 2010). As apical progenitors proliferate, the single-cell thick neuroepithelium rapidly adopts a pseudostratified structure. Apical progenitors undergo interkinetic nuclear migration, which involves the apical-basal migration of their nuclei within the pseudostratified VZ during the cell cycle, with cells undergoing mitosis only at the apical ventricular surface (Kriegstein & Alvarez-Buylla 2009, Taverna & Huttner 2010). Neuroepithelial progenitors tend to divide symmetrically to generate pairs of daughter cells with progenitor cell fate, though some early neurons may be generated as well (Haubensak et al. 2004, Noctor et al. 2004, Noctor et al. 2007). During the peak of neurogenesis, radial glial progenitors favor asymmetric cell division, leading to pairs of daughter cells with distinct progenitor or early neuronal cell fate (Kriegstein & Alvarez-Buylla 2009). Since symmetrical cell divisions producing two proliferative daughter cells provide exponential expansion of cerebral cortical cell numbers (1, 2, 4, 8, 16, 32...), the control of the “symmetry” of division of apical progenitors is a crucial control on ultimate cerebral cortical size.

Basal progenitors (also called intermediate progenitors, subventricular zone (SVZ) progenitors, or non-surface progenitors) are defined by their lack of an apical process. Basal

progenitors are derived from apical progenitors, but they localize their nuclei in the SVZ, and divide almost exclusively in a symmetric fashion to generate pairs of postmitotic daughter neurons (Kriegstein et al. 2006, Martinez-Cerdeno et al. 2006, Noctor et al. 2008, Pontious et al. 2008). As cortical progenitor cells transition from apical and basal progenitors to postmitotic neurons, they sequentially express molecular markers defining their identity including Pax6 (apical progenitors), Tbr2 (basal progenitors), and Tbr1 (neurons) (Englund et al. 2005, Kawaguchi et al. 2008). However, more complex transcriptional profiles have been proposed to underlie these transitions as well (Kawaguchi et al. 2008).

Recently, a third general type of progenitor has been identified that has a modified radial morphology, but localizes to the SVZ. These outer SVZ (OSVZ) progenitors appear to be present in all mammals to varying extents, but are enriched in mammals with larger cerebral cortices, where an expanded outer SVZ (OSVZ) appears during mid-gestation coinciding with the onset of neurogenesis (Fietz et al. 2010, Hansen et al. 2010, Smart et al. 2002). The OSVZ progenitors show radial glial morphology (OSVZ radial-glial like) and express classic markers of radial glial progenitor cells including Pax6, Sox2, phospho-Vimentin, GFAP, and BLBP, distinguishing them from typical basal progenitors. OSVZ progenitors appear to have a random plane of cleavage and undergo proliferative and self-renewing cell divisions (Fietz et al. 2010, Hansen et al. 2010). While OSVZ progenitors do not extend apical processes to the VZ, their basal processes maintain contact with the pial surface throughout mitosis (Fietz et al. 2010, Hansen et al. 2010).

This review focuses mainly on the control of cell proliferation in the apical progenitors, since the ultimate size of the cerebral cortex, both developmentally and evolutionarily, is so dependent upon even small changes in apical progenitor proliferation. Recent research on the

control of proliferation of these cells has focused on the role of cytoplasmic proteins (Notch, Numb, β -catenin, Par3/Par6/aPKC) and short-acting signaling pathways (e.g., Wnt, Delta, Jagged). In addition, we survey recent work that suggests that the apical complex also integrates cell-extrinsic cues carried in the cerebrospinal fluid (CSF), some derived from extremely distant sources, that may provide “global”, age-related controls over neural proliferation.

Establishing progenitor cell polarity

Apical cortical progenitor cells have a highly stereotyped polarity, with their apical membranes forming the ventricular surface of the developing brain, facing the lumen of the lateral ventricle that is filled with CSF, while their basal processes reach all the way to the outer, pial surface of the developing brain (Chenn et al. 1998, Mission et al. 1991, Takahashi et al. 1990). Apical-basal polarity is established and maintained in part by adherens junctions, which are ring-like, cadherin-containing contacts located between cells that regulate calcium-dependent cell-cell adhesion (Aaku-Saraste et al. 1997, Gotz & Huttner 2005, Harris & Tepass 2010, Stoykova et al. 1997). Cadherins connect to the catenins, including β -catenin and α -catenin, and together regulate actin filament dynamics. In this manner, adherens junctions asymmetrically distribute proteins in progenitor cells, while also anchoring the apical end-feet of adjacent radial glial cells to the ventricular surface (**Figure 2**).

The distinctive polarity of the cortical progenitor cells (Chenn et al. 1998) invites comparison to similar polarized protein distributions in other cell division processes. In particular, genetic control of asymmetric cell divisions has been extensively studied in the *C. elegans* zygote and *Drosophila* larval neuroblasts. In the asymmetric cell divisions of the *Drosophila* neuroblast, an apical protein complex, consisting of the Par3/Par6/aPKC proteins, is

distributed in a highly polarized fashion. Some of these asymmetrically expressed proteins, including Par3/Bazooka, Pins, and Inscuteable, then serve to orient the mitotic spindle, with the orientation of the mitotic spindle thereby specifying whether daughter cells inherit similar complements of cytoplasmic determinants (in which case the daughters tend to adopt similar cell fates, a symmetrical cell division), or inherit dissimilar complements of proteins and adopt distinct (asymmetric) fates (Siller & Doe 2008). Principal among the cytoplasmic regulators of cell fate is the Numb protein, which negatively regulates the neurogenic gene, *Notch*. Inheritance of Numb tends to cause cells to adopt a more differentiated fate, whereas absence of Numb causes cells to remain neuroblasts (Rhyu et al. 1994, Wirtz-Peitz et al. 2008). Thus, the mechanism of asymmetric cell division in these systems requires cytoplasmic regulators of cell fate, and controlled orientation of the mitotic spindle in relationship to these cytoplasmic proteins (Siller & Doe 2008, Tajbakhsh et al. 2009). Though mammalian cerebral cortical progenitor cells express many orthologous proteins with similar effects on proliferation and cortical size, in strikingly asymmetrical patterns, a major unanswered question involves the extent to which inheritance of these cytoplasmic determinants is specified by the orientation of the mitotic spindle.

Adherens junctions in progenitor cells

Proteins associated with adherens junctions in the developing cerebral cortex have been well documented to regulate progenitor cell pool and brain size (Bilder et al. 2000, Chae et al. 2004, Chenn & McConnell 1995, Chenn & Walsh 2002, Kim et al. 2010, Lien et al. 2006). Disruption of adherens junctions via α -E-catenin deletion in cortical progenitor cells leads to an activation of Sonic hedgehog (Shh) signaling associated with accelerated cell cycle progression

and decreased apoptosis, that together lead to hyperplasia (Lien et al. 2006). On the other hand, overexpression of truncated, and hence activated, β -catenin, also an integral component of adherens junctions that interacts with TCF/LEF to transduce Wnt signaling, expands the progenitor cell pool by limiting cell cycle exit (Chenn & Walsh 2002). This increased β -catenin activity considerably expands brain size in the horizontal direction, leading to the generation of additional folia in the cortex (Chenn & Walsh 2002). Cortical thickness is markedly reduced in these brains, suggesting that β -catenin overexpression leads, in effect, to an exchange of neurons for progenitors. Interestingly, β -catenin expression is developmentally regulated such that β -catenin signaling decreases in the progenitor pool over development (Mutch et al. 2009). Consistent with the inside-out model of cortical development in which the earliest neurons form the deepest cortical layers, higher β -catenin activity favors the generation of deep layer neurons, while lower β -catenin activity favors the development of upper layer neurons (Mutch et al. 2009, Wrobel et al. 2007). Re-introduction of β -catenin signaling in late neurogenesis at a time when more upper layer neurons are typically generated can partially extend deep-layer neuron production (Mutch et al. 2009). β -catenin signaling is also reduced in intermediate progenitors, suggesting that radial glial progenitors use β -catenin as one mechanism for retaining apical progenitor cell fate (Mutch et al. 2010, Zhang et al. 2010).

Apical complex proteins, Numb/Numbl, and Notch signaling in progenitor cells

The adherens junctions also serve as docking sites for the proteins of the apical complex, also known as the “Par” (for “partitioning defective”) protein complex in invertebrates (Manabe et al. 2002). Highly conserved across species, the Par proteins have a well established role in determining polarity and cell division (Jan & Jan 2001, Kemphues et al. 1988, Knoblich 2008,

Siller & Doe 2008, Wodarz 2005). The mammalian apical complexes assemble as Par3/Par6/aPKC, Crb/Pals/Patj, and Mals/Pals1 (Margolis & Borg 2005). Perhaps the best understood of these, the Par3/Par6/aPKC complex, is assembled by the PDZ-containing scaffold Par3, which then recruits Par6, a CRIB and PDZ domain-containing protein that inhibits atypical aPKC activity. When bound to Cdc42 or Rac1, Par6 no longer inhibits aPKC, allowing aPKC to phosphorylate downstream target proteins, an event that leads to their selective exclusion from the apical domain of the progenitor (Siller & Doe 2008).

Inheritance of an unequal portion of the apical domain and the associated skew in the distribution of polarity proteins is thought to distinguish whether symmetric or asymmetric cell division occurs during mammalian neurogenesis: The daughter cell that inherits more apical surface from the mother cell (Kosodo et al. 2004, Noctor et al. 2008), including Par3/aPKC, (Marthiens & French-Constant 2009) is anticipated to remain a progenitor cell (**Figure 2**). Consistent with this model, aPKC ζ/λ is associated with the apical surface of neural precursors in the developing chick neural tube (Ghosh et al. 2008). Thus, apical complex proteins regulate progenitor proliferation and cell fate in the developing mammalian brain (Bultje et al. 2009, Costa et al. 2008, Kim et al. 2010, Manabe et al. 2002, Srinivasan et al. 2008).

In invertebrates, the apical complex regulates the distribution of a protein called Numb, that regulates neuronal cell fate, and genetic studies in vertebrates have also uncovered a number of roles for the Numb orthologues Numb and Numb-like (Numb/Numbl) in neurogenesis (Li et al. 2003, Petersen et al. 2002, Petersen et al. 2004, Rasin et al. 2007, Tajbakhsh et al. 2009)(Bultje et al. 2009; Nishimura & Kaibuchi, 2007). However, in contrast to their role in invertebrates, Numb/Numbl signaling in mammalian cells appears to promote progenitor cell fate instead of neuronal cell fate. In mammalian neural progenitors, Numb/Numbl localize to the

basolateral regions of progenitor end feet in the VZ (Rasin et al. 2007). Numb/Numbl are required for continued cell division during development as their deletion in the developing brain leads to premature exit from the cell cycle and disrupts cortical development leading to a mature cortex with severe neocortical thinning and diminished hippocampal size (Li et al. 2003, Petersen et al. 2004). At the cellular level, Numb/Numbl interact with several adherens junction components, including Cdh1 (E-cadherin), Cdh2 (N-cadherin), and the catenins (α -E-catenin, β -catenin). In *Numb/Numbl* deficient cells, the cadherins are mistargeted to the apical membrane, leading to a disruption of adherens junctions (Rasin et al. 2007). Numb is an endocytic adaptor protein known to regulate Notch endocytosis (Berdnik et al. 2002), associates directly with recycling endosomes, and is regulated by the Golgi adapter protein ACBD3 (Zhou et al. 2007). Thus, Numb may play an important role in trafficking adherens junction components.

Numb is phosphorylated in a Par3/Par6/aPKC-dependent manner (Klezovitch et al. 2004, Nishimura & Kaibuchi 2007; Smith et al. 2007), and the apical complex proteins are essential for self-renewal of neural progenitors in the developing mammalian cortex. Knockdown and overexpression studies of Par3 and Par6 promote either premature differentiation or excessive cell division, respectively (Costa et al. 2008). Trafficking of Par3 and Par6 is regulated by Cdc42, a Rho GTPase family member, so that conditional removal of Cdc42 leads to loss of adherens junctions and misdirects mitotic progenitors basally, away from the apical surface (Cappello et al. 2006). While these newly relocated progenitors continue to cycle, they do so with an increase in basal progenitor cell fate.

Recent work suggests that Par3 is segregated asymmetrically in some mammalian cortical progenitor cells, and serves as a cell autonomous regulator of Notch signaling (Bultje et al. 2009, Manabe et al. 2002). Par3 is dynamically localized in progenitors, associating with the

lateral membrane domain of ventricular endfeet during interphase, and then dispersing as the cell cycle progresses. Asymmetric Par3 expression during the cell cycle leads to asymmetric Par3 segregation in daughter cells, promoting differential Notch signaling via Numb/Numbl that drives distinct cell fate in daughter cells. Ultimately, the daughter cell with greater Notch signaling remains a radial glial progenitor, while the cell with lesser Notch activity adopts neuronal or intermediate progenitor cell fate (Bultje et al. 2009). The degree to which Notch signaling may also influence the inheritance of the basal process will be interesting to examine. Quantification of the cell fate of dividing progenitors recently revealed that inheritance of both the apical and basal processes by a progenitor cell may be essential for maintaining its apical progenitor identity (Konno et al. 2008) but the signaling proteins present in the basal process that might regulate this effect are not known.

The asymmetric distribution of Notch by Par3 adds an interesting mechanistic twist to the Notch literature in which Notch is already well known for maintaining the neural stem cell pool during mammalian neurogenesis (Chenn & McConnell 1995, Mizutani & Saito 2005, Mizutani et al. 2007, Tajbakhsh et al. 2009). Notch activity maintains progenitor proliferation, and once removed, promotes the generation of upper layer neurons matching the age of the embryos. Thus, while progenitors retain competence to become neurons, they lose the adaptability to generate lower layer fated cells, which essentially are skipped (Mizutani & Saito 2005). Importantly, Notch selectively promotes neural stem cell fate through activation of the canonical Notch effector C-promoter binding factor 1 (CBF1), which is attenuated in intermediate progenitor cells. While decreased CBF1 activity converts neural stem cells to intermediate progenitors, the converse experiment in which CBF1 is artificially activated in intermediate progenitors fails to revert cell fate, indicating that Notch signaling plays a key role in specifying

lineage commitment of prospective neurons (Mizutani et al. 2007). Oscillations in Notch activity via *Hes1*, which modulate expression of proneural genes including *Ngn2* and *Dll1*, Notch ligands, and cell cycle regulators, have also been suggested to promote neural maintenance (Shimojo et al. 2008). Notch was also recently shown to play a dual role in the maintenance of apical mitoses and apical-basal polarity via interactions with the Crumbs-Moe apical complex in zebrafish neuroepithelial cells (Ohata et al. 2011).

Despite these recent advances, the biochemical interactions between adherens junctions and interacting proteins, and the regulation of downstream effector pathways, are complex and remain poorly understood. Surprisingly, deletion of aPKC λ in mice at E15, midway through neurogenesis, does not clearly affect cell fate decisions in a manner comparable to its role in invertebrates (Imai et al. 2006). In addition, mouse *Lgl1* (*Lethal giant larva 1*) mutants showed hyperproliferation of progenitors in the brain, in conjunction with Numb mislocalization (Klezovitch et al. 2004). Therefore, the role of the apical complex proteins in progenitors likely extends well beyond the regulation of proliferation and cell fate to other biological functions as well.

Potential roles of apical complex proteins in cell survival

Recent evidence indicates that the apical complex is essential for survival of progenitors and newly differentiated neurons. Conditional deletion of the apical complex protein Pals1 (Protein associated with Lin7, a member of the MAGUK (Membrane Associated Guanylate Kinase) family of scaffolding proteins) (Kamberov et al. 2000) in progenitor cells causes not only the expected premature withdrawal from the cell cycle that is coupled with excessive generation of early-born postmitotic neurons, but is also followed by the rapid death of these

newly generated neurons (Kim et al. 2010). Together, these two effects lead to the abrogation of essentially the entire cerebral cortex. Loss of *Pals1* blocks essential cell survival signals, including the mammalian target of rapamycin (mTOR) pathway, such that concomitant mTORC1 activation via conditional *Tsc2* deletion partially restores the *Pals1* deficiency (Kim et al. 2010).

The *Pals1* cell death phenotype reveals a previously unidentified role for the apical complex in promoting survival during the transition from progenitor to neuron. Intriguingly, this is akin to conditional deletion of *Notch1* and *Notch3*, which also show a profound death of progenitors and newly differentiating neurons (Mason et al. 2006). While the exact mechanism underlying the Notch phenotypes remains unclear, Notch-dependent survival in the developing brain is thought to occur by a *Hes*-independent mechanism (Mason et al. 2006). The shared phenotype between *Pals1* and *Notch* conditional mutants suggests that *Pals1* and *Notch* may either signal in the same pathway or converge on a shared set of targets to regulate survival of newly differentiating neurons.

Potential roles of apical complex proteins in growth factor signaling

The prominent cell death phenotype of the *Pals1* mutants, and the finding that upregulation of mTOR signaling partially restores the phenotype, suggests that growth factor signaling pathways are disrupted in apical complex mutants (Kim et al. 2010). Growth factor signaling, especially via the type 1 Insulin-like growth factor (Igf) receptor (Igf1R), mediates powerful, age-dependent effects on the development and maintenance of many organ systems including the brain through the regulation of progenitor cell division (Baker et al. 1993, Hodge et al. 2004, Liu et al. 2009, Popken et al. 2004, Randhawa & Cohen 2005), but the mechanisms

coordinating the availability of Igf ligands to cortical progenitors have remained unclear. Interestingly, growth factor receptors including the Igf1R (Lehtinen et al. 2011) and the Epidermal growth factor receptor (EgfR) (Sun et al. 2005), as well as phospho-tyrosine (Chenn et al. 1998) and phospho-ERK1/2 (Toyoda et al. 2010), have enriched expression along the apical ventricular surface of progenitors. Could the apical-basal polarity of progenitors insure the apical localization of receptors for sampling cell-extrinsic growth factors emanating from the CSF?

A direct interaction between Par3 and Pten (Phosphatase and Tensin homolog) (Feng et al. 2008, Pinal et al. 2006, von Stein et al. 2005, Wu et al. 2007) also suggests that the apical complex interacts with growth factor signaling pathways. Disrupting the apical complex via *Pals1* deletion in progenitors abolishes the apical enrichment of Igf1R and attenuates growth factor signaling assessed by pS6 activity (Lehtinen et al. 2011). Consistent with a genetic interaction between the apical complex and growth factor signaling, artificial activation of growth factor signaling by conditional deletion of *Pten* in *Pals1* heterozygous progenitor cells largely restores brain size, in part due to an expansion of the Igf1R signaling domain, which partly restores the proportions of proliferating apical progenitor cells (Lehtinen et al. 2011). Igf1 is thought to promote S-phase commitment of apical progenitors via PI3K signaling (Mairet-Coello et al. 2009). Thus, it will be important to determine the nature of the biochemical interaction between the apical complex and Pten. The disruption of Igf1R localization in *Pals1* conditional mutants is similar to what has been shown for the *C. elegans* LIN-2A, mutation of which disrupts the LET-23 (Egf) receptor and blocks its appropriate cellular localization and signaling (Hoskins et al. 1996, Simske et al. 1996).

Extrinsic regulation of neurogenesis at the apical membrane

How might extrinsic growth factors be received by the apical ventricular surface? In addition to bathing their apical domains in CSF, progenitor cells extend primary cilia directly into the CSF (Cohen et al. 1988, Dubreuil et al. 2007, Han & Alvarez-Buylla 2010, Hinds & Ruffett 1971). Cilia transduce extracellular signals to the cell body, as has been well established for Shh (Han & Alvarez-Buylla 2010, Han et al. 2008, Rohatgi et al. 2007). Though the details of cilia-dependent signaling are not fully understood, primary cilia are critical for normal brain patterning and development (Breunig et al. 2008, Chizhikov et al. 2007, Gorivodsky et al. 2009, Spassky et al. 2008, Stottmann et al. 2009, Willaredt et al. 2008). While vascular sources of secreted proliferative signals are well characterized (Palmer et al. 2000, Shen et al. 2004, Shen et al. 2008, Tavazoie et al. 2008), the apical surfaces of early cortical precursors and their primary cilia directly contact the CSF but not the vasculature (Cohen et al. 1988, Lehtinen et al. 2011). The location of cilia and their immersion in the CSF suggests that the CSF may distribute and synchronize signals regulating neurogenesis, potentially over long distances since the CSF represents a large fluid space without known barriers to diffusion. These possibilities have recently spurred interest in the growth-promoting features of the CSF, and the dynamic regulation of the CSF proteome during the period of neurogenesis.

Several studies have investigated potential developmental roles for the CSF beyond provision of a fluid cushion for the central nervous system (CNS) and maintenance of extracellular ionic balance (Dziegielewska et al. 1981, Parada et al. 2005a, Zappaterra et al. 2007). In the developing chick brain, CSF facilitates the retention of midbrain markers including *Otx2* and *Fgf8* (Parada et al. 2005b), and CSF-Fibroblast growth factor 2 (Fgf2) promotes precursor proliferation (Martin et al. 2006). In the mouse cerebellum, CSF-distributed Shh

stimulates proliferation of cerebellar granule neuron precursors (Huang et al. 2010). Most recently, it was also found that the embryonic CSF, and in particular CSF-Igf2, provide essential growth and survival promoting factors for the developing rodent cortex (Lehtinen et al. 2011). These and other studies support the notion that the CSF contains a large and dynamic library of proteins that may instruct many aspects of neuronal development.

The cerebrospinal fluid during times of neurogenesis

The study of CSF dates back to the Greeks, who provided the first account of fluid in the brain (reviewed in Tascioglu & Tascioglu 2005). Galen of Pergamon proposed that the cerebral fluid provided energy for the entire body, but it was the work of Leonardo DaVinci and Andreas Vesalius during the Renaissance that produced the first accurate models of the mammalian cerebroventricular system. Surprisingly, while the Greeks had originally described a fluid within the ventricles, few accounts of CSF had been actually been made since that time. CSF was finally documented in the 1700's, but it was not until the early 20th century that Harvey Cushing observed secretion of CSF by the choroid plexus (Cushing 1914).

The primitive cerebroventricular system emerges at neural tube closure, occurring at E8.5-9 during mouse development, at which point the amniotic fluid trapped in the neural tube serves as the developing brain's initial CSF (Lowery & Sive 2009). The surrounding neuroepithelium initially secretes additional factors into the CSF and helps to maintain ventricular pressure. The choroid plexus tissues, which actively generate CSF in the mature brain, develop over the course of the following several days to form modified ependyma that project into the brain's ventricles (Zheng & Chodobski 2005). The choroid plexus develops sequentially in each ventricle in the brain such that the hindbrain/fourth ventricle choroid plexus

develops first (mouse E11-E12), is quickly followed by the lateral ventricle choroid plexi (E11-12), while the mesencephalic/third ventricle choroid plexus is the last to develop by E14.5 (Zheng & Chodobski 2005). In the human embryo the choroid plexus begins to develop in the fourth and lateral ventricles at Carnegie Stage (CS) 18-19, approximately 44 days post-ovulation (O'Rahilly & Muller 1994). The first appearance of cerebral cortical neurons in the human embryo occurs at CS 21, shortly following the appearance of the choroid plexus and the production of CSF (O'Rahilly & Muller 1994), and a similar temporal sequence is observed in several species including mice and rats (**Figure 3**). Thus, the secretory choroid plexus of the lateral ventricles ultimately derives from the roof plate of the forebrain, which is the known source of many key secreted patterning factors, including many bone morphogenic proteins (Bmps), Wnt proteins, as well as Fgf8 and other Fgf family members (Grove & Fukuchi-Shimogori 2003).

Early interest in CSF regulation of brain development focused more on its role in transmitting pressure to the brain than on specific CSF constituent proteins (Desmond & Jacobson 1977, Lowery & Sive 2009, Pexieder & Jelinek 1970). For example, artificially disrupting the cerebroventricular system by draining CSF from chick ventricles by intubation decreases intraventricular pressure and globally affects brain size (Desmond & Jacobson 1977). Conversely, increased ventricular pressure may lead to increases in mitotic density (Desmond et al. 2005). Important to note however are observations from naturally occurring rodent models of hydrocephalus, a condition in which there is an excess of CSF in the ventricles (Mashayekhi et al. 2002). In these animals, early-onset hydrocephalus leads to enlarged, dome-shaped heads, ataxia, and depression. Cell-based investigations with CSF obtained from the hydrocephalic Texas rat have found that hydrocephalus-associated CSF inhibits cell division in vitro

(Mashayekhi et al. 2002, Owen-Lynch et al. 2003), suggesting that the CSF either accumulates signals that specifically interfere with normal cell proliferation or lacks the necessary proliferation-inducing signals.

The large literature regarding CSF is home to many hypotheses of potentially active roles for the CSF in the CNS (Zheng & Chodobski 2005), including relaying the body's satiety levels (Martin et al. 1973), and maintaining circadian rhythms (Silver et al. 1996). However, the first compelling evidence that spatial gradients of CSF factors actively guide cell behavior came from an elegant study investigating the biological consequences of CSF flow generated by the beating cilia of the ependymal cells that line the adult ventricles (but are absent in the developing brain) (Sawamoto et al. 2006). A striking similarity was observed between the pattern of CSF flow and the migration of neuroblasts destined for the olfactory bulb, an effect that was disrupted in mouse ciliary mutants with defective ciliary beating and CSF flow (Sawamoto et al. 2006). It had previously been suggested that the choroid plexus provides Slit chemorepellent activity in the developing and adult brain, possibly in order to induce neuronal migration from the ventricular zone to the cortical plate (Hu 1999, Nguyen-Ba-Charvet et al. 2004). While Slits are also expressed by SVZ and rostral migratory stream cells (Nguyen-Ba-Charvet et al. 2004), gain-of-function experiments with intraventricularly injected Slit, loss-of-function experiments with Slit knockout mice, and choroid plexus grafts demonstrated that CSF-borne Slit1/2 plays an important role in guiding neuroblast migration to the olfactory bulb (Sawamoto et al. 2006). Collectively, these studies inspired a renewed interest in understanding how the embryonic CSF proteome might also provide instructive cues for the developing brain.

Signaling from the CSF to developing cortical tissues

Mass spectrometry has offered new approaches for characterizing the highly dynamic CSF proteome (Cavanagh et al. 1983, Dziegielewska et al. 1981, Parada et al. 2005a, Zappaterra et al. 2007, Zheng & Chodobski 2005). Unbiased analyses of human embryonic CSF from Carnegie Stage 19-20 (approximately 48-51 days post ovulation) together with CSF samples obtained from three distinct time points of rat cortical development: E12.5, E14.5, and E17.5, revealed developmental stage-specific similarities in the CSF proteomes of human and rodents based on molecular function and biological process. Of the 188 proteins identified in the human embryonic CSF, 135 human proteins were identified in any one of the four samples of embryonic rat CSF, and 83 of those proteins were present in all four samples of embryonic rat CSF (Zappaterra et al. 2007). The embryonic CSF of the chick and rat also share many similarities, with both containing proteins of the extracellular matrix, regulators of osmotic pressure, ion carriers, hormone binding proteins, regulators of lipid metabolism, and various enzymes and their regulators (Cavanagh et al. 1983, Parada et al. 2005a).

Distinct embryonic CSF protein signatures exist across ventricles, raising the possibility of region-specific function (Cavanagh et al. 1983, Zappaterra et al. 2007). In a comparison between rat lateral and hindbrain ventricle CSF at E14.5, lateral ventricle CSF contained 61 distinct proteins not found in hindbrain ventricle CSF (Zappaterra et al. 2007). Since similar numbers of peptides were recovered from CSF samples in each ventricle, this difference between samples likely represents truly distinct ventricular proteomes. Consistent with this interpretation, evidence for the active secretion of distinct morphogens by the different choroid plexi has been documented. For example, Shh is highly expressed by the hindbrain ventricle choroid plexus

(Awatramani et al. 2003, Huang et al. 2009). These findings raise interesting questions regarding local signaling gradients that may be generated in the CSF.

Fibroblast growth factor signaling in CSF

Based on these clues, recent studies have investigated whether CSF plays an instructive role in CNS development. Primary CSF removed from the lateral ventricles of the mammalian brain and used as culture medium, is sufficient to maintain neural stem cells in neurosphere cultures, and cortical explants, with no other added factors (Lehtinen et al. 2011). This ability to support progenitor cell development was greatest for age-matched CSF samples and tissues. Though all of the factors responsible for the growth-promoting effects of CSF may not yet be known, several strong candidate molecules have been implicated (**Figure 4**). The growth factor Fgf2 was identified in embryonic chick CSF (Finch et al. 1995, Martin et al. 2006, Raballo et al. 2000). Immunodepletion of CSF-Fgf2 reduces progenitor proliferation to the level of negative controls, suggesting that CSF-Fgf2 plays a direct role in promoting the proliferation of chick midbrain progenitors (Martin et al. 2006, Tao et al. 1997). Intriguingly, intravascularly injected FITC-conjugated Fgf2 passes into the embryonic CSF (Martin et al. 2006), suggesting that in addition to choroid plexus-synthesized factors, somatic sources of CSF-distributed signaling cues potentially regulate neurogenesis as well.

The identification of Fgf2 in CSF is particularly intriguing since the Fgf family members play key roles in early CNS patterning, stem cell proliferation, and most recently, in the development of cilia (Iwata & Hevner 2009, Neugebauer et al. 2009). In the developing brain, dorsal/ventral and anterior/posterior axes are initially established by signaling centers that secrete diffusible cues including Fgf, Bmps, and Shh (Grove & Fukuchi-Shimogori 2003). Remarkably,

experimental manipulations that augment, diminish, or introduce entirely new sources of Fgf8 to the developing brain accordingly affect area identity evaluated by barrel columns in the postnatal somatosensory cortex (Fukuchi-Shimogori & Grove 2001). In the classic view of morphogens, the Fgfs are generally believed to exert their effects by diffusing through tissues (Wolpert 1996). This may be the case for Fgf8 signaling (Toyoda et al. 2010). However, the apical enrichment and polarization of phospho-tyrosine (Chenn et al. 1998) and phospho-ERK1/2 (Toyoda et al. 2010) activities in progenitors are highly suggestive that some growth factor signaling originates from the CSF as well.

Insulin and Insulin-like growth factor 1 and 2 signaling in the CSF

The embryonic CSF also contains Insulin and Igf 1 and 2 (Holm et al. 1994, Lehtinen et al. 2011, Margolis & Altszuler 1967). Igf1 and Igf2 regulate prenatal growth and body size (Baker et al. 1993, DeChiara et al. 1991) mainly by binding to the Igf1R, which mediates their proliferative response (Weber et al. 1992). Consistent with its role in the regulation of somatic size, all small dogs inherit a unique small-nucleotide polymorphism in the Igf1R, not found in the genomes of large dogs (Sutter et al. 2007). Furthermore, conditional deletion of Igf1R in neural precursors leads to microcephaly (Kappeler et al. 2008, Lehtinen et al. 2011, Liu et al. 2009). In contrast, Igf1 overexpression promotes S-phase commitment, accelerated cell cycle kinetics, and cell survival, leading to hyperplasia. (Hodge et al. 2004, Liu et al. 2009, Mairet-Coello et al. 2009, Popken et al. 2004). Igf1 can also regulate neuronal differentiation, glial development, and cell size (reviewed in (D'Ercole & Ye 2008)). Downstream of Igf1R signaling, mutations of *Irs2* (Schubert et al. 2003), *Pdk1* (Chalhoub et al. 2009), *Pten* (Groszer et al. 2001), among others, provide genetic tools for manipulating brain size.

The growth and survival promoting effects of embryonic CSF during neurogenesis depends in part on CSF-Igf2 (Lehtinen et al. 2011). A transient spike in Igf2 expression in embryonic CSF stimulates the proliferation of neural precursor cells in explant cultures of the developing cortex (Lehtinen et al. 2011), as well as the growth and maintenance of neurospheres, an *in vitro* model of neural stem cells (Vescovi et al. 1993). CSF-Igf2 binds directly to the apical domain and primary cilia of cortical progenitors. Igf1R expression is also enriched along the apical ventricular surface of progenitors (Lehtinen et al. 2011). The proliferation-induced effects of CSF appear Igf2-dependent, as gain-of-function and loss-of-function experiments *in vitro* in explants and neurospheres produce opposing effects on proliferation. Moreover, *Igf2* deficient mice have a specific defect in neurogenesis of their upper most layers of the cortex (Lehtinen et al. 2011). The choroid plexus expresses *Igf2* and is likely the principal source of Igf2 in the developing CSF. Other sources of Igf ligands exist, including the developing vasculature (Dugas et al. 2008), potentially neighboring cells, and perhaps even extraneural sources. Studies involving intracerebroventricular injections of Igf1, Igf1 neutralizing antibodies, and Igf1R inhibitors have confirmed that Igf signals delivered in the embryonic CSF trigger proliferative events in the cortical ventricular zone (Mairet-Coello et al. 2009). Interestingly, Insulin/Igf-like peptides secreted by glia were recently shown to stimulate quiescent neuroblasts to re-enter the cell cycle in *Drosophila* (Chell & Brand 2010).

Sonic hedgehog signaling in the CSF

The transventricular delivery of secreted proteins with active roles in the developing brain extends to Shh as well. Recent evidence demonstrates that the hindbrain choroid plexus secretes Sonic hedgehog into the CSF (Huang et al. 2010). Shh stimulates the expansion of a

distinct progenitor domain in the hindbrain choroid plexus adjoining the lower rhombic lip that does not itself express Shh but is responsive to it (Huang et al. 2009). In addition to this novel tissue-autonomous role for choroid plexus-secreted Shh, CSF-Shh is a key mitogen for proliferating cerebellar granule precursors. Shh was previously thought to be primarily secreted by the Purkinje cells (Dahmane & Ruiz i Altaba 1999, Wallace 1999, Wechsler-Reya & Scott 1999). However, *Wnt1Cre*-mediated deletion of *Shh* in the hindbrain choroid plexus also impairs proliferation of cerebellar granule neuron precursors (Huang et al. 2010). Thus, choroid plexus-borne Shh acts both in an autocrine and a paracrine manner to instruct choroid plexus and cerebellar development as well.

Igf synergizes with Shh signaling to promote proliferation of healthy cerebellar granule neuron precursors (Fernandez et al. 2010, Ye et al. 1996) as well as tumorigenic cells leading to medulloblastoma (Corcoran et al. 2008, Hartmann et al. 2005, Rao et al. 2004, Tanori et al. 2010), a childhood tumor which can originate from cerebellar granule neuron precursors (Gibson et al. 2010, Gilbertson & Ellison 2008, Pomeroy et al. 2002). While the mechanisms by which Igf2 binding to primary cilia influence downstream signaling events remain to be elucidated, Shh is well-established to signal via binding to primary cilia (Corbit et al. 2005, Han et al. 2008, Rohatgi et al. 2007). Mutations in genes encoding the basal body proteins *Fantom* and *Odf1* cause abnormal cerebellar development (Delous et al. 2007, Ferrante et al. 2006, Vierkotten et al. 2007). Primary cilia are also required for Shh-dependent expansion of the cerebellar progenitor cell pool (Spassky et al. 2008). As well, mice deficient in primary cilia, as the result of *Kif3a*, *Igft88*, or *Stumpy* deletions, have profound hypoplasia and foliation defects (Breunig et al. 2008, Chizhikov et al. 2007, Town et al. 2008). It will be interesting in future studies to elucidate the mechanisms by which Igf and Shh signaling may synergize at primary cilia. Shh-dependent

signaling is also implicated in the formation and maintenance of adult neural stem cells (Breunig et al. 2008, Han et al. 2008), but the source and means of distributing Shh in the adult CSF remains to be fully elucidated.

Retinoic acid in the CSF

Retinoic acid (RA), a hormone signal derived from Vitamin A, also acts on the developing forebrain far from its source (Chambon 1996, Haskell & LaMantia 2005, Ribes et al. 2006). The meninges are an important source of RA (Siegenthaler et al. 2009). Meningeal defects in the *Foxc1* mutant mice are associated with impaired neurogenesis, including reduced intermediate progenitor production and diminished expansion of the neuroepithelium. Interestingly, these deficiencies are due to reduced RA signaling originating from the dorsal forebrain meninges (Siegenthaler et al. 2009). Since RA has been identified in CSF (Lehtinen et al. 2011, Parada et al. 2008, Redzic et al. 2005), and RA synthetic and catabolic enzymes are expressed in the choroid plexus as well as the meninges (Lehtinen et al. 2011, Siegenthaler et al. 2009), meningeally derived RA may reach the neuroepithelial cells via the lateral ventricular CSF as well as by crossing the cerebral mantle. However, the roles of meningeally-derived RA suggest the remarkable range of mechanisms by which signals at both the basal process (Konno et al. 2008, Radakovits et al. 2009) and apical process may play key roles in the maintenance of the apical progenitors, as well as potentially the OSVZ, during neurogenesis (Fietz et al. 2010, Hansen et al).

Other potential signaling activities in the CSF

It remains an open question whether other secreted factors that act on the forebrain also act far from their source by diffusing through the CSF. The Wnt signaling pathway plays a fundamental role in regulating early development of the CNS (Freese et al, Wang & Wynshaw-Boris 2004, Zhou et al. 2006), and Wnt signaling activity has been identified in the embryonic CSF (Lehtinen et al. 2011). Similarly, dynamic levels of Bmp signaling activity (Hebert et al. 2002, Shimogori et al. 2004) is also present in the CSF (Lehtinen et al. 2011). Consistent with the coordination of Bmp signaling by the choroid plexus-CSF system, Growth and differentiation factors 3 and 8 (Gdf3 and Gdf8), both members of the TGF- β superfamily of proteins that can modulate Bmp signaling (Levine & Brivanlou 2006) have been identified in CSF (Lehtinen et al. 2011).

Potential clinical implications

Dysregulated Shh signaling is central to medulloblastoma progression (Gibson et al. 2010, Goodrich et al. 1997, Pomeroy et al. 2002). Shh and Igf signaling synergize to promote cerebellar granule precursor proliferation (Fernandez et al. 2010), for example through stabilization of c-Myc (Kenney et al. 2004). *Pten* deletion in mice with constitutively active Smoothed also enhances medulloblastoma tumorigenesis (Castellino et al. 2010). Since transventricular delivery of Shh supports the proliferation of cerebellar granule neuron progenitors (Huang et al. 2010), and CSF-Igf2 promotes proliferation of neural stem cells, it is possible that CSF-borne Shh and/or Igf ligands, among other factors, may serve to exacerbate medulloblastomas.

Igf2 and other diffusible growth factors that drive PI3K signaling and neural progenitor proliferation during development are upregulated in some glioblastoma (GBM) patient tumors as well (Louis 2006, Soroceanu et al. 2007). Some GBM patients also have elevated CSF IGF2 levels, and high CSF IGF2 levels correlate with advance of disease (Lehtinen et al. 2011). Moreover, GBM patient CSF stimulates neural stem cell proliferation in an IGF2-dependent manner, suggesting that GBM CSF IGF2 may act in a general autocrine as well as paracrine manner on putative tumor cells. The Shh and IGF2 data suggest the intriguing possibility that the protein content of the CSF may represent a brain tumor risk factor. On the other hand, Igf2 was recently shown to promote memory consolidation and enhancement (Chen et al. 2011), raising provocative questions of how the availability of Igf ligands in CSF may regulate postnatal neurogenesis, as well as memory function. Given the active role of CSF in instructing neurogenesis in the developing cortex, modulation of the proteomic composition of the CSF may provide new and unanticipated ways to regulate the CNS in health and disease.

Conclusion

It is time to re-evaluate the role of cell-intrinsic and cell-extrinsic cues during neurogenesis in the developing cerebral cortex. In addition to regulating progenitor proliferation, the apical complex appears to regulate cell survival and primary cilia. The cell polarity imparted by the apical complex allows for an enrichment of growth factor receptors along the apical surface of cortical progenitor cells, strongly suggesting that progenitors selectively sample the CSF for diffusible signals that instruct neurogenesis. Indeed, compelling evidence from several brain regions and different species supports a new model in which diffusible signals distributed by the cerebrospinal fluid support the growth and survival of the developing brain (**Figure 4**).

Collectively, these findings suggest that the embryonic CSF forms an integral component of the embryonic stem cell niche. Since the apical complex polarizes progenitor cells and helps anchor them to the ventricular surface, one possibility is that the apical complex may control neurogenesis by regulating progenitor access to the growth-promoting factors that circulate in the CSF. While initial studies are exploring the active role of the CSF in the adult CNS, it remains an open question whether the CSF plays an essential, active role in maintaining adult neurogenesis, the health of the adult CNS, and contributes to the pathogenesis of disease.

Literature Cited

- Aaku-Saraste E, Oback B, Hellwig A, Huttner WB. 1997. Neuroepithelial cells downregulate their plasma membrane polarity prior to neural tube closure and neurogenesis. *Mech Dev* 69: 71-81
- Agnati LF, Zoli M, Stromberg I, Fuxe K. 1995. Intercellular communication in the brain: wiring versus volume transmission. *Neuroscience* 69: 711-26
- Awatramani R, Soriano P, Rodriguez C, Mai JJ, Dymecki SM. 2003. Cryptic boundaries in roof plate and choroid plexus identified by intersectional gene activation. *Nat Genet* 35: 70-5
- Baker J, Liu JP, Robertson EJ, Efstratiadis A. 1993. Role of insulin-like growth factors in embryonic and postnatal growth. *Cell* 75: 73-82
- Berdnik D, Torok T, Gonzalez-Gaitan M, Knoblich JA. 2002. The endocytic protein alpha-Adaptin is required for numb-mediated asymmetric cell division in *Drosophila*. *Dev Cell* 3: 221-31
- Bilder D, Li M, Perrimon N. 2000. Cooperative regulation of cell polarity and growth by *Drosophila* tumor suppressors. *Science* 289: 113-6
- Boulder Comm. 1970. Embryonic vertebrate central nervous system: revised terminology. *Anat Rec* 166: 257-61
- Breunig JJ, Sarkisian MR, Arellano JI, Morozov YM, Ayoub AE, et al. 2008. Primary cilia regulate hippocampal neurogenesis by mediating sonic hedgehog signaling. *Proc Natl Acad Sci U S A* 105: 13127-32

- Bultje RS, Castaneda-Castellanos DR, Jan LY, Jan YN, Kriegstein AR, Shi SH. 2009. Mammalian Par3 regulates progenitor cell asymmetric division via notch signaling in the developing neocortex. *Neuron* 63: 189-202
- Cappello S, Attardo A, Wu X, Iwasato T, Itohara S, et al. 2006. The Rho-GTPase cdc42 regulates neural progenitor fate at the apical surface. *Nat Neurosci* 9: 1099-107
- Castellino RC, Barwick BG, Schniederjan M, Buss MC, Becher O, et al. 2010. Heterozygosity for Pten promotes tumorigenesis in a mouse model of medulloblastoma. *PLoS One* 5: e10849
- Cavanagh ME, Cornelis ME, Dziegielewska KM, Evans CA, Lorscheider FL, et al. 1983. Comparison of proteins in CSF of lateral and IVth ventricles during early development of fetal sheep. *Brain Res* 313: 159-67
- Chae TH, Kim S, Marz KE, Hanson PI, Walsh CA. 2004. The *hyh* mutation uncovers roles for alpha Snap in apical protein localization and control of neural cell fate. *Nat Genet* 36: 264-70
- Chalhoub N, Zhu G, Zhu X, Baker SJ. 2009. Cell type specificity of PI3K signaling in Pdk1- and Pten-deficient brains. *Genes Dev* 23: 1619-24
- Chambon P. 1996. A decade of molecular biology of retinoic acid receptors. *Faseb J* 10: 940-54
- Chell JM, Brand AH. 2010. Nutrition-responsive glia control exit of neural stem cells from quiescence. *Cell* 143: 1161-73

- Chen DY, Stern SA, Garcia-Osta A, Saunier-Rebori B, Pollonini G, et al. 2011. A critical role for IGF-II in memory consolidation and enhancement. *Nature* 469: 491-7
- Chenn A, McConnell SK. 1995. Cleavage orientation and the asymmetric inheritance of Notch1 immunoreactivity in mammalian neurogenesis. *Cell* 82: 631-41
- Chenn A, Walsh CA. 2002. Regulation of cerebral cortical size by control of cell cycle exit in neural precursors. *Science* 297: 365-9
- Chenn A, Zhang YA, Chang BT, McConnell SK. 1998. Intrinsic polarity of mammalian neuroepithelial cells. *Mol Cell Neurosci* 11: 183-93
- Chizhikov VV, Davenport J, Zhang Q, Shih EK, Cabello OA, et al. 2007. Cilia proteins control cerebellar morphogenesis by promoting expansion of the granule progenitor pool. *J Neurosci* 27: 9780-9
- Cohen E, Binet S, Meininger V. 1988. Ciliogenesis and centriole formation in the mouse embryonic nervous system. An ultrastructural analysis. *Biol Cell* 62: 165-9
- Corbit KC, Aanstad P, Singla V, Norman AR, Stainier DY, Reiter JF. 2005. Vertebrate Smoothed functions at the primary cilium. *Nature* 437: 1018-21
- Corcoran RB, Bachar Raveh T, Barakat MT, Lee EY, Scott MP. 2008. Insulin-like growth factor 2 is required for progression to advanced medulloblastoma in patched1 heterozygous mice. *Cancer Res* 68: 8788-95

- Costa MR, Wen G, Lepier A, Schroeder T, Gotz M. 2008. Par-complex proteins promote proliferative progenitor divisions in the developing mouse cerebral cortex. *Development* 135: 11-22
- Cushing H. 1914. Studies on the cerebro-spinal fluid. *Journal of Medical Research* 31: 1-19
- D'Ercole AJ, Ye P. 2008. Expanding the mind: insulin-like growth factor I and brain development. *Endocrinology* 149: 5958-62
- Dahmane N, Ruiz i Altaba A. 1999. Sonic hedgehog regulates the growth and patterning of the cerebellum. *Development* 126: 3089-100
- DeChiara TM, Robertson EJ, Efstratiadis A. 1991. Parental imprinting of the mouse insulin-like growth factor II gene. *Cell* 64: 849-59
- Delous M, Baala L, Salomon R, Laclef C, Vierkotten J, et al. 2007. The ciliary gene RPGRIP1L is mutated in cerebello-oculo-renal syndrome (Joubert syndrome type B) and Meckel syndrome. *Nat Genet* 39: 875-81
- Desmond ME, Jacobson AG. 1977. Embryonic brain enlargement requires cerebrospinal fluid pressure. *Dev Biol* 57: 188-98
- Desmond ME, Levitan ML, Haas AR. 2005. Internal luminal pressure during early chick embryonic brain growth: descriptive and empirical observations. *Anat Rec A Discov Mol Cell Evol Biol* 285: 737-47

- Dubreuil V, Marzesco AM, Corbeil D, Huttner WB, Wilsch-Brauninger M. 2007. Midbody and primary cilium of neural progenitors release extracellular membrane particles enriched in the stem cell marker prominin-1. *J Cell Biol* 176: 483-95
- Dugas JC, Mandemakers W, Rogers M, Ibrahim A, Daneman R, Barres BA. 2008. A novel purification method for CNS projection neurons leads to the identification of brain vascular cells as a source of trophic support for corticospinal motor neurons. *J Neurosci* 28: 8294-305
- Dziegielewska K, Evans C, Lai P, Lorscheider F, Malinowska D, et al. 1981. Proteins in cerebrospinal fluid and plasma of fetal rats during development. *Dev Biol* 83: 193-200
- Englund C, Fink A, Lau C, Pham D, Daza RA, et al. 2005. Pax6, Tbr2, and Tbr1 are expressed sequentially by radial glia, intermediate progenitor cells, and postmitotic neurons in developing neocortex. *J Neurosci* 25: 247-51
- Feng W, Wu H, Chan LN, Zhang M. 2008. Par-3-mediated junctional localization of the lipid phosphatase PTEN is required for cell polarity establishment. *J Biol Chem* 283: 23440-9
- Fernandez C, Tatard VM, Bertrand N, Dahmane N. 2010. Differential modulation of Sonic-hedgehog-induced cerebellar granule cell precursor proliferation by the IGF signaling network. *Dev Neurosci* 32: 59-70
- Ferrante MI, Zullo A, Barra A, Bimonte S, Messaddeq N, et al. 2006. Oral-facial-digital type I protein is required for primary cilia formation and left-right axis specification. *Nat Genet* 38: 112-7

- Fietz SA, Huttner WB. 2010. Cortical progenitor expansion, self-renewal and neurogenesis-a polarized perspective. *Curr Opin Neurobiol*
- Fietz SA, Kelava I, Vogt J, Wilsch-Brauninger M, Stenzel D, et al. 2010. OSVZ progenitors of human and ferret neocortex are epithelial-like and expand by integrin signaling. *Nat Neurosci* 13: 690-9
- Finch PW, Cunha GR, Rubin JS, Wong J, Ron D. 1995. Pattern of keratinocyte growth factor and keratinocyte growth factor receptor expression during mouse fetal development suggests a role in mediating morphogenetic mesenchymal-epithelial interactions. *Dev Dyn* 203: 223-40
- Freese JL, Pino D, Pleasure SJ. Wnt signaling in development and disease. *Neurobiol Dis* 38: 148-53
- Fukuchi-Shimogori T, Grove EA. 2001. Neocortex patterning by the secreted signaling molecule FGF8. *Science* 294: 1071-4
- Gal JS, Morozov YM, Ayoub AE, Chatterjee M, Rakic P, Haydar TF. 2006. Molecular and morphological heterogeneity of neural precursors in the mouse neocortical proliferative zones. *J Neurosci* 26: 1045-56
- Ghosh S, Marquardt T, Thaler JP, Carter N, Andrews SE, et al. 2008. Instructive role of aPKCzeta subcellular localization in the assembly of adherens junctions in neural progenitors. *Proc Natl Acad Sci U S A* 105: 335-40

- Gibson P, Tong Y, Robinson G, Thompson MC, Currle DS, et al. 2010. Subtypes of medulloblastoma have distinct developmental origins. *Nature* 468: 1095-9
- Gilbertson RJ, Ellison DW. 2008. The origins of medulloblastoma subtypes. *Annu Rev Pathol* 3: 341-65
- Goodrich LV, Milenkovic L, Higgins KM, Scott MP. 1997. Altered neural cell fates and medulloblastoma in mouse patched mutants. *Science* 277: 1109-13
- Gorivodsky M, Mukhopadhyay M, Wilsch-Braeuninger M, Phillips M, Teufel A, et al. 2009. Intraflagellar transport protein 172 is essential for primary cilia formation and plays a vital role in patterning the mammalian brain. *Dev Biol* 325: 24-32
- Gotz M, Huttner WB. 2005. The cell biology of neurogenesis. *Nat Rev Mol Cell Biol* 6: 777-88
- Groszer M, Erickson R, Scripture-Adams DD, Lesche R, Trumpp A, et al. 2001. Negative regulation of neural stem/progenitor cell proliferation by the Pten tumor suppressor gene in vivo. *Science* 294: 2186-9
- Grove EA, Fukuchi-Shimogori T. 2003. Generating the cerebral cortical area map. *Annu Rev Neurosci* 26: 355-80
- Han YG, Alvarez-Buylla A. 2010. Role of primary cilia in brain development and cancer. *Curr Opin Neurobiol* 20: 58-67
- Han YG, Spassky N, Romaguera-Ros M, Garcia-Verdugo JM, Aguilar A, et al. 2008. Hedgehog signaling and primary cilia are required for the formation of adult neural stem cells. *Nat Neurosci* 11: 277-84

- Hansen DV, Lui JH, Parker PR, Kriegstein AR. 2010. Neurogenic radial glia in the outer subventricular zone of human neocortex. *Nature* 464: 554-61
- Harris TJ, Tepass U. 2010. Adherens junctions: from molecules to morphogenesis. *Nat Rev Mol Cell Biol* 11: 502-14
- Hartmann W, Koch A, Brune H, Waha A, Schuller U, et al. 2005. Insulin-like growth factor II is involved in the proliferation control of medulloblastoma and its cerebellar precursor cells. *Am J Pathol* 166: 1153-62
- Haskell GT, LaMantia AS. 2005. Retinoic acid signaling identifies a distinct precursor population in the developing and adult forebrain. *J Neurosci* 25: 7636-47
- Haubensak W, Attardo A, Denk W, Huttner WB. 2004. Neurons arise in the basal neuroepithelium of the early mammalian telencephalon: a major site of neurogenesis. *Proc Natl Acad Sci U S A* 101: 3196-201
- Hebert JM, Mishina Y, McConnell SK. 2002. BMP signaling is required locally to pattern the dorsal telencephalic midline. *Neuron* 35: 1029-41
- Hinds JW, Ruffett TL. 1971. Cell proliferation in the neural tube: an electron microscopic and golgi analysis in the mouse cerebral vesicle. *Z Zellforsch Mikrosk Anat* 115: 226-64
- Hodge RD, D'Ercole AJ, O'Kusky JR. 2004. Insulin-like growth factor-I accelerates the cell cycle by decreasing G1 phase length and increases cell cycle reentry in the embryonic cerebral cortex. *J Neurosci* 24: 10201-10

- Holm NR, Hansen LB, Nilsson C, Gammeltoft S. 1994. Gene expression and secretion of insulin-like growth factor-II and insulin-like growth factor binding protein-2 from cultured sheep choroid plexus epithelial cells. *Brain Res Mol Brain Res* 21: 67-74
- Hoskins R, Hajnal AF, Harp SA, Kim SK. 1996. The *C. elegans* vulval induction gene *lin-2* encodes a member of the MAGUK family of cell junction proteins. *Development* 122: 97-111
- Hu H. 1999. Chemorepulsion of neuronal migration by Slit2 in the developing mammalian forebrain. *Neuron* 23: 703-11
- Huang X, Ketova T, Fleming JT, Wang H, Dey SK, et al. 2009. Sonic hedgehog signaling regulates a novel epithelial progenitor domain of the hindbrain choroid plexus. *Development* 136: 2535-43
- Huang X, Liu J, Ketova T, Fleming JT, Grover VK, et al. 2010. Transventricular delivery of Sonic hedgehog is essential to cerebellar ventricular zone development. *Proc Natl Acad Sci USA* 107: 8422-7
- Imai F, Hirai S, Akimoto K, Koyama H, Miyata T, et al. 2006. Inactivation of aPKC λ results in the loss of adherens junctions in neuroepithelial cells without affecting neurogenesis in mouse neocortex. *Development* 133: 1735-44
- Iwata T, Hevner RF. 2009. Fibroblast growth factor signaling in development of the cerebral cortex. *Dev Growth Differ* 51: 299-323

- Jan YN, Jan LY. 2001. Asymmetric cell division in the *Drosophila* nervous system. *Nat Rev Neurosci* 2: 772-9
- Kamberov E, Makarova O, Roh M, Liu A, Karnak D, et al. 2000. Molecular cloning and characterization of Pals, proteins associated with mLin-7. *J Biol Chem* 275: 11425-31
- Kappeler L, De Magalhaes Filho C, Dupont J, Leneuve P, Cervera P, et al. 2008. Brain IGF-1 receptors control mammalian growth and lifespan through a neuroendocrine mechanism. *PLoS Biol* 6: e254
- Kawaguchi A, Ikawa T, Kasukawa T, Ueda HR, Kurimoto K, et al. 2008. Single-cell gene profiling defines differential progenitor subclasses in mammalian neurogenesis. *Development* 135: 3113-24
- Kemphues KJ, Priess JR, Morton DG, Cheng NS. 1988. Identification of genes required for cytoplasmic localization in early *C. elegans* embryos. *Cell* 52: 311-20
- Kenney AM, Widlund HR, Rowitch DH. 2004. Hedgehog and PI-3 kinase signaling converge on Nmyc1 to promote cell cycle progression in cerebellar neuronal precursors. *Development* 131: 217-28
- Kim S, Lehtinen MK, Sessa A, Zappaterra MW, Cho SH, et al. 2010. The apical complex couples cell fate and cell survival to cerebral cortical development. *Neuron* 66: 69-84
- Klezovitch O, Fernandez TE, Tapscott SJ, Vasioukhin V. 2004. Loss of cell polarity causes severe brain dysplasia in Lgl1 knockout mice. *Genes Dev* 18: 559-71
- Knoblich JA. 2008. Mechanisms of asymmetric stem cell division. *Cell* 132: 583-97

- Konno D, Shioi G, Shitamukai A, Mori A, Kiyonari H, et al. 2008. Neuroepithelial progenitors undergo LGN-dependent planar divisions to maintain self-renewability during mammalian neurogenesis. *Nat Cell Biol* 10: 93-101
- Kosodo Y, Roper K, Haubensak W, Marzesco AM, Corbeil D, Huttner WB. 2004. Asymmetric distribution of the apical plasma membrane during neurogenic divisions of mammalian neuroepithelial cells. *Embo J* 23: 2314-24
- Kriegstein A, Alvarez-Buylla A. 2009. The glial nature of embryonic and adult neural stem cells. *Annu Rev Neurosci* 32: 149-84
- Kriegstein A, Noctor S, Martinez-Cerdeno V. 2006. Patterns of neural stem and progenitor cell division may underlie evolutionary cortical expansion. *Nat Rev Neurosci* 7: 883-90
- Lehtinen MK, Zappaterra MW, Chen X, Yang Y, Hill A, et al. 2011. The cerebrospinal fluid provides a proliferative niche for neural progenitor cells. *Neuron* In press
- Levine AJ, Brivanlou AH. 2006. GDF3 at the crossroads of TGF-beta signaling. *Cell Cycle* 5: 1069-73
- Li HS, Wang D, Shen Q, Schonemann MD, Gorski JA, et al. 2003. Inactivation of Numb and Numlike in embryonic dorsal forebrain impairs neurogenesis and disrupts cortical morphogenesis. *Neuron* 40: 1105-18
- Lien WH, Klezovitch O, Fernandez TE, Delrow J, Vasioukhin V. 2006. alphaE-catenin controls cerebral cortical size by regulating the hedgehog signaling pathway. *Science* 311: 1609-12

- Liu W, Ye P, O'Kusky JR, D'Ercole AJ. 2009. Type 1 insulin-like growth factor receptor signaling is essential for the development of the hippocampal formation and dentate gyrus. *J Neurosci Res* 87: 2821-32
- Louis DN. 2006. Molecular pathology of malignant gliomas. *Annu Rev Pathol* 1: 97-117
- Lowery LA, Sive H. 2009. Totally tubular: the mystery behind function and origin of the brain ventricular system. *Bioessays* 31: 446-58
- Mairet-Coello G, Tury A, DiCicco-Bloom E. 2009. Insulin-like growth factor-1 promotes G(1)/S cell cycle progression through bidirectional regulation of cyclins and cyclin-dependent kinase inhibitors via the phosphatidylinositol 3-kinase/Akt pathway in developing rat cerebral cortex. *J Neurosci* 29: 775-88
- Manabe N, Hirai S, Imai F, Nakanishi H, Takai Y, Ohno S. 2002. Association of ASIP/mPAR-3 with adherens junctions of mouse neuroepithelial cells. *Dev Dyn* 225: 61-9
- Manzini MC, Walsh CA. 2011. What disorders of cortical development tell us about the cortex: one plus one does not always make two. *Curr Opin Genet Dev*
- Margolis B, Borg JP. 2005. Apicobasal polarity complexes. *J Cell Sci* 118: 5157-9
- Margolis RU, Altszuler N. 1967. Insulin in the cerebrospinal fluid. *Nature* 215: 1375-6
- Marthiens V, French-Constant C. 2009. Adherens junction domains are split by asymmetric division of embryonic neural stem cells. *EMBO Rep* 10: 515-20

- Martin C, Bueno D, Alonso MI, Moro JA, Callejo S, et al. 2006. FGF2 plays a key role in embryonic cerebrospinal fluid trophic properties over chick embryo neuroepithelial stem cells. *Dev Biol* 297: 402-16
- Martin FH, Seoane JR, Baile CA. 1973. Feeding in satiated sheep elicited by intraventricular injections of CSF from fasted sheep. *Life Sci* 13: 177-84
- Martinez-Cerdeno V, Noctor SC, Kriegstein AR. 2006. The role of intermediate progenitor cells in the evolutionary expansion of the cerebral cortex. *Cereb Cortex* 16 Suppl 1: i152-61
- Mashayekhi F, Draper CE, Bannister CM, Pourghasem M, Owen-Lynch PJ, Miyan JA. 2002. Deficient cortical development in the hydrocephalic Texas (H-Tx) rat: a role for CSF. *Brain* 125: 1859-74
- Mason HA, Rakowiecki SM, Gridley T, Fishell G. 2006. Loss of notch activity in the developing central nervous system leads to increased cell death. *Dev Neurosci* 28: 49-57
- Mission JP, Takahashi T, Caviness VS, Jr. 1991. Ontogeny of radial and other astroglial cells in murine cerebral cortex. *Glia* 4: 138-48
- Mizutani K, Saito T. 2005. Progenitors resume generating neurons after temporary inhibition of neurogenesis by Notch activation in the mammalian cerebral cortex. *Development* 132: 1295-304
- Mizutani K, Yoon K, Dang L, Tokunaga A, Gaiano N. 2007. Differential Notch signalling distinguishes neural stem cells from intermediate progenitors. *Nature* 449: 351-5

- Mutch CA, Funatsu N, Monuki ES, Chenn A. 2009. Beta-catenin signaling levels in progenitors influence the laminar cell fates of projection neurons. *J Neurosci* 29: 13710-9
- Mutch CA, Schulte JD, Olson E, Chenn A. 2010. Beta-catenin signaling negatively regulates intermediate progenitor population numbers in the developing cortex. *PLoS One* 5: e12376
- Neugebauer JM, Amack JD, Peterson AG, Bisgrove BW, Yost HJ. 2009. FGF signalling during embryo development regulates cilia length in diverse epithelia. *Nature* 458: 651-4
- Nguyen-Ba-Charvet KT, Picard-Riera N, Tessier-Lavigne M, Baron-Van Evercooren A, Sotelo C, Chedotal A. 2004. Multiple roles for slits in the control of cell migration in the rostral migratory stream. *J Neurosci* 24: 1497-506
- Nishimura T, Kaibuchi K. 2007. Numb controls integrin endocytosis for directional cell migration with aPKC and PAR-3. *Dev Cell* 13: 15-28
- Noctor SC, Martinez-Cerdeno V, Ivic L, Kriegstein AR. 2004. Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. *Nat Neurosci* 7: 136-44
- Noctor SC, Martinez-Cerdeno V, Kriegstein AR. 2007. Contribution of intermediate progenitor cells to cortical histogenesis. *Arch Neurol* 64: 639-42
- Noctor SC, Martinez-Cerdeno V, Kriegstein AR. 2008. Distinct behaviors of neural stem and progenitor cells underlie cortical neurogenesis. *J Comp Neurol* 508: 28-44

- O'Rahilly R, Muller F. 1994. *The Embryonic Human Brain: An Atlas of Developmental Stages*. New York: Wiley-Liss, Inc.
- Ohata S, Aoki R, Kinoshita S, Yamaguchi M, Tsuruoka-Kinoshita S, et al. 2011. Dual Roles of Notch in Regulation of Apically Restricted Mitosis and Apicobasal Polarity of Neuroepithelial Cells. *Neuron* 69: 215-30
- Owen-Lynch PJ, Draper CE, Mashayekhi F, Bannister CM, Miyan JA. 2003. Defective cell cycle control underlies abnormal cortical development in the hydrocephalic Texas rat. *Brain* 126: 623-31
- Palmer TD, Willhoite AR, Gage FH. 2000. Vascular niche for adult hippocampal neurogenesis. *J Comp Neurol* 425: 479-94
- Parada C, Gato A, Bueno D. 2005a. Mammalian embryonic cerebrospinal fluid proteome has greater apolipoprotein and enzyme pattern complexity than the avian proteome. *J Proteome Res* 4: 2420-8
- Parada C, Gato A, Bueno D. 2008. All-trans retinol and retinol-binding protein from embryonic cerebrospinal fluid exhibit dynamic behaviour during early central nervous system development. *Neuroreport* 19: 945-50
- Parada C, Martin C, Alonso MI, Moro JA, Bueno D, Gato A. 2005b. Embryonic cerebrospinal fluid collaborates with the isthmic organizer to regulate mesencephalic gene expression. *J Neurosci Res* 82: 333-45

- Petersen PH, Zou K, Hwang JK, Jan YN, Zhong W. 2002. Progenitor cell maintenance requires numb and numbl like during mouse neurogenesis. *Nature* 419: 929-34
- Petersen PH, Zou K, Krauss S, Zhong W. 2004. Continuing role for mouse Numb and Numbl in maintaining progenitor cells during cortical neurogenesis. *Nat Neurosci* 7: 803-11
- Pexieder T, Jelinek R. 1970. Pressure of the CSF and the morphogenesis of the CNS. II. Pressure necessary for normal development of brain vesicles. *Folia Morphol (Praha)* 18: 181-92
- Pinal N, Goberdhan DC, Collinson L, Fujita Y, Cox IM, et al. 2006. Regulated and polarized PtdIns(3,4,5)P₃ accumulation is essential for apical membrane morphogenesis in photoreceptor epithelial cells. *Curr Biol* 16: 140-9
- Pomeroy SL, Tamayo P, Gaasenbeek M, Sturla LM, Angelo M, et al. 2002. Prediction of central nervous system embryonal tumour outcome based on gene expression. *Nature* 415: 436-42
- Pontious A, Kowalczyk T, Englund C, Hevner RF. 2008. Role of intermediate progenitor cells in cerebral cortex development. *Dev Neurosci* 30: 24-32
- Popken GJ, Hodge RD, Ye P, Zhang J, Ng W, et al. 2004. In vivo effects of insulin-like growth factor-I (IGF-I) on prenatal and early postnatal development of the central nervous system. *Eur J Neurosci* 19: 2056-68
- Raballo R, Rhee J, Lyn-Cook R, Leckman JF, Schwartz ML, Vaccarino FM. 2000. Basic fibroblast growth factor (Fgf2) is necessary for cell proliferation and neurogenesis in the developing cerebral cortex. *J Neurosci* 20: 5012-23

- Radakovits R, Barros CS, Belvindrah R, Patton B, Muller U. 2009. Regulation of radial glial survival by signals from the meninges. *J Neurosci* 29: 7694-705
- Rakic P. 2009. Evolution of the neocortex: a perspective from developmental biology. *Nat Rev Neurosci* 10: 724-35
- Randhawa R, Cohen P. 2005. The role of the insulin-like growth factor system in prenatal growth. *Mol Genet Metab* 86: 84-90
- Rao G, Pedone CA, Del Valle L, Reiss K, Holland EC, Fults DW. 2004. Sonic hedgehog and insulin-like growth factor signaling synergize to induce medulloblastoma formation from nestin-expressing neural progenitors in mice. *Oncogene* 23: 6156-62
- Rasin MR, Gazula VR, Breunig JJ, Kwan KY, Johnson MB, et al. 2007. Numb and Numbl are required for maintenance of cadherin-based adhesion and polarity of neural progenitors. *Nat Neurosci* 10: 819-27
- Redzic ZB, Preston JE, Duncan JA, Chodobski A, Szmydynger-Chodobska J. 2005. The choroid plexus-cerebrospinal fluid system: from development to aging. *Curr Top Dev Biol* 71: 1-52
- Rhyu MS, Jan, LY, Jan, YN. 1994. Asymmetric distribution of numb protein during division of the sensory organ presensory cell confers distinct fates to daughter cells. *Cell* 76: 477-491
- Ribes V, Wang Z, Dolle P, Niederreither K. 2006. Retinaldehyde dehydrogenase 2 (RALDH2)-mediated retinoic acid synthesis regulates early mouse embryonic forebrain development by controlling FGF and sonic hedgehog signaling. *Development* 133: 351-61

- Rohatgi R, Milenkovic L, Scott MP. 2007. Patched1 regulates hedgehog signaling at the primary cilium. *Science* 317: 372-6
- Sawamoto K, Wichterle H, Gonzalez-Perez O, Cholfin JA, Yamada M, et al. 2006. New neurons follow the flow of cerebrospinal fluid in the adult brain. *Science* 311: 629-32
- Schubert M, Brazil DP, Burks DJ, Kushner JA, Ye J, et al. 2003. Insulin receptor substrate-2 deficiency impairs brain growth and promotes tau phosphorylation. *J Neurosci* 23: 7084-92
- Shen Q, Goderie SK, Jin L, Karanth N, Sun Y, et al. 2004. Endothelial cells stimulate self-renewal and expand neurogenesis of neural stem cells. *Science* 304: 1338-40
- Shen Q, Wang Y, Kokovay E, Lin G, Chuang SM, et al. 2008. Adult SVZ stem cells lie in a vascular niche: a quantitative analysis of niche cell-cell interactions. *Cell Stem Cell* 3: 289-300
- Shimogori T, Banuchi V, Ng HY, Strauss JB, Grove EA. 2004. Embryonic signaling centers expressing BMP, WNT and FGF proteins interact to pattern the cerebral cortex. *Development* 131: 5639-47
- Shimojo H, Ohtsuka T, Kageyama R. 2008. Oscillations in notch signaling regulate maintenance of neural progenitors. *Neuron* 58: 52-64
- Siegenthaler JA, Ashique AM, Zarbalis K, Patterson KP, Hecht JH, et al. 2009. Retinoic acid from the meninges regulates cortical neuron generation. *Cell* 139: 597-609

- Siller KH, Doe CQ. 2008. Lis1/dynactin regulates metaphase spindle orientation in *Drosophila* neuroblasts. *Dev Biol* 319: 1-9
- Silver R, LeSauter J, Tresco PA, Lehman MN. 1996. A diffusible coupling signal from the transplanted suprachiasmatic nucleus controlling circadian locomotor rhythms. *Nature* 382: 810-3
- Simske JS, Kaech SM, Harp SA, Kim SK. 1996. LET-23 receptor localization by the cell junction protein LIN-7 during *C. elegans* vulval induction. *Cell* 85: 195-204
- Smart IH, Dehay C, Giroud P, Berland M, Kennedy H. 2002. Unique morphological features of the proliferative zones and postmitotic compartments of the neural epithelium giving rise to striate and extrastriate cortex in the monkey. *Cereb Cortex* 12: 37-53
- Smith CA, Lau KM, Rahmani Z, Dho SE, Brothers G, et al. 2007. aPKC-mediated phosphorylation regulates asymmetric membrane localization of the cell fate determinant Numb. *EMBO J* 26:468-80
- Soroceanu L, Kharbanda S, Chen R, Soriano RH, Aldape K, et al. 2007. Identification of IGF2 signaling through phosphoinositide-3-kinase regulatory subunit 3 as a growth-promoting axis in glioblastoma. *Proc Natl Acad Sci U S A* 104: 3466-71
- Spassky N, Han YG, Aguilar A, Strehl L, Besse L, et al. 2008. Primary cilia are required for cerebellar development and Shh-dependent expansion of progenitor pool. *Dev Biol* 317: 246-59

- Srinivasan K, Roosa J, Olsen O, Lee SH, Brecht DS, McConnell SK. 2008. MALS-3 regulates polarity and early neurogenesis in the developing cerebral cortex. *Development* 135: 1781-90
- Stancik EK, Navarro-Quiroga I, Sellke R, Haydar TF. 2010. Heterogeneity in ventricular zone neural precursors contributes to neuronal fate diversity in the postnatal neocortex. *J Neurosci* 30: 7028-36
- Stottmann RW, Tran PV, Turbe-Doan A, Beier DR. 2009. Ttc21b is required to restrict sonic hedgehog activity in the developing mouse forebrain. *Dev Biol* 335: 166-78
- Stoykova A, Gotz M, Gruss P, Price J. 1997. Pax6-dependent regulation of adhesive patterning, R-cadherin expression and boundary formation in developing forebrain. *Development* 124: 3765-77
- Sun Y, Goderie SK, Temple S. 2005. Asymmetric distribution of EGFR receptor during mitosis generates diverse CNS progenitor cells. *Neuron* 45: 873-86
- Sutter NB, Bustamante CD, Chase K, Gray MM, Zhao K, et al. 2007. A single IGF1 allele is a major determinant of small size in dogs. *Science* 316: 112-5
- Tajbakhsh S, Rocheteau P, Le Roux I. 2009. Asymmetric cell divisions and asymmetric cell fates. *Annu Rev Cell Dev Biol* 25: 671-99
- Takahashi T, Misson JP, Caviness VS, Jr. 1990. Glial process elongation and branching in the developing murine neocortex: a qualitative and quantitative immunohistochemical analysis. *J Comp Neurol* 302: 15-28

- Tanori M, Santone M, Mancuso M, Pasquali E, Leonardi S, et al. 2010. Developmental and oncogenic effects of insulin-like growth factor-I in Ptc1^{+/-} mouse cerebellum. *Mol Cancer* 9: 53
- Tao Y, Black IB, DiCicco-Bloom E. 1997. In vivo neurogenesis is inhibited by neutralizing antibodies to basic fibroblast growth factor. *J Neurobiol* 33: 289-96
- Tascioglu AO, Tascioglu AB. 2005. Ventricular anatomy: Illustrations and concepts from antiquity to Renaissance. *Neuroanatomy* 4: 57-63
- Tavazoie M, Van der Veken L, Silva-Vargas V, Louissaint M, Colonna L, et al. 2008. A specialized vascular niche for adult neural stem cells. *Cell Stem Cell* 3: 279-88
- Taverna E, Huttner WB. 2010. Neural progenitor nuclei IN motion. *Neuron* 67: 906-14
- Thornton GK, Woods CG. 2009. Primary microcephaly: do all roads lead to Rome? *Trends Genet* 25: 501-10
- Town T, Breunig JJ, Sarkisian MR, Spilianakis C, Ayoub AE, et al. 2008. The stumpy gene is required for mammalian ciliogenesis. *Proc Natl Acad Sci U S A* 105: 2853-8
- Toyoda R, Assimacopoulos S, Wilcoxon J, Taylor A, Feldman P, et al. 2010. FGF8 acts as a classic diffusible morphogen to pattern the neocortex. *Development* 137: 3439-48
- Vescovi AL, Reynolds BA, Fraser DD, Weiss S. 1993. bFGF regulates the proliferative fate of unipotent (neuronal) and bipotent (neuronal/astroglial) EGF-generated CNS progenitor cells. *Neuron* 11: 951-66

- Vierkotten J, Dildrop R, Peters T, Wang B, Ruther U. 2007. Ftm is a novel basal body protein of cilia involved in Shh signalling. *Development* 134: 2569-77
- von Stein W, Ramrath A, Grimm A, Muller-Borg M, Wodarz A. 2005. Direct association of Bazooka/PAR-3 with the lipid phosphatase PTEN reveals a link between the PAR/aPKC complex and phosphoinositide signaling. *Development* 132: 1675-86
- Wallace VA. 1999. Purkinje-cell-derived Sonic hedgehog regulates granule neuron precursor cell proliferation in the developing mouse cerebellum. *Curr Biol* 9: 445-8
- Wang J, Wynshaw-Boris A. 2004. The canonical Wnt pathway in early mammalian embryogenesis and stem cell maintenance/differentiation. *Curr Opin Genet Dev* 14: 533-9
- Weber MM, Melmed S, Rosenbloom J, Yamasaki H, Prager D. 1992. Rat somatotroph insulin-like growth factor-II (IGF-II) signaling: role of the IGF-I receptor. *Endocrinology* 131: 2147-53
- Wechsler-Reya RJ, Scott MP. 1999. Control of neuronal precursor proliferation in the cerebellum by Sonic Hedgehog. *Neuron* 22: 103-14
- Willaredt MA, Hasenpusch-Theil K, Gardner HA, Kitanovic I, Hirschfeld-Warneken VC, et al. 2008. A crucial role for primary cilia in cortical morphogenesis. *J Neurosci* 28: 12887-900

- Wirtz-Peitz, F, Nishimura, T, Knoblich, JA. 2008. Linking cell cycle to asymmetric division: Aurora-A phosphorylates the Par complex to regulate Numb localization. *Cell* 135: 161-173
- Wodarz A. 2005. Molecular control of cell polarity and asymmetric cell division in *Drosophila* neuroblasts. *Curr Opin Cell Biol* 17: 475-81
- Wolpert L. 1996. One hundred years of positional information. *Trends Genet* 12: 359-64
- Wrobel CN, Mutch CA, Swaminathan S, Taketo MM, Chenn A. 2007. Persistent expression of stabilized beta-catenin delays maturation of radial glial cells into intermediate progenitors. *Dev Biol* 309: 285-97
- Wu H, Feng W, Chen J, Chan LN, Huang S, Zhang M. 2007. PDZ domains of Par-3 as potential phosphoinositide signaling integrators. *Mol Cell* 28: 886-98
- Ye P, Xing Y, Dai Z, D'Ercole AJ. 1996. In vivo actions of insulin-like growth factor-I (IGF-I) on cerebellum development in transgenic mice: evidence that IGF-I increases proliferation of granule cell progenitors. *Brain Res Dev Brain Res* 95: 44-54
- Zappaterra MD, Lisgo SN, Lindsay S, Gygi SP, Walsh CA, Ballif BA. 2007. A comparative proteomic analysis of human and rat embryonic cerebrospinal fluid. *J Proteome Res* 6: 3537-48
- Zhang J, Woodhead GJ, Swaminathan SK, Noles SR, McQuinn ER, et al. 2010. Cortical neural precursors inhibit their own differentiation via N-cadherin maintenance of beta-catenin signaling. *Dev Cell* 18: 472-9

Zheng W, Chodobski, A, eds. 2005. *The blood-cerebrospinal fluid barrier*. Boca Raton: Taylor & Francis Group.

Zhou CJ, Borello U, Rubenstein JL, Pleasure SJ. 2006. Neuronal production and precursor proliferation defects in the neocortex of mice with loss of function in the canonical Wnt signaling pathway. *Neuroscience* 142: 1119-31

Zhou Y, Atkins JB, Rompani SB, Bancescu DL, Petersen PH, et al. 2007. The mammalian Golgi regulates numb signaling in asymmetric cell division by releasing ACBD3 during mitosis. *Cell* 129: 163-78

KEYWORDS

1. Adherens junctions
2. Apical complex
3. Cerebral cortex
4. Neural progenitor cell
5. Polarity
6. Signaling

ABSTRACT

Cerebral cortical progenitor cells integrate cell-intrinsic and cell-extrinsic cues to regulate neurogenesis. Cell-intrinsic mechanisms depend largely upon appropriate apical-basal polarity established by adherens junctions and apical complex proteins. These protein complexes regulate mitotic spindle orientation, and patterns of inheritance of these asymmetrically localized proteins regulate cell fate. However, remarkably little has been known about how cell-extrinsic cues signal to progenitors, and couple with cell-intrinsic mechanisms to instruct neurogenesis. Recent research shows that the cerebrospinal fluid, which contacts apical progenitors at the ventricular surface, provides growth and survival promoting cues for the developing brain. This review addresses how apical-basal polarity of progenitor cells regulates cell fate and allows progenitors to sample diffusible signals distributed by the cerebrospinal fluid. We also review several classes of signaling factors that are distributed by the CSF to the developing brain to instruct neurogenesis.

TERMS/DEFINITIONS

1. Adherens junctions – ring-like, cadherin containing, contacts between cells that regulate cell-cell adhesion
2. Apical complex – Par polarity proteins that in mammalian neural precursors assemble as 3 complexes: Crb/Pals1/Patj, Par3/Par6/aPKC, and Mals/Pals1.
3. Cerebrospinal fluid – fluid that fills the ventricles, spinal canal, and subarachnoid space surrounding the central nervous system.
4. Neural progenitor cell – neural stem cell that gives rise to future neurons in the brain
5. Neurogenesis – the process of generating neurons from progenitor cells

ACRONYMS LIST

1. VZ – ventricular zone
2. SVZ – subventricular zone
3. OSVZ – outer subventricular zone
4. CSF – cerebrospinal fluid
5. Par3/Par6/aPKC – Partitioning defective “Par” protein complex known as apical complex in mammalian cells
6. Fgf – Fibroblast growth factor
7. Igf – Insulin-like growth factor
8. RA – Retinoic acid
9. Shh – Sonic hedgehog
10. GBM – glioblastoma multiforme

SUMMARY POINTS

1. The apical complex proteins are necessary for progenitor cell proliferation.
2. Apical complex is also critical for cell survival.
3. The apical complex allows for the integration of cell-intrinsic and cell-extrinsic signals by progenitor cells.
4. The apical complex may regulate neuronal differentiation by controlling progenitor access to the CSF.
5. The CSF is a rich and dynamic source of proteins for the brain.
6. The enrichment of growth factor receptors along the ventricular surface of the developing brain allows progenitors to receive signals distributed in the CSF.
7. Embryonic CSF provides growth and survival promoting factors including Igf2 to the developing brain.
8. The active distribution of diffusible signals in the CNS may extend to the regulation of CNS diseases, including brain tumors.

FUTURE ISSUES

1. How can we better understand the sources and regulation of CSF-distributed factors?
2. How might local gradients and ventricle-specific CSF composition influence the development and functioning of distinct brain regions?
3. Will CSF-distributed factors play an active role in instructing the adult neural stem cells as well?
4. Brain development is typically considered to take place independent of the rest of the body. Given the role of CSF in guiding development and the distribution of extracellular

signals therein, to what extent do signals originating from outside the CNS actually influence CNS development and maintenance?

ANNOTATED REFERENCES

1. Martin et al. 2006, *Dev Biol*
This study showed that CSF-FGF2 stimulates proliferation of chick midbrain progenitor cells.
2. Sawamoto et al. 2006, *Science*
This study provided the first compelling evidence that gradients of CSF-distributed factors actively influence the brain.
3. Costa et al., 2008, *Development*
This study demonstrated that Par3 and Par6 are key players in promoting progenitor polarity and proliferation in the developing cerebral cortex.
4. Bultje et al. 2009, *Neuron*
This study showed that Par3 distributes unequally in cells and acts as a cell-autonomous regulator of Notch activity.
5. Siegenthaler et al. 2009, *Cell*
This study demonstrated that meningeally derived Retinoic acid regulates neurogenesis.
6. Huang et al. 2010, *PNAS*
This study demonstrated that hindbrain Shh signaling, distributed in the CSF, regulates choroid plexus and cerebellar development.
7. Kim et al. 2010, *Neuron*

This study showed that Pals1 and the apical complex are indispensable for progenitor proliferation, survival of newly generated neurons, and the development of the cerebral cortex.

8. Toyoda et al. 2010, *Development*

This study showed that Fgf8 generates classical diffusion gradients in the developing brain.

9. Lehtinen et al. In press, *Neuron*

This study showed that the CSF, and in particular CSF-Igf2, provides essential growth and survival promoting cues for neural stem cells. It also uncovered that CSF-Igf2 levels associate with advanced tumor progression in glioblastoma multiforme.

RELATED RESOURCES

1. He, S, Nahada, D, Morrison, SJ. 2009. Mechanisms of stem cell self-renewal. *Ann Rev Cell Dev Biol* 25:377-406.
2. Nachury, MV, Seeley, ES, Jin H. 2010. Trafficking to the ciliary membrane: How to get across the periciliary diffusion barrier? *Ann Rev Cell Dev Biol* 26:59-87.
3. <http://jaxmice.jax.org/jaxnotes/archive/445q.html>
4. <http://jaxmice.jax.org/jaxnotes/archive/490f.html>

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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FIGURE LEGENDS

Figure 1 – Progenitor cells in the developing mammalian cerebral cortex.

Cerebral cortical progenitor cells divide in three principal locations in the developing mammalian brain: The apical ventricular zone (VZ), the inner subventricular zone (SVZ), and the outer subventricular zone (OSVZ). Progenitors in the apical progenitor pool can be divided into two principal types of progenitor cells: neuroepithelial cells and radial glial cells. Progenitors in the SVZ consist of the basal progenitor cell pool. The newest class of progenitors constituting the OSVZ are more apparent in mammals with larger brains.

Figure 2 – Apical-basal polarity in cortical progenitor cells.

Apical progenitor cells have a distinct apical-basal polarity. Their apical surface contacts the cerebrospinal fluid (CSF) that fills the ventricles, while their basal processes extend to and contact the meninges, basal lamina, and vasculature. The adhesion of adjacent progenitor cells to each other is maintained by adherens junctions, which are cadherin-containing contacts between cells. The adherens junctions also define the border of the apical membrane domain that contacts the CSF. The adherens junctions and apical membrane are home to the apical complex proteins, which play an active role in polarizing cellular proteins. The unequal inheritance of the apical membrane and associated proteins is thought to determine whether dividing cells generate pairs of daughter cells with the same, symmetric, cell fate (two progenitor cells) or cells with distinct, asymmetric, cell fates (i.e. one progenitor and one neuron).

Figure 3 – Cerebrospinal fluid flow during embryonic brain development and in adulthood. Schematic of the cerebroventricular system during early human brain development

and in the mature adult brain. **(A)** Upon anterior neural tube closure, the three primary brain vesicles (Telencephalic, Mesencephalic, and Rhombencephalic vesicles) serve as the rudimentary cerebroventricular system for the developing CNS. Human Carnegie Stages (CS) 11 corresponds to approximately E8.5-E9.75 during mouse embryogenesis, and CS13-15 corresponds to approximately E10-E11.25 during mouse embryogenesis. **(B)** In the mature CNS, CSF generated primarily by the choroid plexus tissues located in each ventricle in the brain fills the ventricles, the subarachnoid space, and spinal canal. CSF flows from the lateral ventricles via the Foramen of Munro/intraventricular foramen into the mesencephalic/third ventricle, and then via the aqueduct of Sylvius/cerebral aqueduct into the hindbrain/fourth ventricle. The CSF then continues through the foramina of Magendie/Median aperture and foramina of Luschka/Lateral apertures into the subarachnoid space and spinal canal, and is finally resorbed into the venous system via arachnoid villi. An adult human circulates approximately 150ml CSF within the cerebroventricular system. The CSF is estimated to turn over approximately 3-4 times per day, indicating that the healthy CNS produces close to 500ml CSF daily. Thus, the choroid-plexus-CSF system is poised to act as a spatially synchronized medium for triggering local and global changes in molecular signaling across vast distances in the mammalian CNS.

Figure 4 – The cerebrospinal fluid distributes diffusible factors during brain development.

(A) Igf2 is secreted by the choroid plexus and delivered by the CSF to targets on the apical ventricular surface of the developing cerebral cortex. CSF-Fgf2 has been shown to also promote proliferation of midbrain progenitor cells. Whether similar rules apply to CSF-borne Retinoic acid, Wnt, and Bmp signaling, as well as other as yet uncharacterized signals, remains to be elucidated. **(B)** Shh secreted by the hindbrain/fourth ventricle choroid plexus signals in an

autocrine manner to instruct choroid plexus development. CSF-Shh also signals in a paracrine manner to stimulate the proliferation of cerebellar granule neuron precursors located in the external granule cell layer.